Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 68 (2004) 1041–1047
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Modulation of poly(ADP-ribosylation) in apoptotic cells

A. Ivana Scovassi[a,*], Marc Diederich[b]

[a]*Istituto di Genetica Molecolare CNR, Via Abbiategrasso 207, I-27100 Pavia, Italy*
[b]*Laboratoire de Biologie Moléculaire et Cellulaire du Cancer (LBMCC), Hôpital Kirchberg, 9 rue Edward Steichen L-2540, Luxembourg*

Received 29 March 2004; accepted 23 April 2004

Abstract

Poly(ADP-ribosylation) is a post-translational modification of proteins playing a crucial role in DNA repair, replication, transcription and cell death. In this paper, the main features of this process have been reviewed, focusing on the best known poly(ADP-ribose) polymerizing enzyme, PARP-1, a DNA nick-sensor protein that uses β-NAD^+ to form polymers of ADP-ribose. The modulation of poly(ADP-ribosylation) during apoptosis and the possible effects of its inhibition on cell metabolism are discussed.

Keywords: Poly(ADP-ribosylation); PARP-1; PARG; Apoptosis; Anticancer therapy; NF-κB

1. Poly(ADP-ribosylation): a sensor of DNA damage

The poly(ADP-ribosylation) reaction, discovered 40 years ago [1], is a post-translational modification of proteins involved in DNA repair, replication, transcription and cell death [2–6]. Poly(ADP-ribose) metabolism is regulated by the concerted action of PARP and PARG. In the presence of DNA strand breaks, PARP-1 uses the substrate β-NAD^+ for transferring ADP-ribose polymers to itself and to nuclear acceptor proteins. Polymers are rapidly removed by PARG, which catalyzes the hydrolysis of the ribosyl–ribose glycosidic bonds of linear and branched polymers. This enzyme displays both endo- and exo-glycosidic activities, thus producing short polymers and free ADP-ribose [7]. The basic features of poly(ADP-ribosylation) reactions are shown in Fig. 1.

The best known poly(ADP-ribose) polymerase, PARP-1, is a 113 kDa zinc-finger nuclear protein activated by DNA breaks and using the NAD^+ molecule to catalyze the synthesis of polymers of ADP-ribose on nuclear proteins. PARP-1 has three functional domains: the DNA binding domain (DBD, 46 kDa), located at the N-terminus, which contains the bipartite nuclear localization sequence (NLS) and two Zn fingers; the automodification domain (22 kDa) which mediates PARP-1 autoribosylation; the C-terminus catalytic domain (54 kDa), which is essential for the conversion of NAD^+ into ADP-ribose. In addition to the classical nuclear PARP-1, which has for decades been the only PARP known, novel nuclear and extra-nuclear ADP-ribose-polymerizing enzymes have been recently described, including PARP-2, PARP-3, Tankyrase-1 and -2, VPARP and TiPARP [2–6].

PARP-1 acts as an endogenous detection system for DNA breaks induced by a variety of environmental stimuli, including free radicals and oxidant molecules [8]. As shown in Fig. 2, PARP-1 binds to DNA breaks and generates polymers of ADP-ribose bound to chromatin-associated proteins, including itself. The negative charge of polymers allows the dissociation of automodified PARP-1 from DNA, thus making it accessible to DNA repair enzymes. PARP-1 inhibitors in combination with DNA damaging agents were found to increase DNA damage, thus suggesting the involvement of PARP-1 in DNA repair [9]. These data were further supported by the observation that mouse strains with a disrupted PARP-1 gene [10–12] exhibit genomic instability after ionizing radiation and alkylating agents [13]. The role of PARP-1 in the base excision repair (BER) mechanism was demonstrated by different approaches [14,15]; the conversion of NAD^+ to ADP-ribose during DNA repair may account for the generation of ATP, which is required for the final ligation step

Abbreviations: NAD, nicotinamide adenine dinucleotide; PARG, poly(-ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerase

* Corresponding author. Tel.: +39 0382 546334/8; fax: +39 0382 422286.
E-mail address: scovassi@igm.cn.it (A. Ivana Scovassi).

0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.04.023

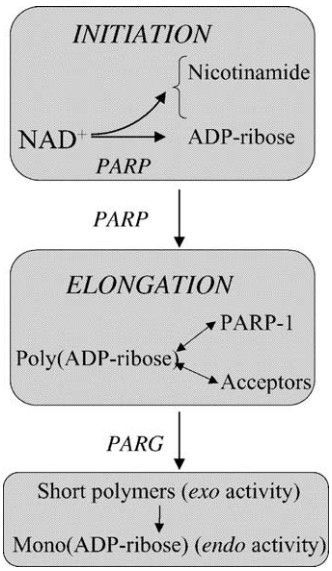

Fig. 1. Poly(ADP-ribosylation) reactions. Initiation step: poly(ADP-ribose) polymerase converts NAD^+ into ADP-ribose, with the release of nicotinamide and protons. Elongation step: PARP-1 produces polymers of ADP-ribose and binds them covalently to itself and to nuclear acceptor proteins. Poly(ADP-ribose) metabolism is controlled by poly(ADP-ribose) glycohydrolase (PARG), which displays both an exoglycosidic activity, thus detaching ADP-ribose from the distal portion of polymers, and an endoglycosidic activity that shorten polymers.

of BER [16]. In cells with inhibited PARP-1, a defective p53-mediated response to DNA damage was found, dependent on the nature of the damaging agent [17–23]. Moreover, it has been shown that P53 acts as an acceptor of poly(ADP-ribose) and that this event modulates its DNA binding and oligomerization functions [24–28]. The recent development of mice deficient for both PARP-1 and p53 contributed to a better elucidation of the cooperation between the two proteins in maintaining chromosome stability and in protecting from tumor development [23,29–31]. As an active member of the surveillance network against DNA damage, PARP-1 also interacts with the Werner syndrome protein [32–34] in a complex, which includes Ku70/80 [35], a component of DNA-PK holoenzyme able to recognize and bind DNA double strand breaks. PARP-1 was described to act synergistically with another factor involved in the response to DNA strand breaks, i.e. ATM [36,37].

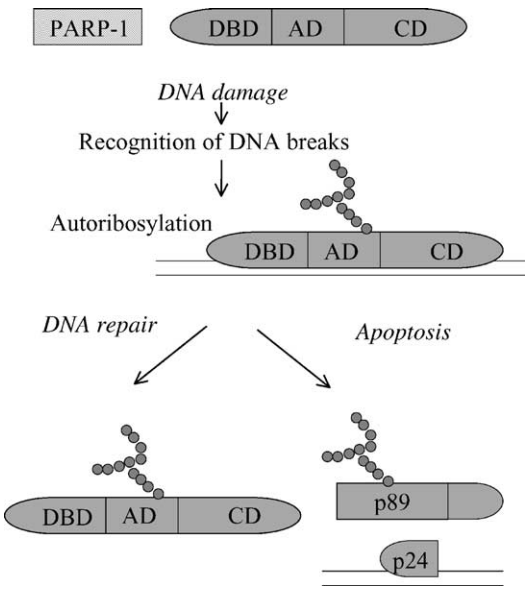

Fig. 2. Involvement of PARP-1 in DNA repair and apoptosis. PARP-1, a 113 kDa protein, has three functional domains: at the N-terminus, the DNA-binding domain (DBD); the central automodification domain (AD); the C-terminal catalytic domain (CD). PARP-1 is a DNA nick sensor recruited to DNA damage sites where it poly(ADP-ribosylates) itself. During DNA repair, autoribosylated PARP-1 is detaching from DNA, thus allowing DNA damage accessible to DNA repair factors. During apoptosis, to avoid excessive NAD consumption, autoribosylated PARP-1 is cleaved by caspases into the p24 and p89 inactive fragments.

2. Poly(ADP-ribosylation) and apoptosis

Once activated, PARP-1 produces polymers of ADP-ribose, thus allowing the depletion of intracellular NAD^+ and a fall in ATP. Cellular NAD^+ regulates several vital cellular processes, and serves also as precursor for NADP, a cofactor for different synthetic pathways [6]. In this respect, years ago it was proposed that NAD consumption may lead to cell suicide [38]; in fact, intracellular ATP is relevant in driving the apoptotic response and to prevent necrosis [39–41].

During apoptosis, a precocious and transient stimulation of PARP-1 causes poly(ADP-ribose) accumulation in early apoptotic cells [42–46]. Excessive NAD consumption is prevented by the cleavage of PARP-1 by caspases (reviewed in [47]), i.e. cysteine proteases able to cleave after an aspartic acid [48]. As shown in Fig. 2, PARP-1 cleavage generates two inactive fragments of 24 and 89 kDa. The N-terminal fragment (p24) remains in the nucleolus, retains its DNA-binding activity and inhibits the catalytic activity of uncleaved PARP-1, and also impairs DNA repair [49,50]. The p89 fragment migrates from the nucleus to the cytoplasm in late apoptotic cells with advanced nuclear fragmentation [46,51,52], becoming a potential target of autoimmunity [53].

A further evidence for the involvement of poly(ADP-ribosylation) in cell death machinery is provided by the observation that PARP-1 is required for the translocation of the AIF (Apoptosis-Inducing Factor) protein from the mitochondria to the nucleus, with the consequent activation of a caspase-independent apoptotic pathway [54]. The molecular mechanisms triggering the release of AIF are still obscure [55], even if the recent evidence of an intramitochondrial PARP could support its direct effect on AIF [56]. Remarkably, during apoptosis poly(ADP-ribosylation) regulates the activity of DNAS1L3, a Ca^{2+}/Mg^{2+}-dependent nuclease that is normally repressed by poly(-ADP-ribose). PARP-1 inhibition was shown to promote its release, with a consequent DNA fragmentation and cell death [57,58].

3. Protective effect of PARP inhibitors

Although poly(ADP-ribosylation) may be beneficial, a massive PARP-1 activation under damage conditions can be detrimental to the tissue because of energy depletion and consequent occurrence of necrotic death. PARP-1 inhibition by chemicals, by the antisense strategy, by creating dominant negative mutants or PARP-$1^{-/-}$ mice, could interfere with apoptosis to a different extent [10–13,59–65], possibly depending on the nature of the apoptogenic stimulus.

During inflammation, ischemia/reperfusion or shock, the generation of free radicals, reactive oxygen species, and peroxynitrite can activate PARP-1, leading to NAD depletion [66–69]. Pharmacological inactivation of PARP-1 represents a novel therapeutical strategy to limit cellular injury and to improve the outcome of a variety of inflammatory conditions. Genetic deletion of PARP-1 supports the critical role of poly(ADP-ribosylation) in the pathogenesis of different diseases (reviewed in [70]). Accordingly, it has been shown that PARP-1 inhibition by chemical compounds exerts a protective effect toward a number of diseases, including cancer [71,72]. PARP-1 inhibitors can be used as chemosensitizing/radiosensitizing agents, being effective against several tumors [73–80]. Remarkably, they reduce nephrotoxicity [81] and acute photodamage caused by chemotherapeutic drugs [82]. Also the inhibition of tankyrase activity, which regulates the function of TRF-1 and TRF-2 telomeric proteins via poly(ADP-ribosylation), could represent a good strategy to repress telomere maintenance in tumor cells, thus promoting chemotherapy-induced apoptosis (reviewed in [83]).

4. Protective effect of PARG inhibitors

PARG regulates poly(ADP-ribose) turnover by releasing monomers of ADP-ribose and by shortening long polymers (exo/endo activity, Fig. 1) [84]. During apoptosis, PARG is a target of caspases [85,86]. Since PARG inhibition could influence PARP-1-mediated cell death either by making poly(ADP-ribose) turnover slower or causing PARP-1 inhibition by massive PARP-1 autoribosylation, PARG is now considered as a cell death mediator [87]. Accordingly to this view, some compounds that inhibit PARG activity were found to be effective in rescuing neuronal cell death [87–89]. Remarkably, experiments carried out on *Drosophila* revealed that the genetic deletion of PARG allows neurodegeneration [90].

5. Future perspectives

The assumption that poly(ADP-ribosylation) plays a crucial role in carcinogenesis (reviewed in [91]) legitimates the growing interest in developing PARP-1 and PARG inhibitors to improve the efficacy of radio- and chemo-therapy. Since PARP-1 shares the role of guardian of genome integrity with other factors, e.g. DNA-PK, a promising strategy for tumor sensitization could be represented by the combined use of inhibitors of PARP-1 and DNA-PK [92]. On the other hand, it is well known that many agents, including chemotherapic drugs, induce DNA damage via the production of oxygen radicals, thus generating oxidatively modified proteins, which can be degraded by the 20S proteasome to facilitate a correct DNA repair. Since proteasome is activated by PARP-1 [93], the efficacy in removing oxidized proteins can be impaired by PARP-1 inhibitors, thus favoring damage accumulation and possibly driving tumoral cells to death [94]. Similarly, it has been reported that PARP-1 interacts with transcription factors of the NF-κB family [95–98]. Since NF-κB activity can confer resistance to apoptosis, the inhibition of NF-κB-dependent gene expression by PARP-1 could be a potentially efficient strategy to promote apoptosis. Accordingly, proteasome inhibitors, such as PS-341, but also natural NF-κB inhibitors including curcumin [99] could modulate the anti-apoptotic effects.

The present renaissance of PARP-1 inhibitors, based on the modeling of new compounds, may be helpful in promoting the pharmacological inactivation of PARP-1 as a novel therapeutical strategy to limit cellular injury, to improve the outcome of a variety of inflammatory conditions and to enhance the efficacy of anticancer therapies. However, long-term studies are necessary to estimate the risks and benefits associated with therapeutic PARP-1 and/or PARG inhibition. This aspect is particularly relevant if one considers the crucial role of poly(ADP-ribosylation) in the maintenance of chromatin structure [100,101] and in regulating transcription [5,100].

Finally, it is important to remind that the molecular mechanisms at the origin of PARP-1 gene expression largely remain to be elucidated. It is known that human PARP-1 promoter lacks a functional consensus TATA box, is GC-rich, and contains a consensus initiator sequence surrounding the transcription start site. Moreover, it has potential binding sites for several transcription factors, including Sp1, AP-2, YY1, and Ets [102]. Additional sequence analyzes give hints about regulatory pathways, which remain yet to be characterized by molecular and cellular technologies (Fig. 3). Novel ways of PARP-1 regulation would then promise additional therapeutic perspectives aiming at the reduction of PARP-1 expression by the way of lowering the constitutive gene expression mechanisms.

The following recent monographs have been devoted to poly(ADP-ribosylation):

- G. de Murcia, S. Shall (Eds.), From DNA Damage and Stress Signalling to Cell Death. Poly ADP Ribosylation Reactions, Oxford University Press, 2000.

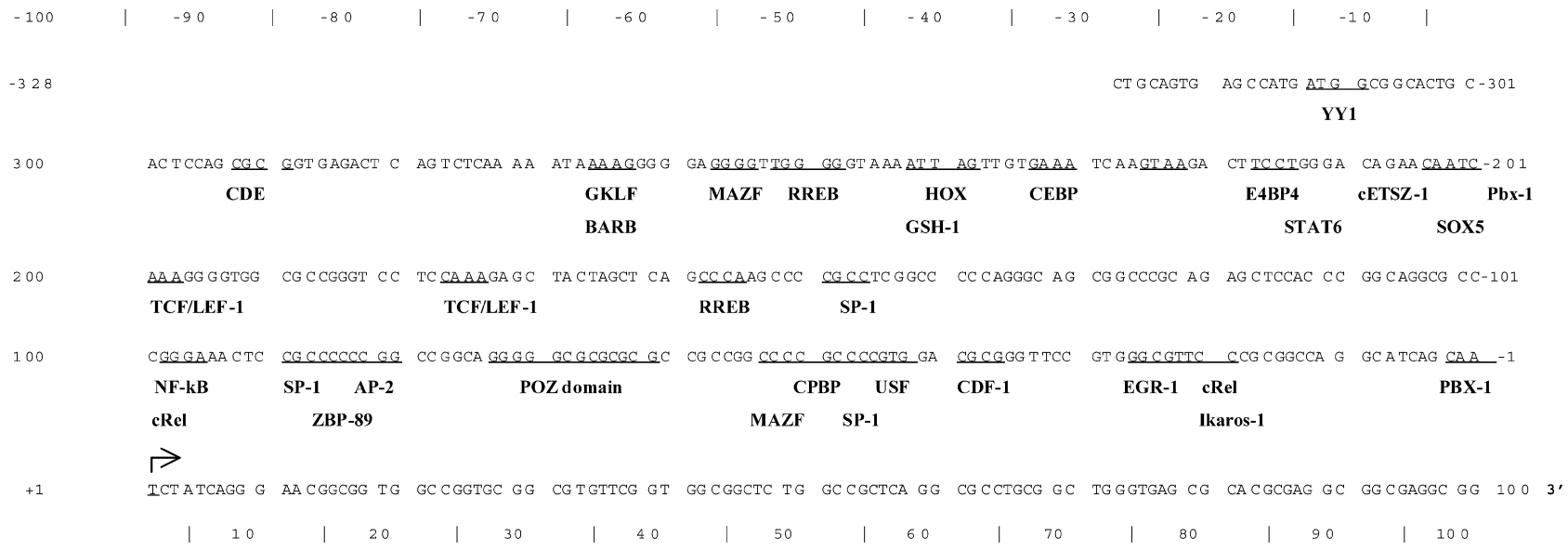

Fig. 3. PARP-1 gene promoter. Putative transcription factor binding sites within the PARP-1 promoter gene are represented. The arrow designates the transcriptional start site. YY1, Yin and Yang 1; CDE, cell cycle-dependent element; GKLF, gut-enriched Krueppel-like factor; BARB, barbiturate-inducible element; MAZF, MYC-associated zinc finger protein related transcription factor; RREB, Ras-responsive element binding protein 1; HOX, muscle segment homeo box 2 homologue of Drosophila (HOX 8); GSH-1, homeobox transcription factor Gsh-1; CEBP, CCAAT/enhancer binding protein beta; E4BP4, bZIP domain, transcriptional repressor; STAT6, signal transducer and activator of transcription 6; cETSZ-1, c-Ets-1 binding site; Pbx-1, homeo domain factor Pbx-1; TCF/LEF-1, involved in the Wnt signal transduction pathway; SP-1, GC box elements; NF-kappaB, nuclear factor-kappa B; AP-2, Activator protein 2; c-Rel, NF-kB related; ZBP-89, zinc finger transcription factor ZBP-89; POZ, zinc finger; SP-1, stimulating protein 1; CPBP, core promoter-binding protein with three Krueppel-type zinc fingers; MAZF, MYC-associated zinc finger protein related transcription factor; USF, ubiquitous zinc finger transcription factor, Upstream stimulating factor; CDF-1, cell cycle-dependent element; EGR-1, Egr-1/Krox-24/NGFI-A immediate-early gene product; Ikaros 1, potential regulator of lymphocyte differentiation.

- C. Szabó (Ed.), Cell Death. The Role of PARP, CRC Press, Boca Raton, FL, 2000.
- J. Zhang (Ed.), PARP as a Therapeutic Target, CRC Press, Boca Raton, FL, 2002.
- A. Bürkle (Ed.), Poly(ADP-ribosyl)ation, Landes Bioscience, 2004.

Acknowledgments

Research at the laboratory of AIS is supported by the Italian CNR and MIUR (FIRB Project RBNE0132MY). MD's work is supported by the ''Fondation de Recherche Cancer et Sang'', the ''Recherches Scientifiques Luxembourg'' Association, Télévie (grant 7.4577.02) and ''Een Häerz fir kriibskrank Kanner'' Association.

References

[1] Chambon P, Weill JD, Mandel P. Nicotinamide mononucleotide activation of a new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. Biochem Biophys Res Commun 1963;11:39–43.
[2] Bürkle A. Physiology and pathophysiology of poly(ADP-ribosyl)ation. BioEssays 2001;23:795–806.
[3] Smith S. The world according to PARP. Trends Biochem Sci 2001;26:174–9.
[4] Chiarugi A. Poly(ADP-ribose) polymerase: killer or conspirator? The 'suicide hypothesis' revisited. Trends Pharmacol Sci 2002;23:122–9.
[5] Kraus WL, Lis JT. PARP goes transcription. Cell 2003;113:677–83.
[6] Berger F, Ramirez-Hernandez MH, Ziegler M. The new life of a centenarian: signaling functions of NAD(P). Trends Biochem Sci 2004;29:111–8.
[7] D'Amours D, Desnoyers S, D'Silva I, Poirier GG. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J 1999;342:249–68.
[8] Bürkle A. Poly(ADP-ribosyl)ation, a DNA damage-driven protein modification and regulator of genomic instability. Cancer Lett 2001;63:1–5.
[9] Durkacz BW, Omidiji O, Gray DA, Shall S. (ADP-ribose)$_n$ participates in DNA excision repair. Nature 1980;283:593–6.
[10] Wang ZQ, Auer B, Stingl L, Berghammer H, Haidacher D, Schweiger M, et al. Mice lacking ADPRT and poly(ADP-ribosyl)ation develop normally but are susceptible to skin disease. Genes Dev 1995;9:509–20.
[11] Ménissier-de Murcia JM, Niedergang C, Trucco C, Ricoul M, Dutrillaux B, Mark M, et al. Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. Proc Natl Acad Sci USA 1997;94:7303–7.
[12] Masutani M, Suzuki H, Kamada N, Watanabe M, Ueda O, Nozaki T, et al. Function of poly(ADP-ribose) polymerase in response to DNA damage: gene-disruption study in mice. Proc Natl Acad Sci USA 1999;96:2301–4.
[13] Shall S, de Murcia G. Poly(ADP-ribose) polymerase-1: what have we learned from the deficient mouse model? Mutat Res 2000;460:1–15.
[14] Dantzer F, Schreiber V, Niedergang C, Trucco C, Flatter E, de la Rubia G, et al. Involvement of poly(ADP-ribose) polymerase in base excision repair. Biochimie 1999;81:69–75.
[15] Prasad R, Lavrik OI, Kim S-J, Kedar P, Yang X-P, Vande Berg BJ, et al. DNA polymerase beta-mediated long patch base excision repair. Poly(ADP-ribose)polymerase-1 stimulates strand displacement DNA synthesis. J Biol Chem 2001;276:32411–4.
[16] Oei SL, Ziegler M. ATP for the DNA ligation step in base excision repair is generated from poly(ADP-ribose). J Biol Chem 2000;275:23234–9.
[17] Whitacre CM, Hashimoto H, Tsai M-L, Chatterjee S, Berger SJ, Berger NA. Involvement of NAD-poly(ADP-ribose) metabolism in p53 regulation and its consequences. Cancer Res 1995;55:3697–701.
[18] Agarwal ML, Agarwal A, Taylor WR, Wang Z-Q, Wagner EF, Stark GR. Defective induction but normal activation and function of p53 in mouse cells lacking poly-ADP-ribose polymerase. Oncogene 1997;15:1035–41.
[19] Simbulan-Rosenthal CM, Rosenthal DS, Ding R-C, Bhatia K, Smulson ME. Prolongation of the p53 response to DNA strand breaks in cells depleted of PARP by antisense RNA expression. Biochem Biophys Res Commun 1998;253:864–8.
[20] Wesierska-Gadek J, Wang Z-Q, Schmid G. Reduced stability of regularly spliced but not alternatively spliced p53 protein in PARP-deficient mouse fibroblasts. Cancer Res 1999;59:28–34.
[21] Beneke S, Geisen C, Zevnik B, Bauch T, Müller WU, Küpper JH, et al. DNA excision repair and DNA damage-induced apoptosis are linked to poly(ADP-ribosyl)ation but have different requirements for p53. Mol Cell Biol 2000;20:6695–703.
[22] Valenzuela MT, Guerrero R, Núñez MI, Ruiz de Almodóvar JM, Sarker M, de Murcia G, et al. PARP-1 modifies the effectiveness of p53-mediated DNA damage response. Oncogene 2002;21:1108–16.
[23] Conde C, Mark M, Oliver FJ, Huber A, de Murcia G, Ménissier-de Murcia J. Loss of poly(ADP-ribose) polymerase-1 causes increased tumour latency in p53-deficient mice. EMBO J 2001;20:3535–43.
[24] Malanga M, Pleschke JM, Kleczkowska HE, Althaus FR. Poly(ADP-ribose) binds to specific domains of p53 and alters its DNA binding functions. J Biol Chem 1998;273:11839–43.
[25] Mendoza-Alvarez H, Alvarez-Gonzalez R. Regulation of p53 sequence-specific DNA-binding by covalent poly(ADP-ribosyl)ation. J Biol Chem 2001;276:36425–30.
[26] Mandir AS, Simbulan-Rosenthal CM, Poitras MF, Lumpkin JR, Dawson VL, Smulson ME, et al. A novel in vivo post-translational modification of p53 by PARP-1 in MPTP-induced parkinsonism. J Neurochem 2002;83:186–92.
[27] Wesierska-Gadek J, Wojciechowski J, Schmid G. Central and carboxy-terminal regions of human p53 protein are essential for interaction and complex formation with PARP-1. J Cell Biochem 2003;89:220–32.
[28] Wieler S, Gagné JP, Vaziri H, Poirier GG, Benchimol S. Poly(ADP-ribose) polymerase-1 is a positive regulator of the p53-Mediated G1 arrest response following ionizing radiation. J Biol Chem 2003;278:18914–21.
[29] Tong W-M, Hande MP, Lansdorp PM, Wang Z-Q. DNA strand break-sensing molecule poly(ADP-ribose) polymerase cooperates with p53 in telomere function, chromosome stability, and tumor suppression. Mol Cell Biol 2001;21:4046–54.
[30] Beneke R, Moroy T. Inhibition of poly(ADP-ribose) polymerase activity accelerates T-cell lymphomagenesis in p53 deficient mice. Oncogene 2001;20:8136–41.
[31] Eberhart CG. Medulloblastoma in mice lacking p53 and PARP: all roads lead to Gli. Am J Pathol 2003;62:7–10.
[32] Adelfak C, Kontou M, Hirsch-Kauffmann M, Schweiger M. Physical and functional interaction of the Werner syndrome protein with poly-ADP ribosyl transferase. FEBS Lett 2003;554:55–8.
[33] Lebel M, Lavoie J, Gaudreault I, Bronsard M, Drouin R. Genetic cooperation between the Werner syndrome protein and poly(ADP-ribose) polymerase-1 in preventing chromatid breaks, complex chromosomal rearrangements, and cancer in mice. Am J Pathol 2003;162:1559–69.
[34] von Kobbe C, Harrigan JA, May A, Opresko PL, Dawut L, Cheng WH, et al. Central role for the Werner syndrome protein/poly(ADP-

ribose) polymerase 1 complex in the poly(ADP-ribosyl)ation pathway after DNA damage. Mol Cell Biol 2003;23:8601–13.
[35] Li B, Navarro S, Kasahara N, Comai L. Identification and biochemical characterization of a Werner syndrome protein complex with Ku70/80 and poly(ADP-ribose) polymerase-1. J Biol Chem 2004;279:13659–67.
[36] Ménissier-de Murcia J, Mark M, Wendling O, Wynshaw-Boris A, de Murcia G. Early embryonic lethality in PARP-1 Atm double-mutant mice suggest a functional synergy in cell proliferation during development. Mol Cell Biol 2001;21:1828–32.
[37] Marecki JC, McCord JM. The inhibition of poly(ADP-ribose) polymerase enhances growth rates of ataxia telangiectasia cells. Arch Biochem Biophys 2002;402:227–34.
[38] Berger NA. Poly(ADP-ribose) in the cellular response to DNA damage. Radiat Res 1985;101:4–15.
[39] Eguchi Y, Shimizu S, Tsujimoto Y. Intracellular ATP levels determine cell death fate by apoptosis or necrosis. Cancer Res 1997;57:1835–40.
[40] Ha HC, Snyder SH. Poly(ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion. Proc Natl Acad Sci USA 1999;96:13978–82.
[41] Leist M, Single B, Naumann H, Fava E, Simon B, Kühnle S, et al. Inhibition of mitochondrial ATP generation by nitric oxide switches apoptosis to necrosis. Exp Cell Res 1999;249:396–403.
[42] Negri C, Bernardi R, Braghetti A, Astaldi Ricotti GCB, Scovassi AI. The effect of the chemotherapeutic drug VP-16 on poly(ADP-ribosylation) in apoptotic HeLa cells. Carcinogenesis 1993; 14:2559–64.
[43] Donzelli M, Negri C, Mandarino A, Rossi L, Prosperi E, Frouin I, et al. Poly(ADP-ribose) synthesis: a useful parameter to identify apoptotic cells. Histochem J 1997;29:831–7.
[44] Negri C, Donzelli M, Bernardi R, Rossi L, Bürkle A, Scovassi AI. Multiparametric staining to identify apoptotic human cells. Exp Cell Res 1997;234:174–7.
[45] Rosenthal DS, Ding R, Simbulan-Rosenthal CMG, Vaillancourt JP, Nicholson DW, Smulson ME. Intact cell: evidence for the early synthesis, and subsequent late apopain-mediated suppression, of poly(ADP-ribose) during apoptosis. Exp Cell Res 1997;232:313–21.
[46] Soldani C, Lazzè MC, Bottone MG, Tognon G, Biggiogera M, Pellicciari CE, et al. Poly(ADP-ribose) polymerase cleavage during apoptosis: when and where? Exp Cell Res 2001;269:193–201.
[47] Soldani C, Scovassi AI. Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: an update. Apoptosis 2002;7:321–8.
[48] Scovassi AI, Torriglia A. Activation of DNA-degrading enzymes during apoptosis. Eur J Histochem 2003;47:185–94.
[49] Alvarez-Gonzalez R, Spring H, Müller M, Bürkle A. Selective loss of poly(ADP-ribose) and the 85-kDa fragment of poly(ADP-ribose) polymerase in nucleoli during alkylation-induced apoptosis in HeLa cells. J Biol Chem 1999;274:32122–6.
[50] D'Amours D, Sallmann FR, Dixit VM, Poirier GG. Gain-of-function of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis. J Cell Sci 2001;114:3771–8.
[51] Soldani C, Bottone MG, Pellicciari C, Scovassi AI. Two-color fluorescence detection of poly(ADP-ribose) polymerase-1 (PARP-1) cleavage and DNA strand breaks in etoposide-treated apoptotic cells. Eur J Histochem 2001;45:389–92.
[52] Mi Y, Thomas SD, Xu X, Casson LK, Miller DM, Bates PJ. Apoptosis in leukemia cells is accompanied by alterations in the levels and localization of nucleolin. J Biol Chem 2003;278:8572–9.
[53] Rodenburg RJT, Raats JMH, Prujin GJM, van Venrooij WJ. Cell death: a trigger of autoimmunity? BioEssays 2000;22:627–36.
[54] Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, et al. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 2002;297:259–63.
[55] Cregan SP, Dawson VL, Slack RS. Role of AIF in caspase-dependent and caspase-independent cell death. Oncogene 2004;23:2785–96.
[56] Du L, Zhang X, Han YY, Burke NA, Kochanek PM, Watkins SC, et al. Intra-mitochondrial poly(ADP-ribosylation) contributes to NAD^+ depletion and cell death induced by oxidative stress. J Biol Chem 2003;278:18426–33.
[57] Yakovlev AG, Wang G, Stoica BA, Boulares HA, Spoonde AY, Yoshihara K, et al. A role of the Ca^{2+}/Mg^{2+}-dependent endonuclease in apoptosis and its inhibition by poly(ADP-ribose) polymerase. J Biol Chem 2000;275:21302–8.
[58] Boulares AH, Zoltosky AJ, Contreras FJ, Yakovlev AG, Yoshihara K, Smulson ME. Regulation of DNAS1L3 endonuclease activity by poly(ADP-ribosyl)ation during etoposide-induced apoptosis. J Biol Chem 2002;277:372–8.
[59] Le Rhun Y, Kirkland JB, Shah GM. Cellular responses to DNA damage in the absence of poly(ADP-ribose) polymerase. Biochem Biophys Res Commun 1998;245:1–10.
[60] Oliver FJ, de la Rubia G, Rolli V, Ruiz-Ruiz MC, de Murcia G, Ménissier-de Murcia J. Importance of poly(ADP-ribose) polymerase and its cleavage in apoptosis. Lesson from an uncleavable mutant. J Biol Chem 1998;273:33533–9.
[61] Leist M, Single B, Künstle G, Volbracht C, Hentze H, Nicotera P. Apoptosis in the absence of poly-(ADP-ribose) polymerase. Biochem Biophys Res Commun 1997;233:518–22.
[62] Kühnle S, Nicotera P, Wendel A, Leist M. Prevention of endotoxin-induced lethality, but not of liver apoptosis in poly(ADP-ribose) polymerase-deficient mice. Biochem Biophys Res Commun 1999;263:433–8.
[63] Halappanavar SS, Le Rhun Y, Mounir S, Martins LM, Huot J, Earnshaw WC, et al. Survival and proliferation of cells expressing caspase-uncleavable poly(ADP-ribose) polymerase in response to death-inducing DNA damage by an alkylating agent. J Biol Chem 1999;274:37097–104.
[64] Herceg Z, Wang ZQ. Failure of poly(ADP-ribose) polymerase cleavage by caspases leads to induction of necrosis and enhanced apoptosis. Mol Cell Biol 1999;19:5124–33.
[65] Boulares AH, Yakovlev AG, Ivanova V, Stoica BA, Wang G, Iyer S, et al. Role of poly(ADP-ribose) polymerase (PARP) cleavage in apoptosis. Caspase 3-resistant PARP mutant increases rates of apoptosis in transfected cells. J Biol Chem 1999;274:22932–40.
[66] Zhang J, Dawson VL, Dawson TM, Snyder SH. Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. Science 1994;263:687–9.
[67] Zingarelli B, O'Connor M, Wong H, Salzman AL, Szabó C. Peroxynitrite-mediated DNA strand breakage activates poly-adenosine diphosphate ribosyl synthetase and causes cellular energy depletion in macrophages stimulated with bacterial lipopolysaccharide. J Immunol 1996;156:350–8.
[68] Virág L, Scott GS, Cuzzocrea S, Marmer D, Salzman AL, Szabó C. Peroxynitrite-induced thymocyte apoptosis: the role of caspases and poly(ADP-ribose) synthetase (PARS) activation. Immunology 1998;94:345–55.
[69] Palomba L, Guidarelli A, Scovassi AI, Cantoni O. Different effects of tert-butylhydroperoxide-induced peroxynitrite-dependent and -independent DNA single-strand breakage on PC12 cell poly(ADP-ribose) polymerase activity. Eur J Biochem 2001;68:5223–8.
[70] Scovassi AI. Multiple roles of poly(ADP-ribosylation). Recent Res Dev Prot 2002;1:345–60.
[71] Szabó SC, Dawson VL. Role of poly(ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. Trends Pharmacol Sci 1998;19:287–98.
[72] Meli E, Pangallo M, Baronti R, Chiarugi A, Cozzi A, Pellegrini-Giampietro DE, et al. Poly(ADP-ribose) polymerase as a key player in excitotoxicity and post-ischemic brain damage. Toxicol Lett 2003;139:153–62.
[73] Chiarugi A, Meli E, Calvani M, Picca R, Baronti R, Camaioni E, et al. Novel isoquinolinone-derived inhibitors of poly(ADP-ribose) polymerase-1: pharmacological characterization and neuroprotective

effects in an in vitro model of cerebral ischemia. J Pharmacol Exp Ther 2003;305:943–9.
[74] Virág L, Szabó C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol Rev 2003;54:375–429.
[75] Nicoletti VG, Stella AM. Role of PARP under stress conditions: cell death or protection? Neurochem Res 2003;28:87–94.
[76] Tentori L, Portarena I, Graziani G. Potential clinical applications of poly(ADP-ribose) polymerase (PARP) inhibitors. Pharmacol Res 2002;45:73–85.
[77] Tentori L, Leonetti C, Scarsella M, d'Amati G, Portarena I, Zupi G, et al. Combined treatment with temozolomide and poly(ADP-ribose) polymerase inhibitor enhances survival of mice bearing hematologic malignancy at the central nervous system site. Blood 2002;99: 2241–4.
[78] Tentori L, Leonetti C, Scarsella M, d'Amati G, Vergati M, Portarena I, et al. Systemic administration of GPI 15427, a novel poly(ADP-ribose) polymerase-1 inhibitor, increases the antitumor activity of temozolomide against intracranial melanoma, glioma, lymphoma. Clin Cancer Res 2003;9:5370–9.
[79] Calabrese CR, Almassy R, Barton S, Batey MA, Calvert AH, Canan-Koch S, et al. Anticancer chemosensitization and radiosensitization by the novel poly(ADP-ribose) polymerase-1 inhibitor AG14361. J Natl Cancer Inst 2004;96:56–67.
[80] Curtin NJ, Wang LZ, Yiakouvaki A, Kyle S, Arris CA, Canan-Koch S, et al. Novel poly(ADP-ribose) polymerase-1 inhibitor, AG14361, restores sensitivity to temozolomide in mismatch repair-deficient cells. Clin Cancer Res 2004;10:881–9.
[81] Racz I, Tory K, Berente Z, Osz E, Jaszlits L, Bernath S, et al. BGP-15—a novel poly(ADP-ribose) polymerase inhibitor- protects against nephrotoxicity of cisplatin without compromising its anti-tumor activity. Biochem Pharmacol 2002;63:1099–111.
[82] Farkas B, Magyarlaki M, Csete B, Nemeth J, Rabloczky G, Bernath S, et al. Reduction of acute photodamage in skin by topical application of a novel PARP inhibitor. Biochem Pharmacol 2002;63:921–32.
[83] Mondello C, Scovassi AI. Telomeres, telomerase and apoptosis. Biochem Cell Biol, in press.
[84] Davidovic L, Vodenicharov M, Affar EB, Poirier GG. Importance of poly(ADP-ribose) glycohydrolase in the control of poly(ADP-ribose) metabolism. Exp Cell Res 2001;268:7–13.
[85] Affar EB, Germain M, Winstall E, Vodenicharov M, Shah RG, Salvesen GS, et al. Caspase-3-mediated processing of poly(ADP-ribose) glycohydrolase during apoptosis. J Biol Chem 2001;276:2935–42.
[86] Bonicalzi ME, Vodenicharov M, Coulombe M, Gagne JP, Poirier GG. Alteration of poly(ADP-ribose) glycohydrolase nucleocytoplasmic shuttling characteristics upon cleavage by apoptotic proteases. Biol Cell 2003;95:635–44.
[87] Ying W, Sevigny MB, Chen Y, Swanson RA. Poly(ADP-ribose) glycohydrolase mediates oxidative and excitotoxic neuronal death. Proc Natl Acad Sci USA 2001;98:12227–32.
[88] Ying W, Swanson RA. The poly(ADP-ribose) glycohydrolase inhibitor gallotannin blocks oxidative astrocyte death. Neuroreport 2000;11:1385–8.
[89] Lu XC, Massuda E, Lin Q, Li W, Li JH, Zhang J. Post-treatment with a novel PARG inhibitor reduces infarct in cerebral ischemia in the rat. Brain Res 2003;978:99–103.
[90] Hanai S, Kanai M, Ohashi S, Okamoto K, Yamada M, Takahashi H, et al. Loss of poly(ADP-ribose) glycohydrolase causes progressive neurodegeneration in *Drosophila melanogaster*. Proc Natl Acad Sci USA 2004;101:82–6.
[91] Masutani M, Nakagama H, Sugimura T. Poly(ADP-ribose) and carcinogenesis. Genes Chrom Cancer 2003;38:339–48.
[92] Veuger SJ, Curtin NJ, Richardson CJ, Smith GC, Durkacz BW. Radiosensitization and DNA repair inhibition by the combined use of novel inhibitors of DNA-dependent protein kinase and poly(ADP-ribose) polymerase-1. Cancer Res 2003;63:6008–15.
[93] Ullrich O, Reinheckel T, Sitte N, Hass R, Grune T, Davies KJA. Poly(ADP-ribose) polymerase activates nuclear proteasome to degrade oxidatively damaged histones. Proc Natl Acad Sci USA 1999;96:6223–8.
[94] Ciftci O, Ullrich O, Schmidt CA, Diestel A, Hass R. Regulation of the nuclear proteasome activity in myelomonocytic human leukemia cells after adriamycin treatment. Blood 2001;97:2830–8.
[95] Hassa PO, Hottiger MO. A role of poly(ADP-ribose) polymerase in NF-κB transcriptional activation. Biol Chem 1999;380:953–9.
[96] Hassa PO, Covic M, Hasan S, Imhof R, Hottiger MO. The enzymatic and DNA binding activity of PARP-1 are not required for NF-κB coactivator function. J Biol Chem 2001;276:45588–97.
[97] Hassa PO, Buerki C, Lombardi C, Imhof R, Hottiger MO. Transcriptional coactivation of nuclear factor-κB-dependent gene expression by p300 is regulated by poly(ADP)-ribose polymerase-1. J Biol Chem 2003;278:45145–53.
[98] Carrillo A, Monreal Y, Ramírez P, Marin L, Parrilla P, Oliver FJ, et al. Transcription regulation of TNF-α-early response genes by poly(-ADP-ribose) polymerase-1 in murine heart endothelial cells. Nucl Acids Res 2004;32:757–66.
[99] Duvoix A, Morceau F, Delhalle S, Schmitz M, Schnekenburger M, Galteau MM, et al. Induction of apoptosis by curcumin: mediation by glutathione S-transferase P1-1 inhibition. Biochem Pharmacol 2003;66:1475–83.
[100] Tulin A, Spradling A. Chromatin loosening by poly(ADP)-ribose polymerase (PARP) at Drosophila puff loci. Science 2003;299: 560–2.
[101] Rouleau M, Aubin RA, Poirier GG. Poly(ADP-ribosyl)ated chromatin domains: access granted. J Cell Sci 2004;117:815–25.
[102] Laniel MA, Poirier GG, Guerin SL. Nuclear factor 1 interferes with Sp1 binding through a composite element on the rat poly(ADP-ribose) polymerase promoter to modulate its activity in vitro. J Biol Chem 2001;276:20766–73.