CHAPTER 17

Role of Poly-ADP-Ribosylation in Cancer Development

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Abstract

Elucidation of the relationship between poly-ADP-ribosylation and carcinogenesis has markedly progressed by the recent development of knockout or transgenic mice models of poly(ADP-ribose) polymerase (Parp)-1, Parp-2, and poly(ADP-ribose) glycohydrolase (Parg). Parp-1 is involved in base excision repair (BER), single- and double-strand break repair, and chromosomal stability. These multiple functions explain why *Parp-1* deficiency enhances carcinogenesis induced by alkylating agents and that in aged animals. Parp-1 is also involved in transcriptional regulation through protein-protein interaction as a coactivator and/or poly-ADP-ribosylation reaction and is possibly involved in epigenetic alteration during carcinogenesis and modulation of tumor phenotypes. Parp-1-dependent cell-death accompanying NAD depletion may be another important issue in carcinogenesis because this process could lead to the selection of *Parp-1* deficient cells due to their survival advantage during cancer growth. The relationship of Parp-2, Parp-3, tankyrase and Parg with carcinogenesis is also discussed.

Introduction

Carcinogenesis is a multistage process that involves multiple pathways and each cancer arises though different combinations of a variety of genetic and epigenetic changes.[1,2] Compared to the pathogenesis process of other diseases, carcinogenesis involves a wider range of aberrations of biological processes, including genomic stability, induction of cell death, differentiation, control of cell cycle and proliferation. The poly-ADP-ribosylation reaction and the Parp-1 molecule are involved in each of the above processes and thus should be related to carcinogenesis.[3] In this chapter, we give an overview of the impact of dysfunction of the poly-ADP-ribosylation reaction on carcinogenesis in various experimental models, and discuss their mechanistic bases in relation to human cancer.

Mouse Models of Carcinogenesis

Three types of Parp-1 knockout mice established by disruption of either exon 1[4], 2[5], or 4[6] were examined for their susceptibility to carcinogenesis. As shown in Table 1, an increased frequency of the development of hepatocellular carcinomas (HCC) in *Parp-1*$^{-/-}$ mice, harboring exon 2 disruption of the *Parp-1* gene was observed in aged mice as reported by Tong et al.[7] In developed HCC, frequent occurrence of genomic instability, loss of expression of E-cadherin and accumulation of β-catenin were observed. In our experiments, although spontaneous tumor development was not observed at 7 and 9 months of age,[8,9] the

Poly(ADP-Ribosyl)ation, edited by Alexander Bürkle.

Table 1. Outcome of carcinogenesis experiments carried out in* Parp-1 *knockout and transgenic mouse models

Model	Tumor	Incidence*	References
Spontaneous tumor			
at 9 months old	Not detected	→	Nozaki et al 2003[9]
at 18-24 months old	Hepatocellular carcinoma	↑	Tong et al 2002[7]
at 21-23 months old	Hepatocellular carcinoma	↑	Masutani et al unpublished
BHP treatment	Hemangioma & hemangiosarcoma (liver)	↑	Tsutsumi et al 2000[8]
	Adenoma & adenocarcinoma (lung)	↑	Tsutsumi et al 2000[8]
Azoxymethane treatment	Adenocarcinoma (colon)	↑	Nozaki et al 2003[9]
	Nodule (liver)	↑	Nozaki et al 2003[9]
4NQO treatment	Squamous cell carcinoma (oral, esophagus)	→	Gunji et al unpublished
IQ treatment	Hepatocellular carcinoma	→	Ogawa et al unpublished
	Adenoma (lung)	→	Ogawa et al unpublished
	Papilloma (forestomach)	→	Ogawa et al unpublished
SCID*Parp-1*$^{-/-}$ mice	Thymic lymphoma	↑	Morrison et al 1997[11]
P53$^{-/-}$*Parp-1*$^{-/-}$ mice	Carcinomas (colon & breast)	↑	Tong et al 2001[13]
	Medulloblastoma	↑	Tong et al 2003[15]
P53$^{-/-}$*Parp-1*$^{-/-}$ mice	Thymic lymphoma	↓	Conde et al 2001[17]
Ku80$^{-/-}$*Parp-1*$^{-/-}$ mice	Hepatocellular carcinoma	↑	Tong et al 2002[7]
PARP-DBD *p53*$^{-/-}$mice	T-cell lymphoma	↑	Beneke et al 2001[14]

*(→) no change, (↑) elevated, (↓) reduced incidence, compared to wild-type mice, respectively

frequency of HCC development was increased in 21-23-month-old *Parp-1*$^{-/-}$ mice, whereas no tumors were observed in other tissues (Masutani et al unpublished). Administration of *N*-nitrosobis(2-hydroxypropyl)amine (BHP) resulted in the development of liver hemangioma and hemangiosarcoma at significantly higher frequencies in *Parp-1*$^{-/-}$ than in *Parp-1*$^{+/+}$ mice.[8] Another alkylating agent, azoxymethane also enhanced the frequency of tumor development both in the colon and liver in *Parp-1*$^{-/-}$ compared with that in *Parp-1*$^{+/+}$ mice.[9] In addition to the differences in tumor incidence, the size of tumors, mainly adenocarcinoma, was larger in *Parp-1*$^{-/-}$ than in *Parp-1*$^{+/+}$ mice in the colon, suggesting that loss of Parp-1 affects tumor growth. In sharp contrast, the frequency of hepato- and pulmonary carcinogenesis was not different among *Parp-1*$^{-/-}$, *Parp-1*$^{+/-}$ and *Parp-1*$^{+/+}$ mice administered IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline), a cooked food-borne heterocyclic amine that produces bulky adducts on DNA (Ogawa et al unpublished). 4-Nitrosoquinoline 1-oxide (4NQO) mimics ultraviolet (UV)-induced damage and generates a DNA adduct, which is removed mainly by nucleotide excision repair involving XPA (xeroderma pigmentosum group A). It was reported that development of oral tumors in mice given 4NQO was markedly higher in *XPA*$^{-/-}$ than in wild-type mice.[10] In contrast, there was no difference in tumor incidence between *Parp-1*$^{-/-}$ and *Parp-1*$^{+/+}$ mice after 4NQO administration in drinking water (Gunji et al unpublished). These experiments strongly suggest that susceptibility to carcinogenesis under *Parp-1* deficiency depends substantially on the type of DNA damage and implies the significant contribution of Parp-1 in BER and/or DNA strand break repair pathways to prevent carcinogenesis.

The impact of the combination of deficiency of *Parp-1* and *DNA-PK* or *p53* was also studied in mice. SCID mice harbor a mutation in the gene encoding a catalytic subunit of the DNA-PK complex and show immunodeficiency due to the lack of V[D]J recombination to produce mature T and B cells. In *Parp-1*$^{-/-}$SCID mice, a marked increase of the frequency of T-cell lymphoma was observed from an early age compared to SCID mice, although the frequency of B-cell lymphoma was not increased.[11]

P53 is a major genome guardian and is involved in the regulation of both proper cell cycle and apoptosis after DNA damage. Mice lacking *p53* (*p53*$^{-/-}$) show a high incidence of spontaneous tumors as well as an increase in various types of genomic instabilities after DNA damage.[12] Tong et al reported that the deficiency of *p53* in *Parp-1*$^{-/-}$ mice, harboring exon 2 disruption of *Parp-1*, significantly promotes the development of thymic lymphoma, colon and breast carcinomas.[13] A transgenic mouse, which overexpresses the DNA binding domain of Parp-1, as a dominant negative mutant (PARP-DBD), also showed increased incidence of T-cell lymphomagenesis in *p53*$^{-/-}$ mice with a significantly shorter tumor latency period.[14] It is possible that the suppressive effect of PARP-DBD on DNA repair promotes the accumulation of genomic instability and contributes to lymphomagenesis.

Another intriguing finding is the spontaneous development of medulloblastoma in the cerebellum of 8-week-old *p53*$^{-/-}$*Parp-1*$^{-/-}$ mice and that nearly half of these mice harbor medulloblastoma by 6 months of age.[15] Lee et al reported the spontaneous development of medulloblastoma in the knockout mice harboring both *DNA ligase IV* and *p53* disruption.[16] Since DNA ligase IV is a key enzyme in nonhomologous end joining (NHEJ) repair and accumulating evidence implies that Parp-1 also participates in NHEJ repair, it is possible that the NHEJ-dependent DSB repair is important in prevention of medulloblastoma formation in the cerebellum.

Different consequences of *p53* and *Parp-1* deficiencies were reported by Conde et al in *p53*$^{-/-}$*Parp-1*$^{-/-}$ mice, harboring exon 4 disruption in *Parp-1* gene. These mice show a lower frequency of thymic lymphoma compared to *p53*$^{-/-}$ mice.[17] In relation to this, H-*ras*-transformed fibroblasts derived from *p53*$^{-/-}$*Parp-1*$^{-/-}$ mice showed reduction of *inducible nitric oxide synthase* (*iNOS*) expression, nitric oxide release and decreased potential of cell growth and tumorigenesis compared to those derived from *p53*$^{-/-}$ mice.[17] The diminished cell growth is likely to be related to the decreased level of nitric oxide, since the stimulatory role of nitric oxide in proliferation and its inhibitory role in apoptosis have been reported.[18] We suggest that the role of Parp-1 in cell proliferation significantly affects tumorigenesis under certain conditions. Notably, B-cell lymphoma development was not increased either in SCID*Parp-1*$^{-/-}$[11] or in *p53*$^{-/-}$*Parp-1*$^{-/-}$ mice[13] whereas T-cell lymphoma development was augmented in these cases. Since impairment of S-phase entry of the B-cell population in splenocytes was also observed in *Parp-1*$^{-/-}$ mice,[19] the evidence indicates the possibility that Parp-1 is required for B-cell lymphoma development by supporting its cell proliferation potential. The genetic background of mice may also affect the extent of contribution of Parp-1 in cell proliferation, since we observed that *Parp-1*$^{-/-}$ mice of the C57BL/6 congenic strain show partial lethality during late embryogenesis[19] and reduced body-weight gain (Ogawa et al unpublished), whereas those of ICR/129Sv mixed genetic background do not show such phenotypes.

The elucidation of the relationship of other Parp family members to carcinogenesis awaits the results of further experiments including those using the transgenic animal models. Recent studies reported the involvement of Parp-2 in BER processes,[20] of Parp-3 in centrosome regulation[21] and of tankyrase in telomere length regulation.[22] Hence, dysfunction of these molecules may cause genomic instability and is expected to have certain impacts on carcinogenesis.

Compared to the poly-ADP-ribosylation reaction, the involvement of poly(ADP-ribose) degradation by Parg on carcinogenesis has not been elucidated yet. *Parg*-deficient embryonic stem (ES) cells[23] and *Drosophila*[24] have become available recently. Since *Parg*-deficient ES cells show increased sensitivity to alkylating agents and γ-irradiation and undergo early apoptosis (Fujihhara et al unpublished), dysfunction of the *Parg* gene may also be involved in carcinogenesis possibly through regulation of recovery from DNA damage.

Effect of PARP Inhibitors on Carcinogenesis

Several carcinogenesis experiments have been carried out using Parp inhibitors or modulating NAD level, summarized in Table 2. Parp inhibitors including 3-aminobenzamide and 3-methoxybenzamide augment[25-29] or decrease[30-32] the incidence of tumors depending on the treatment protocol of inhibitors, carcinogens, tissue, or animals used in the studies.[33] The exact explanation for this phenomenon is still not available. Since Parp inhibitors also block activity of Parp family members other than Parp-1, the inhibition of various Parp family proteins should have a substantial influence on the susceptibility to carcinogens. The experimental model of Boyonoski et al manipulated NAD levels in vivo and showed that NAD deficiency increases the incidence of tumors and leukemia,[34] whereas supplementation with the NAD precursor niacin delayed the onset of HCC.[35] These results suggest that maintenance of cellular NAD levels prevents or delays carcinogenesis.

In vitro transformation systems using cultured cells in combination with various types of carcinogens have been also described. The effects of Parp inhibitors exhibit a wide spectrum as depicted in Table 3. Parp inhibitors suppressed transformation induced by various type of carcinogens,[36] not only methylating agents but also one inducing bulky adducts, benzo[a]pyrene,[36] as well as ionizing irradiation (IR)[37,38,42] and UV.[37,39] In contrast, against ethylating agents, such as ethylnitrosourea and ethylmethanesulfonate,[40-42] Parp inhibitors enhanced such transformation. The enhancement of in vitro transformation was observed in a time-specific manner. Simultaneous treatment with carcinogen and Parp-inhibitor was effective whereas Parp inhibitor treatment 24 hr after carcinogen exposure was not.[41] These in vitro transformation systems use immortalized cells and the cell proliferation potential may be the major factor that influences the overall frequency of transformation. It is thus conceivable that Parp inhibitors may reduce the proliferation capacity of cells, leading to a low transformation frequency. However, no plausible explanation is currently available as to why Parp inhibitors exert the opposite effects on transformation induced by methylating and ethylating agents. Parp inhibitors also increase the transformation of NIH3T3 cells by transfection of SV40 DNA and this was further proven to be due to the increase in integration frequency of SV40 DNA into the genome.[43]

Tumorigenesis and Differentiation

Functional loss of Parp modulates tumorigenesis and differentiation of malignant cells as summarized in Table 4. Early studies showed that Parp inhibitors induced differentiation of various types of tumor cell lines.[44-47] In the case of HL-60 cells[48] and H-*ras* transformed NIH3T3 cells,[47] loss of amplified oncogenes, *c-myc* and H-*ras* genes, was induced after treatment with Parp inhibitors and these changes could reduce the proliferation potential of tumor cells. Teratocarcinoma EC-AI cells also showed differentiation to endodermal cells during tumorigenesis in nude mice.[44] During cell differentiation, an increase in poly-ADP-ribosylation activity generally occurs at the commitment stage, which is followed by decrease in its activity. Reversion of the tumorigenic phenotype was also observed in vivo with a different type of Parp inhibitor, 5-iodo-6-amino-1, 2-benzopyrone, which is thought to interfere with zinc-finger function of Parp-1.[49]

When *Parp-1*$^{-/-}$ mouse ES cells were subcutaneously injected into nude mice, the recipient mice developed teratocarcinoma-like tumors, similar to the case with *Parp-1*$^{+/+}$ ES cells.[50] In *Parp-1*$^{-/-}$ tumors, the trophoblast lineage cells, including trophoblast giant cells and spongiotrophoblasts, are preferentially induced and large blood lacuna structures were secondarily induced, probably by the action of trophoblasts. *Parp-1*$^{-/-}$ ES cells in culture showed elevated expression levels of trophoblast marker genes,[51] suggesting that loss of Parp-1 promotes commitment to a trophoblast lineage. Trophoblast giant cells emerged after a repeated endoreduplication process and showed up to 1,000N ploidy. Loss of Parp-1 function may, therefore, also enhance endoreduplication. Overexpression of PARP-DBD in HeLa cells also interfered with tumorigenesis in nude mice, accompanied by an increased frequency of apoptosis.[52] These examples indicate that Parp dysfunction can markedly influence tumor phenotype.

Table 2. Effect of PARP inhibitors on carcinogenesis

Carcinogen	Inhibitor/ Treatment	Species	Tissue	Tumor	Incidence	Refs.
Streptozotocin	Nicotinamide	Rat *1	Kidney	Renal cell tumor	↓ *5	Rakieten, 1971[30]
	Nicotinamide	Rat *1	Pancreas	Insulinoma	↑ *6	Rakieten, 1971[30]
	3-Aminobenzamide	Rat *2	Pancreas	Insulinoma	↑	Yamagami, 1985[25]
	Benzamide	Rat *2	Pancreas	Insulinoma	↑	Yamagami, 1985[25]
Alloxan	3-Aminobenzamide	Rat *2	Pancreas	Insulinoma	↑	Yamagami, 1985[25]
	Benzamide	Rat *2	Pancreas	Insulinoma	↑	Yamagami, 1985[25]
Diethylnitrosamine	3-Aminobenzamide	Rat *2	Liver	γ-GTP positive foci*7	↑	Takahashi, 1982[26]
	Nicotinamide	Rat *3	Kidney	Renal tubular cell tumor	↑	Rosenberg, 1985[27]
Ethylnitrosourea	Supplementation with niacin or nicotinamide	Rat *4	Liver	HCC	↓	Boyonoski, 2002[35]
7β,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzopyrene	3-Aminobenzamide	Rat *8	Liver	γ-GTP positive foci	↑	Denda, 1988[33]
Methylnitrosourea	3-Aminobenzamide	Rat *1	Liver	γ-GTP positive foci	→*9	Denda, 1988[33]
1,2-Dimethylhydrazine	3-Aminobenzamide	Rat *2	Liver	γ-GTP positive foci	↑	Denda, 1988[33]
	3-Aminobenzamide	Rat *3	Liver	γ-GTP positive foci	→	Denda, 1988[33]
N-Nitrosobis(2-hydroxypropyl) amine	3-Aminobenzamide	Rat *2	Liver	γ-GTP positive foci	→	Denda, 1988[33]
Ethylnitrosourea	Niacin deficiency	Rat *4	Liver	GST-P positive foci*10	↑	Boyonoski, 2002[34]
Diethylnitrosamine + phenobarbital	3-Aminobenzamide	Rat *3	Liver	GST-P positive foci	↓	Tsujiuchi, 1990[31]
	Luminol	Rat *3	Liver	GST-P positive foci	↓	Tsujiuchi, 1990[31]
Methylazoxymethanol	3-Aminobenzamide	Rat *3	Colon	Adenocarcinoma	↓	Nakagawa, 1988[32]
	3-Aminobenzamide	Medaka (*Oryzias latipes*)	Liver	Hepatoma	↑	Miwa, 1985[28]
Dimethylbenz[a]anthracene	3-Methoxybenzamide	Hamster	Cheek pouch	Oral squamous cell carcinoma	↑	Miller, 1989[29]

*1 Holtzman rat
*2 Wistar rat
*3 Fischer rat
*4 Long-Evans rat
*5 Suppression
*6 Elevation
*7 γ-Glutamyl transpeptidase
*8 Wistar & Fischer rats
*9 No change
*10 Glutathione *S*-transferase placental form

Table 3. Effect of PARP inhibitors on in vitro transformation

Carcinogen	Inhibitor	Species	Cells	Transformation Frequency	Refs.
1,1-Dimethylhydrazine	Benzamide	Human	Fibroblast	↓*1*2	Kun, 1983[36]
Benzo[a]pyrene	Benzamide	Human	Fibroblast	↓	Kun, 1983[36]
β-Propiolactone	Benzamide	Human	Fibroblast	↓	Kun, 1983[36]
Methylazoxymethanol	Benzamide	Human	Fibroblast	↓	Kun, 1983[36]
MNNG	Benzamide	Human	Fibroblast	↓	Kun, 1983[36]
3-Hydroxy-1-propane-sulfonic acid γ-sulfone	Benzamide	Human	Fibroblast	↓	Kun, 1983[36]
Ionizing radiation (IR)	Benzamide	Mouse	C3H10T/1/2	↓	Borek, 1984[37,42]
	3-Amino-benzamide	Hamster	Embryo cells	↓	
UV	Benzamide	Mouse	C3H10T/1/2	↓	Borek, 1984[37,39]
	3-Amino-benzamide	Hamster	Embryo cells	↓	
MNNG	Benzamide	Mouse	C3H10T/1/2	↓	Borek, 1984[37,42]
	3-Amino-benzamide	Hamster	Embryo cells	↓	
Methylcholanthrene	3-Amino-benzamide	Mouse	BALB/c3T3A31-1	→*3	Lubet, 1984[40]
Aflatoxin B1	3-Amino-benzamide	Mouse	BALB/c3T3A31-1	→	Lubet, 1984[40]
Ethylnitrosourea	3-Amino-benzamide	Mouse	C3H10T1/2	↑*4	Borek, 1984[42]
Ethylmethanesulfonate	3-Amino-benzamide	Mouse	BALB/c3T3A31-1 C3H10T1/2	↑	Lubet, 1984[40], 1986[41]
IR + 12-*O*-tetradecanoyl-phorbol-13-acetate	3-Amino-benzamide	Mouse	C3H10T1/2	↓	Borek, 1986[3?]
SV40 DNA	3-Methoxy-benzamide	Mouse	NIH3T3	↑	Strain, 1985[43]

*1 S-phase treatment was most effective
*2 Suppression
*3 No change
*4 Elevation

Parg$^{-/-}$ ES cells produced tumors as in the case of wild-type ES cells (Fujihara et al unpublished). The differentiation potential of ES cells was not different among *Parg* genotypes. Induction of trophoblast lineage was not observed in *Parg*$^{-/-}$ tumors, suggesting that *Parg* deficiency and the resulting impairment of poly(ADP-ribose) degradation is not related to trophoblast induction.

DNA Repair and Genomic Instability

As mentioned above, the spectrum of susceptibility of *Parp-1*$^{-/-}$ mice to carcinogens implied a significant contribution of Parp-1 in BER and DNA strand break repair. In BER, after removal of damaged bases, such as 8-hydroxy-dG, or alkylated bases by glycosidases, single

Table 4. Consequence of Parp inhibition for tumorigenesis and differentiation of cancer cells

Methodology	Cell or Tissue	Outcome	References
Gene disruption	*Parp-1*$^{-/-}$ mouse ES cells	Induction of trophoblast giant cells in teratocarcinoma-like tumor (in nude mice)	Nozaki et al, 1999[50]
	H-*ras* transformed *Parp-1*$^{-/-}$ MEFs	Decreased tumorigenesis	Conde et al, 2001[17]
Dominant-negative mutant expression	HeLa cells	Decreased tumorigenesis	Hans et al, 1999[52]
Parp inhibitor			
3-Aminobenzamide	Mouse teratocarcioma EC-AI cells	Differentiation into endodermal epitheloid	Ohashi et al, 1984[44]
Benzamide	Mouse Friend erythroleukemic cells	Differentiation into erythrocytes	Terada et al, 1979[45]
Nicotinamide	Mouse Friend erythroleukemic cells	Differentiation into erythrocytes*[1]	Brac et al, 1987[46]
Benzamide	H-*ras* transformed NIH3T3 cells	Loss of transformed phenotype	Nakayasu et al, 1988[47]
Benzamide	Human HL-60 cells	Differentiation into granulocytes	Shima et al, 1989[48]
4-Hydroxyquinazoline 5-Iodo-6-amino-1,2-benzopyrone	H-*ras* transformed endothelial cells	Reversion of tumorigenicity	Bauer et al, 1995[49]
	Prostate carcinoma cells	Reversion of tumorigenicity	Bauer et al, 1995[49]

*[1] Differentiation is inhibited depending on the concentration of the inhibitor.

strand scission is introduced by AP-endonuclease. The recruitment of a molecular scaffold XRCC-1 (X-ray repair cross-complementing factor-1) to the repair site is a critical step in BER because XRCC1 further recruits DNA ligase IIIα, DNA polymerase β, and polynucleotide kinase.[53] Association of certain polymorphisms in *XRCC1* gene with lung and other cancers was reported by Divine et al.[54] It was demonstrated that Parp-1 is necessary for the assembly or stability of XRCC-1 nuclear foci at the site of DNA damage.[55] Dantzer et al showed that Parp-1 acts in the strand displacement step of the DNA fill-in reaction by DNA polymerase β and FEN-1 (flap endonuclease-1) and that long-patch BER is substantially delayed, whereas short-patch repair is only slightly affected in extracts from *Parp-1*$^{-/-}$ MEF.[56] Recently, defective poly-ADP-ribosylation in cells from Werner syndrome (WS) was reported following DNA damages introduced by an oxidative or an alkylating agent.[57] WS protein (WRN) interacts with proteins acting in BER, including polymerase δ, PCNA, FEN-1, replication factor A as well as Parp-1.[58] WRN may facilitate Parp-1 activation in the BER process. WS is characterized by the early onset of cancer, which may be partly explained by the defective poly-ADP-ribosylation activity in the BER process. Malanga et al also reported the repair of stalled DNA topoisomerase I (topoI)-DNA covalent complex through reactivation of topoI by Parp-1.[59] In addition, Parp-2 was also shown to be involved in BER by Schreiber et al.[20]

Parp-1 is also activated by double-strand break (DSB) and participates in NHEJ catalyzed by DNA-PK complex. Parp-1 activates auto-phosphorylation activity of DNA-PK and Ku70/80 complex,[60] whereas Parp-1 activity is suppressed by DNA-PK.[61] In SCID*Parp-1*$^{-/-}$ T-lymphocytes, V[D]J recombination, which is carried out by NHEJ, is partially restored.[11]

Furthermore, in a recombination-inducible SCID cell line, poly(ADP-ribose) formation was shown to occur during the resolution stage of V[D]J recombination where nascent opened coding ends are generated. Poly(ADP-ribose) formation colocalized with foci positive for the recombination protein Mre11 and facilitated coding-end resolution. In contrast, this response was not observed in wild-type cells possessing a functional catalytic subunit of DNA-dependent protein kinase.[62] WRN protein physically interacts also with Parp-1,[63] and Ku70/80-induced stimulation of WRN exonucleolytic activity was interfered with poly-ADP-ribosylation of Ku70/80 by Parp-1.[64] Parp-1 may thus regulate the exonucleolytic activity of WRN and prevent accidental recombination reaction during NHEJ.

Treatment of *Parp-1*$^{-/-}$ mice with BHP, an alkylating agent, did not enhance the frequency of point mutation, but rather increased the deletion frequency compared with *Parp-1*$^{+/+}$ mice (Shibata et al unpublished). This finding supports the current evidence that Parp-1 is involved in the NHEJ process. Lack of elevation in point mutation under *Parp-1* deficiency also suggests that Parp-1 is probably not required in BER, at least until the removal of the damaged base, but may function after DNA strand break introduction by preventing further conversion of single strand breaks into DSBs, which will be predominantly repaired by NHEJ. Thus, Parp-1 is possibly involved in the repair of DSBs that occur during the process of the BER reaction.

Chromosome Instability and Cell-Cycle Checkpoints Controls

Hallmarks of chromosome instability in cancer cells include aneuploidy, hyperploidy, gene amplification, loss of heterozygosity (LOH) and gene rearrangement. Tong et al[7] reported that *Ku80* haploinsufficiency in *Parp-1*$^{-/-}$ mice increased the incidence of HCC and the presence of chromosome instability in those tumors, such as chromatid/chromosome breaks, end-to-end fusions and recurrent nonreciprocal translocations. Hyperploidy was observed in spontaneously immortalized *Parp-1*$^{-/-}$ MEFs.[65-67] Comparative genomic hybridization analysis revealed that chromosome gain and loss were enhanced in *Parp-1*$^{-/-}$ compared with *Parp-1*$^{+/+}$ MEFs.[65] *Parp-1*$^{-/-}$ cells showed increased sister-chromatid exchange frequencies (SCEs) and micronuclei formation.[6,68]

One of the possible mechanisms for these gross chromosome instabilities is deregulation of cell-cycle checkpoints. Parp-1 directly interacts with p53[69] and was shown to be involved in p53-mediated G1 arrest after DNA damage.[70-72] Parp-1 was further found to complex with PCNA and p21 after DNA damage introduced by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) treatment.[73] Moreover, Kanai et al demonstrated that Parp-1 is located in the centrosome and interacts with p53 to regulate centrosome replication and function.[74] Halappanavar et al showed a defective mitotic checkpoint arrest accompanying down-regulation of cyclinB1/cdk-1 kinase activity in *Parp-1*$^{-/-}$ MEFs.[75] They also found that *Parp-1*$^{-/-}$ MEFs with higher ploidy were resistant to apoptosis in the G1 phase compared to wild-type cells, indicating defective post-mitotic checkpoints under *Parp-1* deficiency. Despite the accumulated evidence, it remains to be clarified whether *Parp-1*$^{-/-}$ tumors show higher chromosome instability and a more malignant phenotype compared to *Parp-1*$^{+/+}$ tumors. Another important issue is to understand whether Parp-1 or poly-ADP-ribosylation is involved in the formation of LOH during tumorigenesis.

Epigenetic Instability and Control of Gene Expression

Epigenetic changes in the gene are defined as nongenetic changes inheritable to daughter cells during cell growth. It has been demonstrated that epigenetic alteration of gene expression by hypo- or hyper-methylation substantially contributes to carcinogenesis.[76] Previous studies reported global hypomethylation in cancer cells and that genome hypomethylation induced by DNA methyltransferase mutation increases tumor incidence in mice.[77] In contrast, local hypermethylation of CpG islands in promoter regions in various tumor suppressor genes, including *p16*^{INK4}, was observed frequently in cancer cells.[76] Using the PARP inhibitor

3-aminobenzamide, Zardo et al[78] reported the presence of a genome-wide negative correlation between DNA methylation and poly-ADP-ribosylation. The promoter region of the *Htf9* gene also displayed this negative correlation.[79] Further studies are needed to investigate whether Parp-1 dysfunction leads to hypermethylation of cancer-related genes, and whether it promotes carcinogenesis.

On the other hand, poly-ADP-ribosylation can be involved in the control of gene expression independent of DNA methylation. In *Drosophila*, engineered *Parp*-deficient flies displayed attenuation of the expression of genes located in puff loci, accompanied by the lack of puff formation as well as marked decrease of the induction of immune related genes such as *Diptericine*.[80] Gene expression was also reported to be altered under *Parp-1* deficiency,[81] including those of *iNOS*,[82] and histone acetyltransferase.[83] A function of Parp-1 as a coactivator could be involved in these phenomena. These possible functions of Parp-1 in the regulation of gene expression may lead to the alteration of differentiation potential and may ultimately affect tumor phenotypes.

Cancer Cell Selection through Cell Death

During cancer development, cancer cells may encounter various forms of cell-death pressure, depending on the site of their growth and surrounding microenvironment. During rapid proliferation of cancer cells in limited tissue space, hypoxic/anoxic conditions may prevail, which in turn enhance p53-dependent apoptosis. Such conditions may preferentially select *p53*-deficient cancer cells and cells overexpressing bcl-2, an apoptosis inhibitory protein, as described by Graeber et al.[84] In this regard, *p53* gene alteration is detected in more than one-half of human tumors and *bcl-2* overexpression is also frequently observed in B-cell lymphoma, prostate cancer and colorectal cancer in humans.

On the other hand, oxidative cell death may be also induced in inflammatory conditions during carcinogenesis. Reactive oxygen species and reactive nitrogen species, including nitric oxide, produced by macrophages, induce rapid activation of Parp-1, leading to NAD depletion and apoptosis-inducing factor (AIF)-dependent cell death. Yu et al demonstrated that Parp-1 is necessary for this process.[85] It may thus be speculated that *Parp-1*-deficient cells may be selected out under such oxidative stress conditions (Fig. 1). Previous studies showed that neuronal cell death[86] and streptozotocin-induced pancreatic β-cell death were inhibited by either the Parp inhibitor, 3-aminobenzamide, or *Parp-1* deficiency.[4,87,88] The experiments by Yamagami et al, in which the development of insulinoma in rats treated with streptozotocin was markedly enhanced by treatment with the Parp inhibitor 3-aminobenzamide,[25] adds further support for this scenario.

Role of PARP in Human Carcinogenesis

Molecular and biochemical studies as well as animal model studies suggest that PARP is involved in carcinogenesis, although the relation of the functional loss of PARP to human carcinogenesis is largely undetermined yet. Several pioneering studies investigated the changes in *PARP-1* gene expression and gene structure in human cancers. In a series of studies, Bhatia et al[89-91] demonstrated that a *PARP-1* pseudogene on chromosome 13q33-qter presents a two allele (A/B) polymorphism and that the frequency of the B allele is higher in African Americans and is associated with endemic Burkitt lymphomas (1.7-fold), multiple myeloma and prostate cancers in the African American population. Enhanced activity and expression of *PARP-1* in Ewing's sarcoma cell lines were reported by Prasad et al.[92] The same group later reported that enhancement of *PARP-1* gene expression is due to activation of transcription factors *ets-1* in Ewing's sarcoma cells.[93] Bieche et al[94] showed that weak expression of the *PARP-1* gene is associated with higher genomic instability in breast cancer. They also showed that chromosome 1q41-42, where the *PARP-1* gene is located, is frequently amplified in cancers that overexpress *PARP-1*. Other studies reported low formation of poly(ADP-ribose) induced by bleomycin treatment in peripheral lymphocytes from laryngeal cancer patients,[95] suggesting

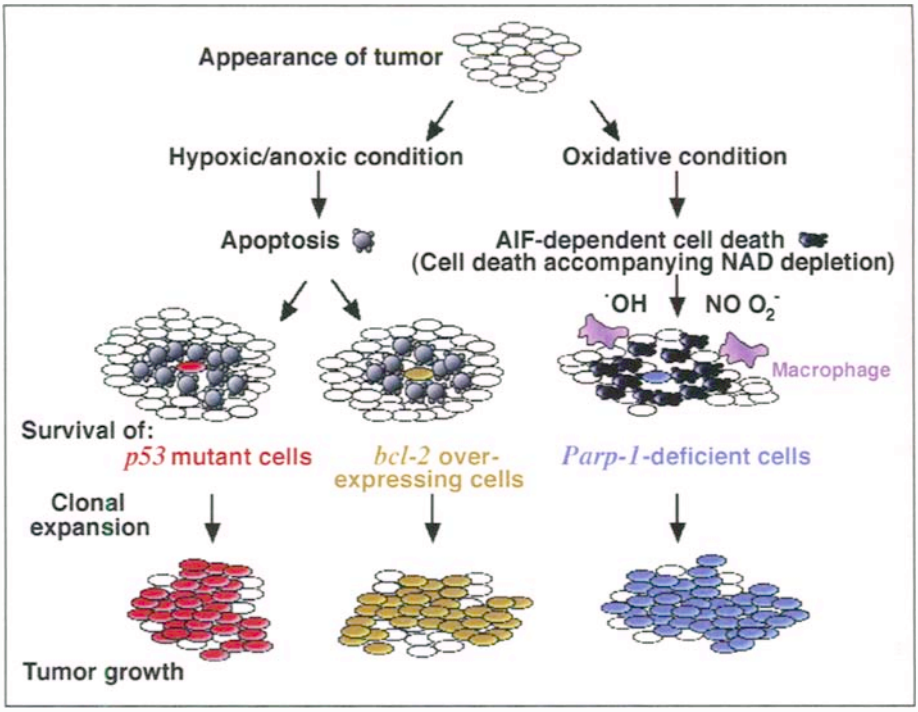

Figure 1. A possible model for selection of *Parp-1*-deficient cells during carcinogenesis. Hypoxic or anoxic conditions often prevail in tumors, which may lead to the preferential selection of *p53*-deficient cancer cells and cells overexpressing the anti-apoptotic protein bcl-2.[84] We speculate that, in contrast, *Parp-1*-deficient cells may be selected under oxidative or nitrosative stress conditions, which also may prevail during cancer formation. For details, see text.

that low PARP activity correlates with a higher risk of laryngeal cancer. We found that the gastric cancer cell line MKN28 harbored a structural alteration in *PARP-1* gene,[96] although it is not known yet whether this affects the function of PARP-1.

Recent biochemical studies suggest that dysfunctions of PARP-2 and PARP-3 are also closely related to carcinogenesis. In this regard, Augustin et al[21] indicated that the *PARP-3* gene is located at chromosome 3p21.1-3p21.31, where LOH is frequently observed in the early stages of lung cancer. Extensive investigation of genetic alterations of these PARP family genes may facilitate our understanding of the role of PARP in human carcinogenesis.

Concluding Remarks

Carcinogenesis in humans generally increases dramatically with age and it is considered that five or more genetic or epigenetic events may be necessary for development of cancer.[1,2] Each event leads to evolution of a certain tumor cell population from the selective pressure given from the microenvironment. Several lines of evidence obtained from research over the years imply that Parp-1 is involved in epithelial carcinogenesis and lymphomagenesis as a tumor suppressor factor. On the other hand, Parp-1 seems to be required for carcinogenesis and lymphomagenesis through its function in promotion of cell proliferation and inflammatory responses. Moolgavkar and Luebeck proposed that intervention strategies aimed at reducing the rate of clonal expansion of initiated/premalignant cells should be more effective than those designed to decrease the rate of early mutational events in the multistage process of human carcinogenesis.[97] In this context, a better understanding of the roles of poly-ADP-ribosylation

in transcription, cell-cycle-control, cell proliferation and modulation of immune responses is also important, especially for cancer development at an advanced age.

It was reported that haploinsufficiency of caretaker genes, such as histone *H2AX*[98,99] and *NBS*[100] genes significantly enhances the susceptibility to carcinogenesis. Since Parp-1 functions as both a caretaker and gate-keeper of the genome, haploinsufficiency of *Parp-1* may also enhance carcinogenesis. In this context, susceptibility of *Parp-1*$^{+/-}$ animals should be further investigated over extended time periods after application of various stimuli. The combination of haploinsufficiency in either caretaker genes or gate-keeper genes may further enhance the carcinogenic process during the long lifespan of human beings.

The identification of various Parp family members as well as Parg evoked intriguing questions on their functions, including whether these proteins are related to carcinogenesis, and further studies using animal models should clarify the impact of their deficiency on susceptibility to carcinogenesis.

References

1. Armitage P, Doll R. The age distribution of cancer and a multi-stage theory of carcinogenesis. Br J Cancer 1954; 8(1):1-12.
2. Sugimura T. Multistep carcinogenesis: A 1992 perspective. Science 1992; 258(5082):603-607.
3. Masutani M, Nakagama H, Sugimura T. Poly(ADP-ribose) and carcinogenesis. Genes Chromosomes Cancer 2003; 38(4):339-348.
4. Masutani M, Suzuki H, Kamada N et al. Poly(ADP-ribose) polymerase gene disruption conferred mice resistant to streptozotocin-induced diabetes. Proc Natl Acad Sci USA 1999; 96(5):2301-2304.
5. Wang ZQ, Auer B, Stingl L et al. Mice lacking ADPRT and poly(ADP-ribosyl)ation develop normally but are susceptible to skin disease. Genes Dev 1995; 9(5):509-520.
6. de Murcia JM, Niedergang C, Trucco C 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(14):7303-7307.
7. Tong WM, Cortes U, Hande MP et al. Synergistic role of Ku80 and poly(ADP-ribose) polymerase in suppressing chromosomal aberrations and liver cancer formation. Cancer Res 2002; 62(23):6990-6996.
8. Tsutsumi M, Masutani M, Nozaki T et al. Increased susceptibility of poly(ADP-ribose) polymerase-1 knockout mice to nitrosamine carcinogenicity. Carcinogenesis 2001; 22(1):1-3.
9. Nozaki T, Fujihara H, Watanabe M et al. Parp-1 deficiency implicated in colon and liver tumorigenesis induced by azoxymethane. Cancer Sci 2003; 94(6):497-500.
10. Ide F, Oda H, Nakatsuru Y et al. Xeroderma pigmentosum group A gene action as a protection factor against 4-nitroquinoline 1-oxide-induced tongue carcinogenesis. Carcinogenesis 2001; 22(4):567-572.
11. Morrison C, Smith GC, Stingl L et al. Genetic interaction between PARP and DNA-PK in V(D)J recombination and tumorigenesis. Nat Genet 1997; 17(4):479-482.
12. Donehower LA, Harvey M, Slagle BL et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 1992; 356(6366):215-221.
13. Tong WM, Hande MP, Lansdorp PM et al. 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(12):4046-4054.
14. Beneke R, Moroy T. Inhibition of poly(ADP-ribose) polymerase activity accelerates T-cell lymphomagenesis in p53 deficient mice. Oncogene 2001; 20(56):8136-8141.
15. Tong WM, Ohgaki H, Huang H et al. Null mutation of DNA strand break-binding molecule poly(ADP-ribose) polymerase causes medulloblastomas in p53(-/-) Mice. Am J Pathol 2003; 162(1):343-352.
16. Lee Y, McKinnon PJ. DNA ligase IV suppresses medulloblastoma formation. Cancer Res 2002; 62(22):6395-6399.
17. Conde C, Mark M, Oliver FJ et al. Loss of poly(ADP-ribose) polymerase-1 causes increased tumour latency in p53-deficient mice. EMBO J 2001; 20(13):3535-3543.
18. Kim PK, Zamora R, Petrosko P et al. The regulatory role of nitric oxide in apoptosis. Int Immunopharmacol 2001; 1(8):1421-1441.
19. Watanabe F, Masutani M, Kamada N et al. Impairment in S-phase entry of splenocytes of Parp-1 knockout mice. Proc Japan Acad 2003; 79 Ser B(8):248-251.
20. Schreiber V, Ame JC, Dolle P et al. Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1. J Biol Chem 2002; 277(25):23028-23036.
21. Augustin A, Spenlehauer C, Dumond H et al. PARP-3 localizes preferentially to the daughter centriole and interferes with the G1/S cell cycle progression. J Cell Sci 2003; 116(Pt 8):1551-1562.

22. Smith S, Giriat I, Schmitt A et al. Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. Science 1998; 282(5393):1484-1487.
23. Gunji A, Fujihara H, Kamada N et al. Lack of altered frequency of sister-chromatid exchanges in poly(ADP-ribose) glycohydrolase-deficient mouse ES cells treated with methylmethanesulfonate. Proc Japan Acad 2003; 79 Ser B(10):305-307.
24. Hanai S, Kanai M, Ohashi S et al. Loss of poly(ADP-ribose) glycohydrolase causes progressive neurodegeneration in Drosophila melanogaster. Proc Natl Acad Sci USA 2004; 101(1):82-86.
25. Yamagami T, Miwa A, Takasawa S et al. Induction of rat pancreatic B-cell tumors by the combined administration of streptozotocin or alloxan and poly(adenosine diphosphate ribose) synthetase inhibitors. Cancer Res 1985; 45(4):1845-1849.
26. Takahashi S, Ohnishi T, Denda A et al. Enhancing effect of 3-aminobenzamide on induction of gamma-glutamyl transpeptidase positive foci in rat liver. Chem Biol Interact 1982; 39(3):363-368.
27. Rosenberg MR, Novicki DL, Jirtle RL et al. Promoting effect of nicotinamide on the development of renal tubular cell tumors in rats initiated with diethylnitrosamine. Cancer Res 1985; 45(2):809-814.
28. Miwa M, Ishikawa T, Kondo T et al. Enhancement by 3-aminobenzamide of methylazoxymethanol acetate-induced hepatoma of the small fish "Medaka" (Oryzias latipes)". In: Althaus FR, Hilz H, Shall S, eds. ADP-Ribosylation of Proteins. Berlin-Heidelberg-New York-Tokyo: Springer-Verag, 1985:480-483.
29. Miller EG, Rivera-Hidalgo F, Binnie WH. 3-Methoxybenzamide, a possible initiator for DMBA-induced carcinogenesis. In: Jacobson MK, Jacobson EL, eds. ADP-Ribose Transfer Reactions. Mechanisms and Biological Significance. New York: Springer-Verlag,1989:287-290.
30. Rakieten N, Gordon BS, Beaty A et al. Pancreatic islet cell tumors produced by the combined action of streptozotocin and nicotinamide. Proc Soc Exp Biol Med 1971; 137(1):280-283.
31. Tsujiuchi T, Tsutsumi M, Denda A et al. Possible involvement of poly ADP-ribosylation in phenobarbital promotion of rat hepatocarcinogenesis. Carcinogenesis 1990; 11(10):1783-1787.
32. Nakagawa K, Utsunomiya J, Ishikawa T. Inhibition of methylazoxymethanol acetate initiation of colon carcinogenesis in rats by treatment with the poly(ADP-ribose)polymerase inhibitor 3-aminobenzamide. Carcinogenesis 1988; 9(7):1167-1171.
33. Denda A, Tsutsumi M, Yokose Y et al. Effects of 3-aminobenzamide on the induction of gamma-glutamyl-transpeptidase-positive foci by various chemicals in rat liver. Cancer Lett 1988; 39(1):29-36.
34. Boyonoski AC, Spronck JC, Gallacher LM et al. Niacin deficiency decreases bone marrow poly(ADP-ribose) and the latency of ethylnitrosourea-induced carcinogenesis in rats. J Nutr 2002; 132(1):108-114.
35. Boyonoski AC, Spronck JC, Jacobs RM et al. Pharmacological intakes of niacin increase bone marrow poly(ADP-ribose) and the latency of ethylnitrosourea-induced carcinogenesis in rats. J Nutr 2002; 132(1):115-120.
36. Kun E, Kirsten E, Milo GE et al. Cell cycle-dependent intervention by benzamide of carcinogen-induced neoplastic transformation and in vitro poly(ADP-ribosyl)ation of nuclear proteins in human fibroblasts. Proc Natl Acad Sci USA 1983; 80(23):7219-7223.
37. Borek C, Ong A, Morgan WF et al. Inhibition of X-ray- and ultraviolet light-induced transformation in vitro by modifiers of poly(ADP-ribose) synthesis. Radiat Res 1984; 99(2):219-227.
38. Borek C, Cleaver JE. Antagonistic action of a tumor promoter and a poly(adenosine diphosphoribose) synthesis inhibitor in radiation-induced transformation in vitro. Biochem Biophys Res Commun 1986; 134(3):1334-1341.
39. Borek C, Morgan WF, Ong A et al. Inhibition of malignant transformation in vitro by inhibitors of poly(ADP-ribose) synthesis. Proc Natl Acad Sci USA 1984; 81(1):243-247.
40. Lubet RA, McCarvill JT, Putman DL et al. Effect of 3-aminobenzamide on the induction of toxicity and transformation by ethyl methanesulfonate and methylcholanthrene in BALB/3T3 cells. Carcinogenesis 1984; 5(4):459-462.
41. Lubet RA, McCarvill JT, Schwartz JL et al. Effects of 3-aminobenzamide on the induction of morphologic transformation by diverse compounds in Balb/3T3 cells in vitro. Carcinogenesis 1986; 7(1):71-75.
42. Borek C, Ong A, Cleaver JE. Methylating and ethylating carcinogens have different requirements for poly(ADP-ribose) synthesis during malignant transformation. Carcinogenesis 1984; 5(12):1573-1576.
43. Strain AJ. Inhibitors of ADP-ribosyl transferase enhance the transformation of NIH3T3 cells following transfection with SV40 DNA. Exp Cell Res 1985; 159(2):531-535.
44. Ohashi Y, Ueda K, Hayaishi O et al. Induction of murine teratocarcinoma cell differentiation by suppression of poly(ADP-ribose) synthesis. Proc Natl Acad Sci USA 1984; 81(22):7132-7136.

45. Terada M, Fujiki H, Marks PA et al. Induction of erythroid differentiation of murine erythroleukemia cells by nicotinamide and related compounds. Proc Natl Acad Sci USA 1979; 76(12):6411-6414.
46. Brac T, Ebisuzaki K. Inhibitors of poly(ADP-ribose) polymerase prevent Friend cell differentiation. In: Althaus FR, Hilz H, Shall S, eds. ADP-Ribosylation of Proteins. Berlin-Heidelberg-New York-Tokyo: Springer, 1985:446-452.
47. Nakayasu M, Shima H, Aonuma S et al. Deletion of transfected oncogenes from NIH 3T3 transformants by inhibitors of poly(ADP-ribose) polymerase. Proc Natl Acad Sci USA 1988; 85(23):9066-9070.
48. Shima H, Nakayasu M, Aonuma S et al. Loss of the MYC gene amplified in human HL-60 cells after treatment with inhibitors of poly(ADP-ribose) polymerase or with dimethyl sulfoxide. Proc Natl Acad Sci USA 1989; 86(19):7442-7445.
49. Bauer PI, Kirsten E, Varadi G et al. Reversion of malignant phenotype by 5-iodo-6-amino-1,2-benzopyrone a noncovalently binding ligand of poly(ADP-ribose) polymerase. Biochimie 1995; 77(5):374-377.
50. Nozaki T, Masutani M, Watanabe M et al. Syncytiotrophoblastic giant cells in teratocarcinoma-like tumors derived from Parp-disrupted mouse embryonic stem cells. Proc Natl Acad Sci USA 1999; 96(23):13345-13350.
51. Hemberger M, Nozaki T, Winterhager E et al. Parp1-deficiency induces differentiation of ES cells into trophoblast derivatives. Dev Biol 2003; 257(2):371-381.
52. Hans MA, Muller M, Meyer-Ficca M et al. Overexpression of dominant negative PARP interferes with tumor formation of HeLa cells in nude mice: Evidence for increased tumor cell apoptosis in vivo. Oncogene 1999; 18(50):7010-7015.
53. Caldecott KW. Protein-protein interactions during mammalian DNA single-strand break repair. Biochem Soc Trans 2003; 31(Pt 1):247-251.
54. Divine KK, Gilliland FD, Crowell RE et al. The XRCC1 399 glutamine allele is a risk factor for adenocarcinoma of the lung. Mutat Res 2001; 461(4):273-278.
55. El-Khamisy SF, Masutani M, Suzuki H et al. A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. Nucleic Acids Res 2003; 31(19):5526-5533.
56. Dantzer F, Schreiber V, Niedergang C et al. Involvement of poly(ADP-ribose) polymerase in base excision repair. Biochimie 1999; 81(1-2):69-75.
57. von Kobbe C, Harrigan JA, May A 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(23):8601-8613.
58. Opresko PL, Cheng WH, von Kobbe C et al. Werner syndrome and the function of the Werner protein; what they can teach us about the molecular aging process. Carcinogenesis 2003; 24(5):791-802.
59. Malagna M, Althaus FR. Poly(ADP-ribose) reactivates stalled DNA topoisomerase I and induces DNA strand break resealing. J Biol Chem 2004; 279(7):5244-5248.
60. Ruscetti T, Lehnert BE, Halbrook J et al. Stimulation of the DNA-dependent protein kinase by poly(ADP-ribose) polymerase. J Biol Chem 1998; 273(23):14461-14467.
61. Ariumi Y, Masutani M, Copeland TD et al. Suppression of the poly(ADP-ribose) polymerase activity by DNA-dependent protein kinase in vitro. Oncogene 1999; 18(32):4616-4625.
62. Brown ML, Franco D, Burkle A et al. Role of poly(ADP-ribosyl)ation in DNA-PKcs-independent V(D)J recombination. Proc Natl Acad Sci USA 2002; 99(7):4532-4537.
63. Adelfalk C, Kontou M, Hirsch-Kauffmann M et al. Physical and functional interaction of the Werner syndrome protein with poly-ADP ribosyl transferase. FEBS Lett 2003; 554(1-2):55-58.
64. Li B, Nacarro S, Kasahara N et al. Identification and biochemical characterization of a Werner syndrome protein complex with Ku70/80 and PARP-1. J Biol Chem 2004; 279(14):13659-13667.
65. Simbulan-Rosenthal CM, Haddad BR, Rosenthal DS et al. Chromosomal aberrations in PARP(-/-) mice: Genome stabilization in immortalized cells by reintroduction of poly(ADP-ribose) polymerase cDNA. Proc Natl Acad Sci USA 1999; 96(23):13191-13196.
66. Nozaki T, Fujihara H, Kamada N et al. Hyperploidy of embryonic fibroblasts derived from Parp-1 knockout mouse. Proc Japan Acad 2001; 77 Ser B(6):121-124.
67. Simbulan-Rosenthal CM, Rosenthal DS, Luo R et al. Inhibition of poly(ADP-ribose) polymerase activity is insufficient to induce tetraploidy. Nucleic Acids Res 2001; 29(3):841-849.
68. Wang ZQ, Stingl L, Morrison C et al. PARP is important for genomic stability but dispensable in apoptosis. Genes Dev 1997; 11(18):2347-2358.
69. Vaziri H, West MD, Allsopp RC et al. ATM-dependent telomere loss in aging human diploid fibroblasts and DNA damage lead to the post-translational activation of p53 protein involving poly(ADP-ribose) polymerase. EMBO J 1997; 16(19):6018-6033.

70. Nozaki T, Masutani M, Akagawa T et al. Suppression of G1 arrest and enhancement of G2 arrest by inhibitors of poly(ADP-ribose) polymerase: Possible involvement of poly(ADP-ribosyl)ation in cell cycle arrest following gamma-irradiation. Jpn J Cancer Res 1994; 85(11):1094-1098.
71. Wieler S, Gagne JP, Vaziri H et al. Poly(ADP-ribose) polymerase-1 is a positive regulator of the p53-mediated G1 arrest response following ionizing radiation. J Biol Chem 2003; 278(21):18914-18921.
72. Agarwal ML, Agarwal A, Taylor WR et al. Defective induction but normal activation and function of p53 in mouse cells lacking poly-ADP-ribose polymerase. Oncogene 1997; 15(9):1035-1041.
73. Frouin I, Maga G, Denegri M et al. Human proliferating cell nuclear antigen, poly(ADP-ribose) polymerase-1, and p21waf1/cip1. A dynamic exchange of partners. J Biol Chem 2003; 278(41):39265-39268.
74. Kanai M, Tong WM, Sugihara E et al. Involvement of poly(ADP-Ribose) polymerase 1 and poly(ADP-Ribosyl)ation in regulation of centrosome function. Mol Cell Biol 2003; 23(7):2451-2462.
75. Halappanavar SS, Shah GM. Defective control of mitotic and post-mitotic checkpoints in poly(ADP-ribose) polymerase-1(-/-)fibroblasts after mitotic spindle disruption. Cell cycle 2004; 3(3):335-342.
76. Sugimura T, Ushijima T. Genetic and epigenetic alterations in carcinogenesis. Mutat Res 2000; 462(2-3):235-246.
77. Gaudet F, Hodgson JG, Eden A et al. Induction of tumors in mice by genomic hypomethylation. Science 2003; 300(5618):489-492.
78. Zardo G, D'Erme M, Reale A et al. Does poly(ADP-ribosyl)ation regulate the DNA methylation pattern? Biochemistry 1997; 36(26):7937-7943.
79. de Capoa A, Febbo FR, Giovannelli F et al. Reduced levels of poly(ADP-ribosyl)ation result in chromatin compaction and hypermethylation as shown by cell-by-cell computer-assisted quantitative analysis. FASEB J 1999; 13(1):89-93.
80. Tulin A, Spradling A. Chromatin loosening by poly(ADP)-ribose polymerase (PARP) at Drosophila puff loci. Science 2003; 299(5606):560-562.
81. Simbulan-Rosenthal CM, Ly DH, Rosenthal DS et al. Misregulation of gene expression in primary fibroblasts lacking poly(ADP-ribose) polymerase. Proc Natl Acad Sci USA 2000; 97(21):11274-11279.
82. Hassa PO, Hottiger MO. A role of poly(ADP-ribose) polymerase in NF-kappaB transcriptional activation. Biol Chem 1999; 380(7-8):953-959.
83. Ota K, Kameoka M, Tanaka Y et al. Expression of histone acetyltransferases was down-regulated in poly(ADP-ribose) polymerase-1-deficient murine cells. Biochem Biophys Res Commun 2003; 310(2):312-317.
84. Graeber TG, Osmanian C, Jacks T et al. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature 1996; 379(6560):88-91.
85. Yu SW, Wang H, Poitras MF et al. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 2002; 297(5579):259-263.
86. Eliasson MJ, Sampei K, Mandir AS et al. Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nat Med 1997; 3(10):1089-1095.
87. Burkart V, Wang ZQ, Radons J et al. Mice lacking the poly(ADP-ribose) polymerase gene are resistant to pancreatic beta-cell destruction and diabetes development induced by streptozotocin. Nat Med 1999; 5(3):314-319.
88. Pieper AA, Brat DJ, Krug DK et al. Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocin-induced diabetes. Proc Natl Acad Sci USA 1999; 96(6):3059-3064.
89. Bhatia KG, Cherney BW, Huppi K et al. A deletion linked to a poly(ADP-ribose) polymerase gene on chromosome 13q33-qter occurs frequently in the normal black population as well as in multiple tumor DNA. Cancer Res 1990; 50(17):5406-5413.
90. Bhatia K, Huppi K, Cherney B et al. Relative predispositional effect of a PADPRP marker allele in B-cell and some non B-cell malignancies. Curr Top Microbiol Immunol 1990; 166:347-357.
91. Lyn D, Cherney BW, Lalande M et al. A duplicated region is responsible for the poly(ADP-ribose) polymerase polymorphism, on chromosome 13, associated with a predisposition to cancer. Am J Hum Genet 1993; 52(1):124-134.
92. Prasad SC, Thraves PJ, Bhatia KG et al. Enhanced poly(adenosine diphosphate ribose) polymerase activity and gene expression in Ewing's sarcoma cells. Cancer Res 1990; 50(1):38-43.
93. Soldatenkov VA, Albor A, Patel BK et al. Regulation of the human poly(ADP-ribose) polymerase promoter by the ETS transcription factor. Oncogene 1999; 18(27):3954-3962.
94. Bieche I, de Murcia G, Lidereau R. Poly(ADP-ribose) polymerase gene expression status and genomic instability in human breast cancer. Clin Cancer Res 1996; 2(7):1163-1167.

95. Rajaee-Behbahani N, Schmezer P, Ramroth H et al. Reduced poly(ADP-ribosyl)ation in lymphocytes of laryngeal cancer patients: Results of a case-control study. Int J Cancer 2002; 98(5):780-784.
96. Masutani M, Nozaki T, Sasaki H et al. Aberration of poly(ADP-ribose) polymerase-1 gene in human tumor cell lines: Its expression and structural alterations. Proc Japan Acad 2004; 80 Ser B(2):114-118.
97. Moolgavkar SH, Luebeck EG. Multistage carcinogenesis and the incidence of human cancer. Genes Chromosomes Cancer 2003; 38(4):302-306.
98. Bassing CH, Suh H, Ferguson DO et al. Histone H2AX: A dosage-dependent suppressor of oncogenic translocations and tumors. Cell 2003; 114(3):359-370.
99. Celeste A, Difilippantonio S, Difilippantonio MJ et al. H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. Cell 2003; 114(3):371-383.
100. Dumon-Jones V, Frappart PO, Tong WM et al. Nbn heterozygosity renders mice susceptible to tumor formation and ionizing radiation-induced tumorigenesis. Cancer Res 2003; 63(21):7263-7269.