CMLS, Cell. Mol. Life Sci. 62 (2005) 769–783
1420-682X/05/080769-015
DOI 10.1007/s00018-004-4509-x

CMLS Cellular and Molecular Life Sciences

Poly(ADP-ribosyl)ation in relation to cancer and autoimmune disease

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Abstract. Carcinogenesis involves multiple steps and pathways with functional alterations in a variety of genes. There is accumulating evidence that a deficiency of poly(ADP-ribose) polymerase (*PARP)-1* leads to DNA repair defects, genomic instability, failure of induction of cell death and modulation of gene transcription. PARP-1 also supports the growth of tumor cells in certain situations. Genetic analyses of the *PARP-1* gene have demonstrated alterations in neoplasms, and a mutation affecting the conserved amino acid E251 in germ cell tumors, as well as an association of a single-nucleotide polymorphism V762A with risk of prostate cancer. Recent development of a selective inhibitor of poly(ADP-ribose) glycohydrolase (PARG), the enzyme primarily responsible for degradation of poly(ADP-ribose), and *PARG*-deficient animals should facilitate studies of the relationship of poly(ADP-ribose) with carcinogenesis. Inhibitors of PARP have also suggested roles in the pathogenesis of autoimmune disease, and a promoter haplotype of *PARP-1* confers a higher risk of rheumatoid arthritis. Further analysis of *PARP-1*, *PARG* and other *PARP* family genes should extend our understanding of the pathogenesis of cancer and autoimmune diseases. Furthermore, there is potential for sensitization to chemo- and radiation therapy of cancers as well as the treatment of autoimmune disease with development of stronger PARP inhibitors.

Key words. Poly(ADP-ribose); PARP; PARG; cancer; autoimmune diseases.

Introduction

Since the discovery of poly(ADP-ribose) and poly(ADP-ribose) polymerase (PARP) in the 1960s, progress in understanding their biological functions has been slow. The first breakthrough was made through the availability of a specific PARP inhibitor, 3-aminobenzamide (3AB), which was found to enhance the effects of DNA damaging agents [1]. The elucidation of the molecular mechanisms of DNA repair, DNA replication and transcription places the science of poly(ADP-ribose) and PARP on a firm basis with detailed information about their biological roles. Involvement of poly(ADP-ribose) and PARP in carcinogenesis has thereby become evident (fig. 1). The usage of appropriately designed inhibitors of PARP and knockout animals has been crucial. We previously published two review articles on carcinogenesis and PARP [2, 3]. In the present review, we include more recent information with relevance to human health, and cover priorities for future work.

In 1977, our group reported the presence of an antibody against poly(ADP-ribose) in the sera of patients with systemic lupus erythematosus (SLE), soon after the discovery of poly(ADP-ribose) and PARP [4]. This was followed by a demonstration of antibodies against poly(ADP-ribose) in the sera of New Zealand mice, which spontaneously develop autoimmune disease. The latter half of this paper reviews current information on research into poly(ADP-ribose) with reference to autoimmunity. Cancer and autoimmune diseases are clearly very different in their clinical features. Nevertheless, to a certain extent, the common involvement of poly(ADP-ribose) and PARP has been found. It can be envisaged that attention to both diseases, focusing on poly(ADP-ribose), would provide new insights. In this article, we summarize and discuss the current understanding of the relation of

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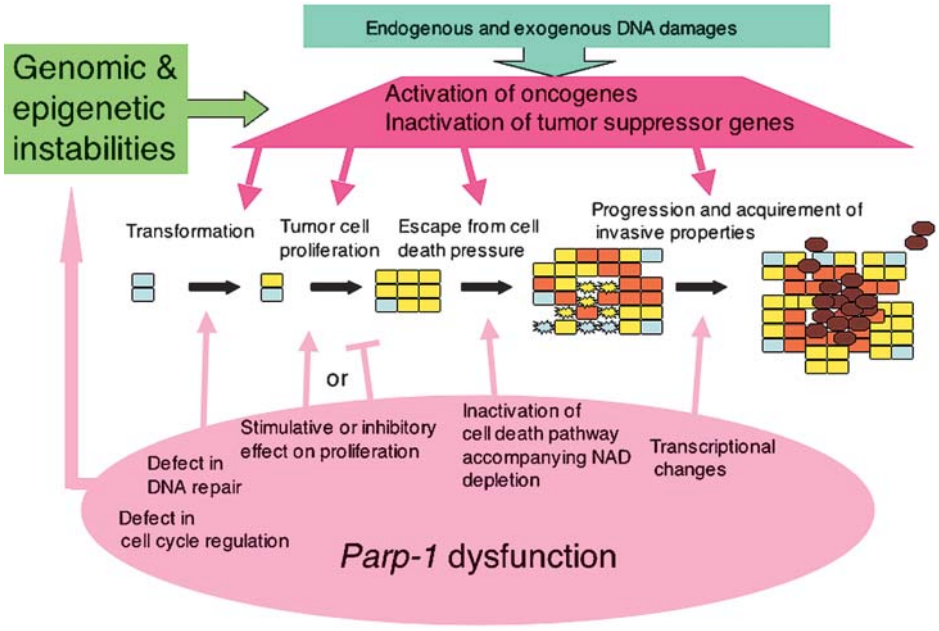

Figure 1. Possible involvement of *Parp-1* dysfunction in multi-step carcinogenesis. Endogenous and exogenous DNA damage, as well as genomic and epigenetic instabilities, induces activation of oncogenes and inactivation of tumor suppressor genes. These events cause cellular transformation (as indicated by yellow) and lead to selection of tumor cells which might accompany potential of survival against cell death pressures (as indicated by orange) and tumor cells which further acquire malignant and invasive properties (as indicated by brown). *Parp-1* dysfunction affects genomic and epigenetic stabilities possibly through defects in DNA repair and cell cycle regulation. Both stimulative and inhibitory effects on proliferation of tumor cells by *Parp-1* dysfunction are observed depending on the types of tissues and tumor cells. *Parp-1* dysfunction may further inactivate a cell death pathway, which accompanies NAD depletion, and may modulate tumor cells to acquire malignant and invasive potential.

poly(ADP-ribose) metabolism to pathogenesis and therapeutic aspects of cancer and autoimmune diseases, focusing on two major molecules, PARP-1 and PARG.

Cancer and autoimmune diseases

The development of cancer is governed by two groups of etiologic factors: heritable and environmental, namely genotoxic agents, such as DNA damaging agents, and non-genotoxic factors, such as hormones [5]. Cancers showing early onset are often associated with a substantial contribution of heritable genetic alteration, while those developing at an advanced age may be triggered by a combination of weak heritable and multiple environmental factors. DNA damage can be induced by chronic or acute inflammatory reactions and cause genetic and epigenetic changes in oncogenes and tumor suppressor genes. When caretaker genes for genomic stability are involved, evolution of tumors would be expected to be markedly accelerated.

Meanwhile, the pathogenesis of autoimmune diseases involves the activation of B and/or T cells, which recognize weak self-antigens and subsequent inflammation, which trigger damage in tissues. Delayed clearance of apoptotic cells by macrophages and dendritic cells is considered to stimulate immune responses to self-antigens, thereby leading to autoimmunity. Substantial involvement of heritable factors is also notable in autoimmune diseases. Hereditary defects may promote failure of T cell regulation, which is critical to support non-responsiveness to weak self-antigens. Environmental factors such as infection by microbes, ultraviolet (UV) radiation, ionizing radiation or drugs that modify weaker antigens might increase specific B or T cell activation.

Inflammation reactions are considered to promote carcinogenesis by inducing genomic instability due to oxidative and nitrosative stress on cellular components [6]. However, in general, organ-specific autoimmune diseases only infrequently predispose to cancer development. Deficiency in poly(ADP-ribose) polymerase (PARP)-1 might contribute to carcinogenesis through induction of genomic and epigenetic instability and alteration of transcriptional regulation and differentiation. In the pathogenesis of autoimmune diseases, PARP-1 dysfunction could modulate immune responses through affecting transcriptional regulation and might also influence cell death induction and clearance of dead cells.

Poly(ADP-ribosyl)ation reactions, PARP and PARG

Poly(ADP-ribosyl)ation reactions function in multiple cellular responses, including DNA damage, inflammation and cell death induction. More than 16 PARP family proteins have been reported, including PARP-1, which has a siginificant function in several DNA repair pathways [7]. PARP uses NAD as a substrate and poly(ADP-ribosyl)ates glutamic acid, aspartic acid and lysine residues on various proteins, including PARP-1 itself. This poly(ADP-ribosyl)ation of proteins affects their conformation through highly negative charges and consequently modulates interactions with DNA.

PARP-1 has a three-domain structure and is activated by binding to DNA strand breaks. The N-terminal DNA binding domain harbors two zinc-finger (ZF) motifs, $CX_2CX_{28,\ 30}HX_2C$, bipartite nuclear localization signals (NLSs) and a conserved cysteine-rich structure, $CX_2CX_{12}CX_9C$. Cleavage sites of PARP-1 during apoptosis by caspase reside in the NLS. ZF-I is necessary for PARP-1 activation, and ZF-II functions in binding to single-strand breaks (SSBs) but is not essential for PARP-1 activation [8]. Both ZF-I and -II have the capacity to bind to SSBs [9]. PARP-1 also binds to cruciform, curved, bent and looped DNA structures. With the latter, the Kd value is equivalent to that for the blunt end of DNA [10]. The ability of PARP-1 to bind to looped and/or curved DNA structures may account for its specific localization to nuclear matrices, chromatin fractions or the promoter sequences of certain genes and should be further investigated.

In the auto-modification domain, 15 glutamic acid and 4 aspartic acid residues are present in human PARP-1, serving as potential acceptor sites for poly(ADP-ribosyl)ation. PARP-1 also poly(ADP-ribosyl)ates other proteins, including histones, DNA topoisomerase I (topo I) and II, DNA polymerases, p53, various transcription factors, such as NF-κB, and proteins involved in DNA repair. A BRCT motif (BRCA1 C-terminus motif) is involved in the interaction with other proteins, including transcription factors, such as YY1 (Ying Yang transcription factor 1) and Oct-1 (Pou-homeodomain-containing octamer transcription factor-1). XRCC1 (X-ray repair cross-complementing factor-1), which is involved in base-excision repair (BER), also interacts with PARP-1. The automodification domain and N-terminal DNA binding domain function in homodimerization of PARP-1 and heterodimerization of PARP-1 and PARP-2 [11].

The C-terminal domain is well conserved among species. Random mutagenesis of the catalytic domain of PARP-1 revealed that mutation at G699, R704, L797, S808, N868, F869, G871, L877, M890, F897, D899, C908, E923, L926, L964, G972, L984, Y986, E988, Y989, L999, Y1001, and L1003 reduced poly(ADP-ribosyl)ation activity [12]. Mutation at Y986 and E988 also affected poly(ADP-ribose) branching and/or elongation [12]. Mutation of some surface residues (R847, E923, G972) also altered the branching of poly(ADP-ribose). The presence of a shorter isoform, sPARP-1, consisting of the C-terminal catalytic domain, has been reported in the mouse [13]. sPARP-1 is localized in the nucleus and is stimulated by genotoxic agents, suggesting an involvement in DNA damage responses.

Poly(ADP-ribose) glycohydrolase (PARG) is the major degradation enzyme for poly(ADP-ribose). A single *PARG* gene has been identified, but three different isoforms of the protein are produced by alternative splicing and located in various cellular organella [14, 15]. These isoforms seem to be responsible for catalyzing the degradation of poly(ADP-ribose) present in different cellular compartments. Human *PARG* complementary DNA (cDNA) encodes a 976-aa protein, comprising N-terminal regulatory and C-terminal catalytic domains [16]. In the N-terminal domain, a putative NLS is present in exon 1. A potential nuclear export signal, LX_2VX_2LXL, is also present in this domain from aa 127–135 [17]. There is also a possible mitochondrial localization signal present in the middle of the protein [14]. When overexpressed, 111-kDa full-length human PARG fused to GFP protein is exclusively present in the nucleus in the interphase and is not associated with chromatin during mitosis, but is present in cytoplasm and centrosomes [18]. The presence of two alternatively spliced variants, 102 kDa, lacking exon 1, and 99 kDa, lacking both exons 1 and 2, has also been demonstrated in cytoplasm [14]. An 80-kDa isoform was also identified by Western blotting [15]. PARG is cleaved by caspase-3 at $DEID_{256}V$ and $MDVD_{307}N$ to yield 85- and 74-kDa C-terminal fragments [19]. The apparent lack of NLS in these fragments may explain their localization in the cytoplasm. The cleaved PARG may have distinct functions and influence cell death processes.

Genomic stability and DNA repair

The impact of *Parp-1* ablation on genomic stability has been analyzed in primary cultures and with immortalized cells, as listed in table 1. Notably, when bone marrow cells were used, chromosome breaks did not show elevation [20, 21], whereas bone marrow cells after treatment with DNA damaging agents or non-treated mouse embryonic fibroblasts (MEFs), even at early passage, exhibited elevated chromosome instability [22, 23]. A plausible explanation is that genomic instability observed in *Parp-1*$^{-/-}$ cells is mostly induced by DNA damage. In fact, the 20% oxygen concentration commonly used for cell culture induces substantial oxidative damage in MEFs, resulting in increased mutation [24].

A higher rate of loss of heterozygosity (LOH) occurred at the *p53* locus derived from *Parp-1*$^{-/-}$ *p53*$^{+/-}$ than from

Table 1. Impact of *Parp-1* deficiency on genomic instability.

Genomic instability	Detailed description of alterations	Treatment	Outcome (observed cells or tissue)
Chromosomal abberation	chromosome/chromatid breaks	none	↑[1] primary MEF[3] [20], →[2]bone marrow [21]
		MNU	↑bone marrow [21]
		γ-irradiation	↑bone marrow [21]
	LOH	*p53* deficiency	↑tumor [20]
	aneuploidy	none	→ES cell[4]
	ploidy increase	none	↑immortalized MEF [108, 109], → splenocyte[4], →ES cell[4]
	SCE	none	↑bone marrow [21], ↑splenocyte [22], ↑immortalized MEF [25]
		mitomycin C	↑ primary MEF [22]
		hydroxyurea	↑ immortalized MEF[25]
		MNU*[5]	↑bone marrow [21]
Micronuclei		none	↑primary MEF [23], → primary MEF [22]
		MMS	↑primary MEF [23]
		mitomycin C	↑splenocyte [22]
		γ-irradiation	↑splenocyte [22]
Elevation of mutation frequency	point mutation	none	→liver [27], →bone marrow [27]
		BHP	→liver [27]
	deletion	none	→liver [27], →bone marrow [27]
		BHP	↑liver [27]
Minisatellite instability		none	→ES cell[4]
Misregulation of transcription		none	↑primary MEF [108], ↑ES cell [55]

[1] No change (→), or [2]elevated (↑), when compared to wild-type cells or tissue.
[3] MEF: mouse embryonic fibroblast.
[4] M. Masutani et al., unpublished.

Parp-1$^{+/+}$*p53*$^{+/-}$ mice [20]. This raises the intriguing possibility that *Parp-1* deficiency induces LOH during tumorigenesis, although the frequency of LOH should be further measured with different loci. LOH could be caused by chromosome breaks, chromosome loss and through homologous recombination (HR) repair. Increased and persistent formation of Rad51 foci and augmented sister chromatid exchange (SCE) frequencies in *Parp-1*$^{-/-}$ cells [25] have indicated that *Parp-1* deficiency causes a higher chance of HR by providing stalled replication forks or increasing DSB formation.

PARP-1 binds specifically with high affinity to the ends of single- and double-strand breaks of DNA. Through this property and also through the capacity of PARP-1 to interact with DNA repair proteins through the BRCT motif, PARP-1 functions in BER, and SSB and double-strand break (DSB) repair, as well as HR [23, 26]. After treatment with an alkylating agent, *N*-nitrosobis(2-hydroxypropyl)amine (BHP), point mutations in the livers of *Parp-1*$^{-/-}$ mice did not increase, but deletions and those accompanying rearrangement/insertions were enhanced [27]. These results suggest that PARP-1 might act to prevent the formation of a stalled complex after excision of damaged bases during BER reactions, and, in its absence, the stalled BER complex may cause DSBs which would require further repair by either non-homologous end-joining repair (NHEJ) or HR. The fidelity of NHEJ itself may also be lower in the absence of PARP-1, resulting in induction of deletions/insertions.

Regarding other members of the PARP family, PARP-2 is involved in BER by interacting with PARP-1 and XRCC1 [11]. PARP-1 and PARP-2 prevent covalent complex formation of topo I with DNA and are able to remove stalled topo I from abortive SSBs [28]. PARP-2 localizes in centromeres, and *Parp-2*$^{-/-}$ cells show enhanced G2/M accumulation, and tetraploidy with kinetochore defects, and an increase of anaphase bridges [29]. PARP-3 localizes in daughter centrioles throughout the cell cycle and interacts with PARP-1, and its overexpression causes G1/S phase arrest [30]. These results indicate the possibility that dysfunction of PARP-2 and PARP-3 could induce mitotic defects leading to genomic instability, thus contributing to carcinogenesis.

During DNA repair, various proteins are poly(ADP-ribosyl)ated. Thus, it is speculated that dysfunction of PARG may also interfere with DNA repair. However, *Parg*$^{-/-}$ embryonic stem (ES) cells did not show an increased frequency of SCE induction in the absence or

presence of an alkylating agent, methylmethanesulfonate (MMS) [30]. Since *Parg*$^{-/-}$ cells [2, 32, 33] and mice show increased lethality on exposure to alkylating agents and ionizing irradiation [34], more detailed analyses should be conducted to clarify the role of PARG in DNA repair. Knockdown of tankyrase 1 resulted in mitotic arrest and non-resolution of sister telomere association [35]. Even partial dysfunction of tankyrase 1 may lead to chromosome aberration and possibly take part in carcinogenesis.

Epigenetic regulation

Epigenetic information in the cell is defined as information that is stably inherited by daughter cells but not coded by DNA sequences. DNA methylation and chromatin modification, including that of histones, are the major known processes underlying epigenetic regulation. Poly(ADP-ribosyl)ation, occurring on histones and other chromatin proteins, may be directly involved in epigenetic regulation and also function indirectly by affecting DNA methylation and histone acetylation. PARP inhibition induced an increase in global DNA methylation in the genome and also showed reduction of *Htf9* gene expression [36]. *Parp-1*-disrupted flies show loss of gene expression in puff loci and a wide range of changes in the gene-expression pattern [37]. These results support some relation of poly(ADP-ribosyl)ation with epigenetic regulation, and further studies are anticipated in this regard.

Genetically manipulated animal models for cancer

PARP inhibitors may enhance chemical-induced carcinogenesis or exert suppressive effects depending on the tissue and carcinogen used. PARP-1 inhibitors, such as 3AB, may impact not only on PARP-1 but also other PARP family proteins, and this might influence the outcome of carcinogenesis experiments. The carcinogenesis experiments of transgenic and knockout models of PARP-1 are summarized in table 2. Transgenic mice expressing the DNA binding domain of PARP-1, which acts as a dominant-negative mutant, show an increased frequency of T cell lymphoma in *p53*-deficient mice [38]. In *Parp-1*$^{-/-}$ mice established by disrupting exon 2 [39] and exon 1[3], an increased frequency of the spontaneous development of hepatocellular carcinoma (HCC) was observed at the ages of 18–24 months. *Parp-1*$^{-/-}$ mice disrupted in exon 1 demonstrated a substantial increase in carcinogenesis after administration of alkylating agents, including BHP, in the liver and lungs [40]. In addition, hemangiosarcomas were observed in the livers of *Parp-1*$^{-/-}$ mice but not at all in wild-type mice, indicating that *Parp-1* deficiency also enhances the malignancy of the tumors. In the case of azoxymethane treatment, *Parp-1*$^{-/-}$ mice also demonstrated increases of the incidences of colon cancer and liver nodules [41]. The sizes of colon adenocarcinomas were larger in *Parp-1*$^{-/-}$ than in *Parp-1*$^{+/+}$ mice, suggesting promotion of tumor growth.

In contrast, *Parp-1*$^{-/-}$ mice did not show an increased incidence of tumors after treatment with the carcinogens 4-nitrosoquinoline 1-oxide (4NQO) [A. Gunji et al, unpublished 43] and IQ (2-amino-3-methylimidazo[4,5-*f*] quinoline) [K. Ogawa et al., unpublished, 43], both of which generate bulky adducts in DNA and would be repaired by nucleotide excision repair. Taken together, these results suggest that the functions of PARP-1 in BER and/or DNA strand break repair that are involved in correction of alkylation-induced DNA lesions are more important than other functions of PARP-1 for suppression of carcinogenesis.

The combination of the *DNA-PKcs* (SCID mutation) [42], *Ku80* [38] or *p53* [20] deficiency all enhanced the incidence of tumor development in exon 2-disrupted *Parp-1*$^{-/-}$ mice, probably through triggering genomic instability. Notably, in *Parp-1*$^{-/-}$*p53*$^{-/-}$ mice, medulloblastomas, cerebellar embryonal tumors, were observed at a 50% frequency from about 16 weeks [43]. All of these tumors accompanied Hedgehog pathway activation by overexpression of the effector *Gli* gene and showed aneuploidy. Further characterization of medulloblastomas in *Parp-1*$^{-/-}$ *p53*$^{-/-}$ mice should be helpful to understand the mechanisms of development of this type of tumor in humans.

When exon 4-disrupted *Parp-1*$^{-/-}$ mice were used to generate *Parp-1*$^{-/-}$*p53*$^{-/-}$ mice, tumor-free survival was extended 50% [44]. Constitutive *iNOS* (*inducible nitric oxide synthase*) upregulation in *p53*$^{-/-}$ mice was previously shown to contribute to carcinogenesis [45], and the *Parp-1* deficiency substantially suppressed *iNOS* expression, causing reduction in the cell proliferation potential in *Parp-1*$^{-/-}$*p53*$^{-/-}$ mice. The incidence of skin papilloma in the *Parp-1*$^{-/-}$mice, harboring exon 4 disruption in *Parp-1* gene induced in a two-step carcinogenesis model with 7,12-dimethylbenz[a]-anthracene (DMBA) and 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), was also decreased with a concomitant reduction in NF-κB activation and proliferation in epidermis after carcinogen treatment [46]. It should be noted that genomic instability is commonly enhanced both with exon 2- and 4-disruption of the *Parp-1* gene in *p53*$^{-/-}$ mice. Therefore, the different results for susceptibility to tumorigenesis between exon 2- and -4-disrupted *Parp-1*$^{-/-}$ mice may not be caused by the difference in genomic instability. PARP-1 may also function to support tumor growth under certain conditions. Interactions of the effects of genetic background with *Parp-1* deficiency and/or the expression of different isoforms of PARP-1 from disrupted alleles may be important in this regard.

Parg knockout mice were recently generated and shown to feature increased sensitivity to streptozotocin and

Table 2. Carcinogenesis studies conducted with *Parp-1* knockout and transgenic mice models.

Method		Disruption of *Parp-1*	Tumors	Incidence	Related features of the *Parp-1*$^{-/-}$ mice and tumors
No treatment		exon 2	liver; hepatocellular carcinoma (HCC) at 18–24 months old	increased [39]	β-catenin accumulation and loss of *E-cadherin* expression[1] [39]
		exon 1	liver; HCC at 21-23 months old	increased [3]	
Chemicals	BHP	exon 1	liver; hemangioma and hemangiosarcoma	increased [40]	increased frequency of hemangiosarcoma[2] [40] and increased deletion mutation in the liver after BHP treatment[3] [27]
			lung; adenoma and adenocarcinoma	increased [40]	
	azoxymethane	exon 1	colon; adenocarcinoma	increased [41]	larger tumor size[2] [41]
			liver; nodule	increased [41]	
	4NQO	exon 1	oral cavity and esophagus; squamous cell carcinoma	no change [3]	not detected
	IQ	exon 1	liver; HCC	no change [3]	not detected
			lung; adenoma	no change [3]	
			forestomach; papilloma	no change [3]	
	DMBA + TPA	exon 4	skin; papilloma	decreased [46]	lack of NF-κB activation[2] [46]
Combined genetic effect	SCID	exon 2	thymus: T cell lymphoma	increased [42]	earlier onset than SCID*Parp-1*$^{+/+}$ mice[2] [42]
	P53$^{-/-}$	exon 2	colon and breast; carcinoma	increased [20]	increase of LOH at the *p53* locus[2] [20]
			brain; medulloblastoma	increased [43]	overexpression of *Gli* and aneuploidy[1] [43]
	P53$^{-/-}$	exon 4	thymus: T-cell lymphoma	decreased [44]	decreased *iNOS* expression[3] [44]
	Ku80$^{+/-}$	exon 2	liver; HCC	increased [39]	increased chromosome breaks and fusions[1] [39] β-catenin mutation and loss of *E-cadherin* expression[1] [39]
	p53$^{-/-}$	PARP-DBD transgenic	thymus, peripheral lymphoid tissues: T cell lymphoma	increased [38]	abrogation of DNA damage checkpoint after γ-irradiation[3] [38]

[1]Features described in *Parp-1*$^{-/-}$ tumors but have not been compared with *Parp-1*$^{+/+}$ tumors.
[2]Comparison between *Parp-1*$^{+/+}$ and *Parp-1*$^{-/-}$ tumors.
[3]Comparison between *Parp-1*$^{+/+}$ and *Parp-1*$^{-/-}$ mice.

γ-irradiation [34]. We have established *Parg*$^{-/-}$ ES cells and found that they exhibit increased lethality to an alkylating agent, MMS, and γ-irradiation [2]. When nude mice were inoculated with *Parg*$^{-/-}$ ES cells, they developed teratocarcinomas with the same spectrum of differentiation as *Parg*$^{+/+}$ ES cells, indicating that the potential for tumorigenesis is not lost by *Parg* disruption [47]. Since several splicing variants have been reported [15], as mentioned earlier, the results of the *Parg* gene-targeting experiment should best be interpreted with information on which splicing variants were actually disrupted. About three fourths of *Parg* knockout *Drosophila* become morbid in the larval stages, and surviving adult flies show progressive neurodegeneration with accumulation of poly(ADP-ribose) in the central nervous system [48]. This experimental system will be also useful for analyzing the impact of *Parg* deficiency on tumorigenesis and genomic stability.

Modulation of tumor phenotypes

Early studies using PARP-1 inhibitors showed induction of differentiation in various types of tumor cells, including mouse teratocarcinoma cells [49], Friend erythroleukemic cells [50] and human HL-60 cells [51]. H-*ras* transformed NIH3T3 cells and endothelial cells showed loss of tumorigenicity on treatment with PARP inhibitors, benzamide 4-hydroxy-quinazoline or 5-iodo-6-amino-1,2-benzopyrone [52]. Overexpression of a dominant-negative mutant of PARP-1 also reduced the tumor-forming ability of HeLa cells with an increase in apoptosis [53]. Although the molecular mechanisms underlying the reduction of tumorigenic potential by PARP-1 inhibition is not fully clarified, a decrease of *iNOS* expression by PARP-1 inhibition may be involved because a decrease of *iNOS* expression causes attenuation of cell proliferation and an increase in apoptosis [44].

When *Parp-1*$^{-/-}$ ES cells were grafted subcutaneously into nude mice, they specifically developed trophoblast giant cells (TGCs) within teratocarcinomas [54]. In culture, induction of expression of the trophoblast markers, including *proliferin*, and *prolactin-like protein A* was observed, indicating that deficiency of *Parp-1* promotes differentiation into the trophoblast lineage [55]. It is known that terminal differentiation to trophoblast giant cells occurs through an endoreduplication process, in which chromosome replication proceeds without cell division and ploidy can reach ~1,000 N. Loss of PARP-1 may also enhance the endoreduplication process. It should be noted that the observed TGCs possess properties similar to syncytiotrophoblastic giant cells (STGCs), which are observed in human germ cell tumors (HGCTs). STGCs show a high potential for invasiveness, and hence their presence correlates with poor prognosis in HGCT of the brain [56]. The above experimental system might be useful for elucidation of the mechanism of STGC induction in HGCTs.

Dysfunction and role of *PARP-1* in human cancers

A number of lines of evidence suggest involvement of PARP-1 in human carcinogenesis. However, genetic alterations and polymorphisms of the *PARP-1* gene have not been extensively studied yet. The *PARP-1* gene consists of 23 exons [57] and is located at chromosome 1q41-42. An augmented expression of the *PARP-1* gene and high PARP-1 activity was demonstrated in Ewing's sarcoma cell lines [58, 59]. They further showed that activation of the transcription factor ETS-1 caused overexpression of the *PARP-1* gene. The structure of *PARP-1* promoter [60, 61] is illustrated in figure 2A. It contains five potential ETS-1 binding sites and two Sp-1 (simian-virus-40-protein-1) binding sites. Binding sites for US-1, YY1 and AP-2 (cAMP responsive elements) are also present [61, 62].

Low *PARP-1* expression levels in breast cancers correlate with higher genomic instability [63]. In addition, breast cancers that overexpress the *PARP-1* gene frequently show amplification of chromosome 1q41-42 [63]. *PARP-1* gene expression in gastric cell lines (KatoIII, OKAJIMA and MKN45), a colon cancer cell line (WiDr) and T-cell leukemia cell lines (Molt-4 and CCRF-CEM) is reported to be substantially lower than in other cancer cell lines [64]. The gastric cancer cell line MKN28 features a structural alteration in the *PARP-1* gene, although precise details and the effect on the cell phenotype have yet to be studied [64]. We have also demonstrated a point mutation of the *PARP-1* gene in a germ cell tumor cell line, NEC8 [M. Masutani et al., unpublished], resulting in the amino acid substitution, E251K, at a conserved peptide stretch (see table 3). A germ cell tumor contained a possible germ line mutation, which causes amino acid substitution of M129T [65]. Several germ cell tumor cell lines show a substantial decrease in the amount of poly(ADP-ribosyl)-ation level [M. Masutani et al., unpublished], suggesting a possible association between dysfunction of PARP and germ cell tumors.

PARP-1 was found to be a coactivator of TCF-4 and to interact with TCF-4, β-catenin and mutated APC (adenomatous polyposis coli) [66]. Furthermore, an association of overexpression of *PARP-1* with accumulation of β-catenin in intestinal adenomatous polyps of familial adenomatous polyposis (FAP) patients, as well as multiple intestinal polyposis (Min) mice, has been reported [66]. Treatment with PARP inhibitor, PJ34, in *APC*$^{min/+}$ mice resulted in a reduction of the incidence of intestinal polyposis [67]. Also, in the early phase of endometrial cancer, *PARP-1* expression appears to be increased.

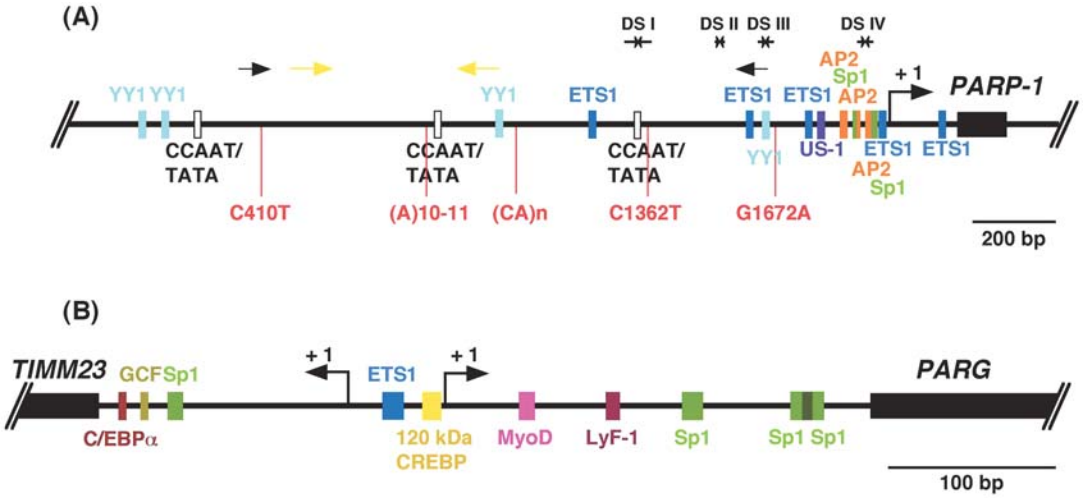

Figure 2. Schematic structures of upstream regulatory sequences for human *PARP-1* and *PARG* genes. (*A*) Human *PARP-1* gene. The positions of polymorphisms are indicated with orange lines. Binding sites for YY1, ETS1, US-1, AP-2 and Sp1 are shown with boxes. Inverted repeat motifs are indicated with arrows. Internal base pairing of these motifs is reported to give rise to quadruplex-DNA-loop structures. Four possible dyad symmetry elements (DS) I–IV are also located at –635/–586, –418/-402, –325/–290 and –71/–46, which may affect transcription by binding to PARP-1 protein [107]. (*B*) Human *PARG* gene [15]. Binding sites for C/EBPa, CREBP, ETS1, GCF (glucocorticoid receptor binding site), LyF-1, MyoD, Sp1 and SRF (serum response factor) are shown with boxes. Coding exons are shown with black boxes in (*A*) and (*B*).

Expression of *PARP-1* in endometrial cancer was highest in grade I lesions, namely in non-atypical and atypical endometrial hyperplasia, and reduced in accordance with histopathological progression towards grade III, paralleling downregulation of the progesterone receptor [68]. These results suggest that PARP-1 may be positively involved in the early phase of colon and endometrial cancer development.

Determination of the human genome sequence accelerated the identification of several single-nucleotide polymorphisms (SNPs) in the human *PARP-1* gene, as listed in table 3. A study of the V762A SNP with reference to prostate cancer risk was carried out in Caucasian subjects, and a significant increase observed with the A/A genotype (odds ratio = 2.5) [69]. In addition, proteomic analysis showed the presence of a PARP-1 molecule with a less basic pI value specifically in human breast cancer cells [70]. Since PARP-1 is present in DNA synthesomes and these breast cancer cells show low DNA replication fidelity, the relationship between the PARP-1 modification and its effect on the DNA replication fidelity should be clarified. The presence of processed pseudogenes for *PARP-1* on chromosome 13q33-qter and chromosome 14 has been reported [71], and a polymorphism for the pseudogene on chromosome 13q33-ter, namely a 193-bp duplication within the A allele and its absence in the B allele, was found [71]. An association of the B allele with endemic Burkitt's lymphoma, multiple myeloma, and colon and prostate cancers in African American populations has been observed [72, 73].

PARG and carcinogenesis

The human *PARG* gene has been mapped to chromosome 10q11.23 [74] and is organized into 18 exons [15]. Exons 9–14 form the catalytic center, while exons 1–4 encode the putative regulatory domains. Notably, PARG structure variants, some of which may be due to polymorphisms, are clustered from exons 1 to 3 [15], as shown in table 4, although the relevance of these polymorphisms to cancer still needs careful analysis. The *PARG* gene shares a 470-bp common promoter region with the *mitochondrial membrane translocase 23* (*TIMM23*) gene. A bidirectional mode of promoter activity was demonstrated [15]. The *PARG* promoter contains three overlapping Sp1 binding sites, binding sites for lymphocyte specific factor I (LyF-1), MyoD (myogenic determination factor D), ETS-1 binding sites and a putative inhibitory 120-kDa cyclic AMP (cAMP) responsive element binding protein (CREBP) [15]. Because the activity of these transcription factors might change during carcinogenesis, resultant alteration in expression levels of the *PARG* gene may cause the alteration in poly(ADP-ribosyl)ation of proteins.

Therapeutic potential

Since PARP-1 is involved in BER, SSB and DSB repair, use of PARP-1 inhibitors as sensitizers for chemotherapeutic agents or radiotherapy for cancer has long been considered as an option. However, there is a limitation in

Table 3. SNPs and sequence alterations in the *PARP-1* gene.

Amino acid substitution	SNP/sequence alteration	Exon no.	Sequence (variable base underlined)	NCBI SNP database no
F54L	SNP	2	TT<u>G</u>*	
			TT<u>C</u>	rs3738708
M129T	sequence alteration	3	A<u>T</u>G	
			A<u>C</u>G	our work
A188T	SNP	4	<u>G</u>CT*	
			<u>A</u>CT[1]	rs1805409
E251K	sequence alteration	6	<u>G</u>AG	
			<u>A</u>AG	our work
V334I	SNP	7	<u>G</u>TA[2]*	
			<u>A</u>TA	rs3219057
S383Y	SNP	8	T<u>C</u>T[3]*	
			T<u>A</u>T	rs3219062
H613Q	SNP	13	CA<u>C</u>*	
			CA<u>G</u>	rs1059011
V762A	SNP	17	G<u>T</u>G[4]*	
			G<u>C</u>G	rs1136410
N827S	SNP	18	A<u>A</u>T*	
			A<u>G</u>T	rs1136420
C908Y	SNP	20	T<u>G</u>C*	
			T<u>A</u>C	rs1059040
K940R	SNP	21	A<u>A</u>G[5]*	
			A<u>G</u>G	rs3219145

* Major allele [65].
Frequency of the heterozygosity is [1]0.006, [2]0.065, [3]0.21, [4]0.45, [5]0.44 in NCBI (National Center for Biotechnology Information) database.

clinical use of the classical PARP inhibitors because of concern of weak activity and side effects. The potent PARP inhibitor AG14361 did show effectiveness in combination with an alkylating agent, temozolomide, a topo I inhibitor, or ionizing irradiation against in vitro growth and also with xenografts of human cancer cell lines [75]. Notably, AG14361 restored sensitivity to temozolomide, possibly by inhibiting BER through PARP-1 inhibition, in mismatch repair (MMR)-deficient cells [76]. MMR defects are frequent in various cancers, suggesting that PARP inhibition might have significant benefit in overcoming resistance to chemo- and radiotherapy.

As mentioned earlier, *Parg* deficiency also can enhance alkylating agent- and γ-irradiation-induced lethality and stimulate early induction of apoptosis after treatment with alkylating agents. Therefore, Parg inhibitors might also be promising sensitizers to cancer chemo- and radiotherapy. Enhancement of lethal effects of γ-irradiation and an alkylating agent on germ cell tumors derived from ES cells with *Parg* deficiency has in fact been observed [A. Gunji et al., unpublished]. There are only a few PARG inhibitors so far reported, including ADP-HPD [adenosine diphosphate (hydroxymethyl)-pyrrolidinediol] [77], 8-octylamino-ADP-HPD [78] and pargamicin [79], but other forms with clinical applications may be developed as derivatives of these and through studies of the structure and function relationships of the enzyme.

Anti-poly(ADP-ribose) antibodies in autoimmune disease

Naturally occurring antibodies to poly(ADP-ribose) have been reported in the sera of patients with SLE [4]. The presence of antibodies to nucleic acids, including DNA, is well known, but the observed antibodies to poly(ADP-ribose) were able to be distinguished from those to DNA. Furthermore, some SLE patients retained high levels of antibodies to poly(ADP-ribose) when the levels of their antibodies to DNA had already decreased in sera [4]. Probably after increase of necrosis, release of PARP and poly(ADP-ribose) occurs systemically and triggers antibody production. Much higher titers of poly(ADP-ribose) have been observed in pregnant women suffering from SLE, suffering abortion after 13 weeks of gestation or a premature delivery when compared with normal pregnant women with SLE [80]. The results indicate that high titers of poly(ADP-ribose) may be a useful indicator of obstetric complications in pregnant SLE patients.

Lupus-prone MRL/Mp-lpr/lpr (MRL/l) mice also show an elevated level of antibodies against poly(ADP-ribose), in addition to those against single-strand and double-strand DNA [81]. When a PARP inhibitor, benadrostin, was administrated, the antibodies to poly(ADP-ribose) were selectively reduced [82], increasing the litter size of healthy neonates from pregnant SLE mice [82], indicating the possibility of prevention of fetal loss in SLE patients with PARP inhibitors.

Table 4. Candidate polymorphisms in the PARG gene*

Position of amino acid	Amino acid	Exon no.	Sequence (variable base underlined)	NCBI database number
13	A	1	GCG	AF005043
	R		CGA	AY258587, ESTs†
14	T	1	ACC	AF005043
	P		CCC	AY258587, ESTs†
61	Q	1	CAG	AF005043
	R		CGG	AY258587, ESTs†
127	V	3	GTA	AF005043
	E		GAA	AY258587, ESTs†
138	L	3	CTC	AF005043
	P		CCC	AY258587, ESTs†
227	H	3	CAC	AF005043
	Q		CAG	AY258587, ESTs†
242	H	3	CAT	AF005043
	D		GAT	AY258587, ESTs†
260	K	3	AAG	AF005043
	E		GAG	AY258587, ESTs†
275	S	3	TCA	AF005043
	P		CCA	AY258587, ESTs†
282	I	3	ATT	AF005043
	T		ACT	AY258587, ESTs†
414	L	3	TTG	AF005043
	F		TTC	AY258587, ESTs†
814	C	15	TGC	AF005043
	W		TGG	AY258587, ESTs†
815	E	15	GAG	AF005043
	Q		CAG	AY258587, ESTs†

* Data described in [15].
† EST clones, BU429712.1, AW861896.1, BG719380.1, BU430589.1 and BU601350.1.

Effects of PARP inhibition on autoimmune disease

Reactive oxygen species, including superoxide anions, hydroxyl radicals, hydrogen peroxide and singlet oxygen, as well as NO and peroxynitrite, are extensively generated in inflammation associated with arthritis. Inhibitors of PARP, including biogenic nicotinamide, are known to reduce arthritic symptoms in a mouse model of arthritis [83]. PARP inhibition also suppresses infiltration of neutrophils, which may be attributable to effects of PARP-1 on the gene expression control of cell adhesion molecules, but this process has not been fully elucidated. A PARP-1 inhibitor also reduced progression of arthritis in a rat model induced by collagen [84]. In the model of inflammatory bowel disease induced by chemicals or in *interleukin (IL)-10* deficiency in mice, PARP-1 inhibition or *Parp-1* deficiency markedly improved various inflammation indexes, including neutrophil infiltration and production of nitrotyrosine [85]. In a rat model of encephalomyelitis, PARP inhibitors increased the latency of the pathogenesis as well as affecting the incidence and progression [86]. During the pathogenesis of encephalomyelitis, the breakdown of the blood-brain barrier, and infiltration of inflammatory cells into the central nervous system (CNS) occur with the subsequent cell death induction of oligodendrocytes and astrocytes and with microglial migration. PARP-1 inhibition may interfere with these processes. The PARP inhibitor, PJ34, also prevented diabetes development in NOD mice [87]. PARP inhibitor reduced autoimmune β-cell destruction in NOD mice by inducing apoptosis of islet-infiltrating leukocytes and decreasing interferon-γ expression in the islets [87].

PARP-1 polymorphism and predisposition to autoimmune diseases

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic disease with chronic inflammation and autoimmune features. Although genetic analysis has revealed that the human leukocyte antigen (HLA) complex gene on chromosome 6 has a major contribution to its pathogenesis, links with several non-HLA genes have been reported [88]. A case control study with 213 Spanish RA patients and 242 healthy controls was conducted to determine associations with *PARP-1* gene promoter polymorphisms [89]. Only two haplotypes, namely, haplotype A (410T-$[A]_{10}$-$[CA]_{10\text{-}12}$-1362C) and haplotype B (410C-$[A]_{11}$-$[CA]_{13\text{-}20}$-1362T) were observed, and haplotype B was preferentially present in the RA patient group (see fig. 2A). Within haplotype B, the *PARP-1* CA 97-bp allele was further found to be a RA-predisposing marker (odds ratio 2.2) [89]. Polymorphic CA repeats reside close to the binding site of transcription factor YY1 (see fig. 2A) and the authors postulated that longer CA repeats in haplotype B may promote the expression of the *PARP-1* gene by enhancing the activity of YY1.

Type I diabetes

Nicotinamide, a PARP-1 inhibitor, has been reported to be protective against inflammatory insult and improve the function of residual β cells [90]. *Parp-1* knockout mice are resistant to streptozotocin-induced diabetes [91–93], and in non-obese diabetic mice nicotinamide was shown to protect islet cells against cytotoxic reagents and reduce the incidence of type I diabetes [94]. One of the genes involved in the inflammatory response, CTLA-4, was previously reported to be associated with type I diabetes [95]. An association study of CA repeat polymorphisms in the *PARP-1* gene promoter in French Caucasian patients with type I diabetes and ethnically matched healthy

controls demonstrated no significant differences in *PARP-1* alleles [96]. Further studies on the effects of polymorphisms in the coding region of *PARP-1* gene are important to understand the relation of PARP-1 and type I diabetes.

SLE and autoimmune thyroiditis

A case-control study of the previously mentioned 85-bp allele of CA repeats in the *PARP-1* promoter (fig. 2A) in 124 SLE families revealed a significant difference in *PARP-1* allele frequencies between the patients and controls [97]. On the other hand, the 85-bp allele was not associated with the risk of SLE among a multiethnic group of patients [98]. One interpretation is that the *PARP-1* gene may be located in physical proximity to the SLE susceptibility locus within the 1q41-42 region. Further studies using other *PARP-1* polymorphic markers are required to clarify the involvement of *PARP-1* in the genetic susceptibility to SLE.

Perspectives

Use of inhibitors and transgenic and knockout mice models of PARP and PARG is greatly extending our understanding of the links between poly(ADP-ribose) metabolism and pathogenesis as well as the therapeutic potential for cancer and autoimmune disease. PARP-1 is involved in DNA repair, maintenance of genetic and epigenetic stability, and transcriptional regulation. It functions also in cell death induction after DNA damage, which might be important in removing cells retaining DNA lesions. Treatment with streptozotocin causes pancreatic β cell death through PARP-1 activation and NAD depletion [99], and rats develop insulinomas whose incidence was markedly increased by the administration of 3AB [100], indicating that the PARP-1-dependent cell death pathway substantially contributes to prevention of carcinogenesis. Knockout mice models showed higher susceptibility to carcinogenesis with *Parp-1* deficiency. PARP-1 also acts as a coactivator of growth regulatory proteins encoded by a retrovirus [101] and a papilloma virus [102], and from the available evidence possible involvement of PARP in viral carcinogenesis can also be envisaged. With the advance of knowledge on the human genome and proteome, this decade is predicted to be most fruitful in revealing influences of genetic, epigenetic and proteomic aberrations of poly(ADP-ribose) metabolism on disease processes. As detailed above, a mutation in the *PARP-1* gene and an association of a SNP in the *PARP-1* gene with the risk of prostate cancer have been found [69]. Sequence analysis of coding exons as well as the regulatory sequences of the *PARP* family and *PARG* genes will be performed in the near future. Random mutagenesis analysis, such as the one carried out with *PARP-1* cDNA [12], as mentioned earlier, should provide useful information on the relation of sequence alterations and the functional changes.

From the viewpoint of prevention, it is also notable that NAD deficiency can cause an increase of the incidence of tumors and leukemia [103], while niacin supplementation increased the latency of ethylnitrosourea-induced HCC development with elevation in the amount of poly(ADP-ribose) in bone marrow [104]. Niacin supplementation may have a protective effect against development of cancer, where its shortage is predictable.

Understanding the molecular basis of autoimmune diseases may also facilitate development of therapeutic strategies for cancers. Paraneoplastic neurological degeneration (PND) is a cancer associated autoimmune disease characterized by anti-tumor autoimmunity [105]. It is reported to be associated with small-cell lung cancer, ovarian and breast cancer and the development of immunity against antigens, such as CDR2 and NOVA1, which are expressed in cancer cells, but which are restricted to the CNS normally. Activated T and B cells specific for the PND antigen are considered to cross the blood-brain barrier, and immune reactions against neurons might induce neuronal degeneration. Spontaneous regression of small-cell lung carcinoma in PND patients has been reported [106]. Since the pathogenesis of PND involves cell death and immune and inflammatory responses, it is of interest to examine whether poly(ADP-ribose) metabolism might make some contribution. The analysis of mechanisms underlying autoimmune disease, including PND, may provide new therapeutic possibilities taking advantage of the immune response and poly(ADP-ribose) metabolism for cancer control.

Acknowledgements. We appreciate the help and suggestions provided by A. Shibata, and H. Ogino. This work was supported in part by a Grant-in-Aid for the Third Term Comprehensive 10-Year Strategy for Cancer Control, a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan and a Grant-in-Aid for Scientific Research on Priority Areas from the ministry of Education, Science, Sports and Culture of Japan (16023271).

1 Purnell M. R. and Whish W. J. (1980) Novel inhibitors of poly(ADP-ribose) synthetase. Biochem. J. **185:** 775–777
2 Masutani M., Nakagama H. and Sugimura T. (2003) Poly(ADP-ribose) and carcinogenesis. Genes Chromosomes Cancer **38:** 339–348
3 Masutani M., Gunji A., Tsutsumi M., Ogawa K., Kamada N., Shirai T. et al. (2004) Role of poly-ADP-ribosylation in cancer development. In: Landes Bioscience Intelligence Unit Series, Poly(ADP-ribosyl)ation, Buerkle A. (ed.), Landes Bioscience/Eurekah.com, Georgetown, in press
4 Kanai Y., Kawaminami Y., Miwa M., Matsushima T. and Sugimura T. (1977) Naturally-occurring antibodies to

poly(ADP-ribose) in patients with systemic lupus erythematosus. Nature **265:** 175–177
5 Lichtenstein P., Holm N. V., Verkasalo P. K., Iliadou A., Kaprio J., Koskenvuo M. et al. (2000) Environmental and heritable factors in the causation of cancer-analyses of cohorts of twins from Sweden, Denmark and Finland. N. Engl. J. Med. **343:** 78–85
6 Ohshima H., Tatemichi M. and Sawa T. (2003) Chemical basis of inflammation-induced carcinogenesis. Arch. Biochem. Biophys. **417:** 3–11
7 Ame J.C., Spenlehauer C. and de Murcia G. (2004) The PARP superfamily. Bioessays **26:** 882–893
8 Ikejima M., Yamashita R., Ogura T., Sugimura T., Gill D. M. and Miwa M. (1990) The zinc fingers of human poly(ADP-ribose) polymerase are differentially required for the recognition of DNA breaks and nicks and the consequent enzyme activation. Other structures recognize intact DNA. J. Biol. Chem. **265:** 21907–21913
9 Rolli V., Ruf A., Augustin A., Schuls G. E., Menissier-de Murcia J. and de Murcia G. (2000) Poly(ADP-ribose) polymerase**:** structure and function. In: From DNA Damage and Stress Signaling to Cell Death. PolyADP-Ribosylation Reactions, de Murcia G. and Shall S. (eds), pp. 35–79, Oxford University Press, New York
10 Lonskaya I. and Soldatenkov V. (2004) Structure-specific DNA binding of PARP-1. Proc. Am. Assoc. Cancer Res. **45:** 532
11 Schreiber V., Ame J. C., Dolle P., Schultz I., Rinaldi B., Fraulob V. et al. (2002) 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. **277:** 23028–23036
12 Rolli V., O'Farrell M., Menissier-de Murcia J. and de Murcia G. (1997) Random mutagenesis of the poly(ADP-ribose) polymerase catalytic domain reveals amino acids involved in polymer branching. Biochemistry **36:** 12147–12154
13 Sallmann F. R., Vodenicharov M. D., Wang Z. Q. and Poirier G.G. (2000) Characterization of sPARP-1. An alternative product of PARP-1 gene with poly(ADP-ribose) polymerase activity independent of DNA strand breaks. J. Biol. Chem. **275**: 15504–15511
14 Meyer-Ficca M. L., Meyer R. G., Coyle D. L., Jacobson E. L. and Jacobson M. K. (2004) Human poly(ADP-ribose) glycohydrolase is expressed in alternative splice variants yielding isoforms that localize to different cell compartments. Exp. Cell Res. **297:** 521–532
15 Meyer R. G., Meyer-Ficca M. L., Jacobson E. L. and Jacobson M. K. (2003) Human poly(ADP-ribose) glycohydrolase (PARG) gene and the common promoter sequence it shares with inner mitochondrial membrane translocase 23 (TIM23). Gene **314:** 181–190
16 Lin W., Ame J. C., Aboul-Ela N., Jacobson E. L. and Jacobson M. K. (1997) Isolation and characterization of the cDNA encoding bovine poly(ADP-ribose) glycohydrolase. J. Biol. Chem. **272:** 11895–11901
17 Shimokawa T., Masutani M., Nagasawa S., Nozaki T., Ikota N., Aoki Y. et al. (1999) Isolation and cloning of rat poly(ADP-ribose) glycohydrolase: presence of a potential nuclear export signal conserved in mammalian orthologs. J. Biochem. (Tokyo) **126:** 748–755
18 Ohashi S., Kanai M., Hanai S., Uchiumi F., Maruta H., Tanuma S. et al. (2003) Subcellular localization of poly(ADP-ribose) glycohydrolase in mammalian cells. Biochem. Biophys. Res. Commun. **307:** 915–921
19 Affar E. B., Germain M., Winstall E., Vodenicharov M., Shah R. G., Salvesen G. S. et al. (2001) Caspase-3-mediated processing of poly(ADP-ribose) glycohydrolase during apoptosis. J. Biol. Chem. **276:** 2935–2942
20 Tong W. M., Hande M. P., Lansdorp P. M. and Wang Z. Q. (2001) DNA strand break-sensing molecule poly(ADP-ribose) polymerase cooperates with p53 in telomere function, chromosome stability and tumor suppression. Mol. Cell. Biol. **21:** 4046–4054
21 de Murcia J. M., Niedergang C., Trucco C., Ricoul M., Dutrillaux B., Mark M. et al. (1997) Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. Proc. Natl. Acad. Sci. USA **94:** 7303–7307
22 Wang Z. Q., Stingl L., Morrison C., Jantsch M., Los M., Schulze-Osthoff K. et al. (1997) PARP is important for genomic stability but dispensable in apoptosis. Genes Dev. **11:** 2347–2358
23 Trucco C., Oliver F. J., de Murcia G. and Menissier-de Murcia J. (1998) DNA repair defect in poly(ADP-ribose) polymerase-deficient cell lines. Nucleic Acids Res. **26:** 2644–2649
24 Busuttil R. A., Rubio M., Dolle M. E., Campisi J. and Vijg J. (2003) Oxygen accelerates the accumulation of mutations during the senescence and immortalization of murine cells in culture. Aging Cell **2:** 287–294
25 Yang Y. G., Cortes U., Patnaik S., Jasin M. and Wang Z. Q. (2004) Ablation of PARP-1 does not interfere with the repair of DNA double-strand breaks, but compromises the reactivation of stalled replication forks. Oncogene **23:** 3872–3882
26 Buerkle A., Schreiber V., Dantzer F., Oliver F. J., Niedergang C. and de Murcia G. et al. (2000) Biological significance of poly(ADP-ribosyl)ation reactions: molecular and genetic approaches. In: From DNA Damage and Stress Signaling to Cell Death. PolyADP-Ribosylation Reactions, pp. 80–124, de Murcia G. and Shall S. (eds), Oxford University Press, New York
27 Shibata A., Kamada N., Masumura K., Nohmi T., Kobayashi S., Teraoka H. et al. (2005) Parp-1 deficiency causes an increase of deletion mutations and insertions/rearrangements *in vivo* after treatment with an alkylating agnet. Oncogene, in press
28 Malanga M. and Althaus F. R. (2004) Poly(ADP-ribose) reactivates stalled DNA topoisomerase I and induces DNA strand break resealing. J. Biol. Chem. **279:** 5244–5248
29 Menissier de Murcia J., Ricoul M., Tartier L., Niedergang C., Huber A., Dantzer F. et al. (2003) Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. EMBO J. **22:** 2255–2263
30 Augustin A., Spenlehauer C., Dumond H., Menissier-De Murcia J., Piel M., Schmit A. C. et al. (2003) PARP-3 localizes preferentially to the daughter centriole and interferes with the G1/S cell cycle progression. J. Cell Sci. **116:** 1551–1562
31 Gunji A., Fujihara H., Kamada N., Omura K., Jishage K., Nakagama H. et al. (2003) Lack of altered frequency of sister-chromatid exchanges in poly(ADP-ribose) glycohydrolase-deficient mouse ES cells treated with methylmethanesulfonate, Proc. Jpn. Acad. **79 Ser. B:** 305–307
32 Nozaki T., Fujihara H., Gunji A., Shibata A., Kamada N., Jishage K. et al. (2002) Increased sensitivity of poly(ADP-ribose) glycohydrolase-deficient ES cell lines to alkylating agent and gamma-irradiation. Cancer Sci. **93 Suppl.:** 51
33 Gunji A., Fujihara H., Kamada N., Jishage K., Omura K., Nakagama H. et al. (2003) Early induction and increase of apoptotic cells in Parp-/- ES cells after methylmethanesulfonate treatment. Cancer Sci. **94 Suppl.:** 93
34 Cortes U., Tong W. M., Coyle D. L., Meyer-Ficca M. L., Meyer R. G., Petrilli V. et al. (2004) Depletion of the 110-kilodalton isoform of poly(ADP-ribose) glycohydrolase increases sensitivity to genotoxic and endotoxic stress in mice. Mol. Cell. Biol. **24:** 7163–7178
35 Dynek J. N. and Smith S. (2004) Resolution of sister telomere association is required for progression through mitosis. Science **304:** 97–100
36 Zardo G., Reale A., Passananti C., Pradhan S., Buontempo S., De Matteis G. et al. (2002) Inhibition of poly(ADP-ribosyl)ation induces DNA hypermethylation: a possible molecular mechanism. FASEB. J. **16:** 1319–1321

37 Tulin A. and Spradling A. (2003) Chromatin loosening by poly(ADP)-ribose polymerase (PARP) at *Drosophila* puff loci. Science **299:** 560–562

38 Beneke R. and Moroy T. (2001) Inhibition of poly(ADP-ribose) polymerase activity accelerates T-cell lymphomagenesis in p53 deficient mice. Oncogene **20:** 8136–8141

39 Tong W. M., Cortes U., Hande M. P., Ohgaki H., Cavalli L. R., Lansdorp P. M. et al. (2002) Synergistic role of Ku80 and poly(ADP-ribose) polymerase in suppressing chromosomal aberrations and liver cancer formation. Cancer Res. **62:** 6990–6996

40 Tsutsumi M., Masutani M., Nozaki T., Kusuoka O., Tsujiuchi T., Nakagama H. et al. (2001) Increased susceptibility of poly(ADP-ribose) polymerase-1 knockout mice to nitrosamine carcinogenicity. Carcinogenesis **22:** 1–3

41 Nozaki T., Fujihara H., Watanabe M., Tsutsumi M., Nakamoto K., Kusuoka O. et al. (2003) Parp-1 deficiency implicated in colon and liver tumorigenesis induced by azoxymethane. Cancer Sci. **94:** 497–500

42 Morrison C., Smith G. C., Stingl L., Jackson S. P., Wagner E. F. and Wang Z. Q. (1997) Genetic interaction between PARP and DNA-PK in V(D)J recombination and tumorigenesis. Nat. Genet. **17:** 479–482

43 Tong W. M., Ohgaki H., Huang H., Granier C., Kleihues P. and Wang Z. Q. (2003) Null mutation of DNA strand break-binding molecule poly(ADP-ribose) polymerase causes medulloblastomas in p53(–/–) Mice. Am. J. Pathol. **162:** 343–352

44 Conde C., Mark M., Oliver F. J., Huber A., de Murcia G. and Menissier-de Murcia J. (2001) Loss of poly(ADP-ribose) polymerase-1 causes increased tumour latency in p53-deficient mice. EMBO J. **20:** 3535–3543

45 Ambs S., Ogunfusika M. O., Merriam W. G., Bennett W. P., Billiar T. R., and Harris C. C. (1998) Up-regulation of inducible nitric oxide synthase expression in cancer-prone p53 knockout mice. Proc. Natl. Acad. Sci. USA **95:** 8823–8828

46 Martin-Oliva D., O'Valle F., Munoz-Gamez J. A., Valenzuela M. T., Nunez M. I., Aguilar M. et al. (2004) Crosstalk between PARP-1 and NF-kappaB modulates the promotion of skin neoplasia. Oncogene **23:** 6684–6692

47 Masutani M., Gunji A., Kamada N., Watanabe M., Jishage K., Nakagama H. et al. (2004) Involvement of poly(ADP-ribose) glycohydrolase in recovery from DNA damage response and modulation of apoptosis. Proc. Am. Assoc. Cancer Res. **45:**1146

48 Hanai S., Kanai M., Ohashi S., Okamoto K., Yamada M., Takahashi H. et al. (2004) Loss of poly(ADP-ribose) glycohydrolase causes progressive neurodegeneration in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **101:** 82–86

49 Ohashi Y., Ueda K., Hayaishi O., Ikai K. and Niwa O. (1984) Induction of murine teratocarcinoma cell differentiation by suppression of poly(ADP-ribose) synthesis. Proc. Natl. Acad. Sci. USA **81:** 7132–7136

50 Terada M., Fujiki H., Marks P. A. and Sugimura T. (1979) Induction of erythroid differentiation of murine erythroleukemia cells by nicotinamide and related compounds. Proc. Natl. Acad. Sci. USA **76:** 6411–6414

51 Shima H., Nakayasu M., Aonuma S., Sugimura T. and Nagao M. (1989) 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 **86:** 7442–7445

52 Bauer P. I., Kirsten E., Varadi G., Young L. J., Hakam A., Comstock J. A. et al. (1995) Reversion of malignant phenotype by 5-iodo-6-amino-1,2-benzopyrone a non-covalently binding ligand of poly(ADP-ribose) polymerase. Biochimie **77:** 374–377

53 Hans M. A., Muller M., Meyer-Ficca M., Buerkle A. and Kupper J. H. (1999) Overexpression of dominant negative PARP interferes with tumor formation of HeLa cells in nude mice: evidence for increased tumor cell apoptosis in vivo. Oncogene **18:** 7010–7015

54 Nozaki T., Masutani M., Watanabe M., Ochiya T., Hasegawa F., Nakagama H. et al. (1999) Syncytiotrophoblastic giant cells in teratocarcinoma-like tumors derived from *Parp*-disrupted mouse embryonic stem cells. Proc. Natl. Acad. Sci. USA **96:** 13345–13350

55 Hemberger M., Nozaki T., Winterhager E., Yamamoto H., Nakagama H., Kamada N. et al. (2003) *Parp1*-deficiency induces differentiation of ES cells into trophoblast derivatives. Dev. Biol. **257:** 371–381

56 von Hochstetter A. R., Sigg C., Saremaslani P. and Hedinger C. (1985) The significance of giant cells in human testicular seminomas. A clinico-pathological study. Virchows Arch. A. Pathol. Anat. Histopathol. **407:** 309–322

57 Auer B., Nagl U., Herzog H., Schneider R. and Schweiger M. (1989) Human nuclear NAD+ ADP-ribosyltransferase(polymerizing): organization of the gene. DNA **8:** 575–580

58 Prasad S. C., Thraves P. J., Bhatia K. G., Smulson M. E. and Dritschilo A. (1990) Enhanced poly(adenosine diphosphate ribose) polymerase activity and gene expression in Ewing's sarcoma cells. Cancer Res. **50:** 38–43

59 Soldatenkov V. A., Albor A., Patel B. K., Dreszer R., Dritschilo A. and Notario V. (1999) Regulation of the human poly(ADP-ribose) polymerase promoter by the ETS transcription factor. Oncogene **18:** 3954–3962

60 Ogura T., Nyunoya H., Takahashi-Masutani M., Miwa M., Sugimura T. and Esumi H. (1990) Characterization of a putative promoter region of the human poly(ADP-ribose) polymerase gene: structural similarity to that of the DNA polymerase beta gene. Biochem. Biophys. Res. Commun. **167:** 701–710

61 Oei S. L., Herzog H., Hirsch-Kauffmann M., Schneider R., Auer B. and Schweiger M. (1994) Transcriptional regulation and autoregulation of the human gene for ADP-ribosyltransferase. Mol. Cell Biochem. **138:** 99–104

62 Schweiger M., Oei S. L., Herzog H., Menardi C., Schneider R., Auer B. et al. (1995) Regulation of the human poly(ADP-ribosyl) transferase promoter via alternative DNA racket structures. Biochimie **77:** 480–485

63 Bieche I., de Murcia G. and Lidereau R. (1996) Poly(ADP-ribose) polymerase gene expression status and genomic instability in human breast cancer. Clin. Cancer Res. **2:** 1163–1167

64 Masutani M., Nozaki T., Sasaki H., Yamada T., Kohno T., Shimizu K. et al. (2004) Aberration of *poly(ADP-ribose)-polymerase-1* gene in human tumor cell lines: its expression and structural alterations. Proc. Jpn. Acad. **80 Ser. B:** 114–118

65 Shiokawa M., Masutani M., Ueki K., Nishikawa R., Sugimura T., Kubo H. et al. (2005) Genetic alteration of poly(ADP-ribose) polymerase-1 in human germ cell tumors. Jpn. J. Clin. Oncol., in press

66 Idogawa M., Yamada T., Honda K., Imai K. and Hirohashi S. (2004) Proteomic analysis of the T-cell factor (TCF)-4/b-catenin transcriptional complex. Proc. Am. Assoc. Cancer Res. **45:** 817

67 Mabley J. G., Pacher P., Bai P., Wallace R., Goonesekera S., Virag L. et al. (2004) Suppression of intestinal polyposis in $Apc^{min/+}$ mice by targeting the nitric oxide or poly(ADP-ribose) pathways. Mutat. Res. **548:** 107–116

68 Ghabreau L., Roux J. P., Frappart P. O., Mathevet P., Patricot L. M., Mokni M. et al. (2004) Poly(ADP-ribose) polymerase-1, a novel partner of progesterone receptors in endometrial cancer and its precursors. Int. J. Cancer **109:** 317–321

69 Lokett K. L., Hall M. C., Xu J., Zheng S. L., Berwick M., Chuang S. C. (2004) The ADPRT V762A genetic variant contributes to prostate cancer susceptibility and deficient enzyme function. Cancer Res. **64:** 6344–6348

70 Abdel-Aziz W., Malkas L., Hoelz D. and Hickey R. (2004) A modified form of poly(ADP-ribose) polymerase (PARP) is

present in breast cancer cells. Proc. Am. Assoc. Cancer Res. **45:** 606
71 Bhatia K. G., Cherney B. W., Huppi K., Magrath I. T., Cossman J., Sausville E. et al. (1990) 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. **50:** 5406–5413
72 Bhatia K., Huppi K., Cherney B., Raffeld M., Smulson M. and Magrath I. (1990) Relative predispositional effect of a PADPRP marker allele in B-cell and some non B-cell malignancies. Curr. Top Microbiol. Immunol. **166:** 347–357
73 Lyn D., Cherney B. W., Lalande M., Berenson J. R., Lichtenstein A., Lupold S. et al. (1993) 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. **52:** 124–134
74 Shimokawa T., Masutani M., Nozaki T., Nakagama H., Araki S., Aoki Y. et al. (1998) The human poly(ADP-ribose) glycohydrolase maps to chromosome 10q11.23-21.1 by fluorescence in situ hybridization. Human Cell **11:** 243–246
75 Calabrese C. R., Almassy R., Barton S., Batey M. A., Calvert A. H., Canan-Koch S. et al. (2004) Anticancer chemosensitization and radiosensitization by the novel poly(ADP-ribose) polymerase-1 inhibitor AG14361. J. Natl. Cancer. Inst. **96:** 56–67
76 Curtin N. J., Wang L. Z., Yiakouvaki A., Kyle S., Arris C. A., Canan-Koch S. et al. (2004) Novel poly(ADP-ribose) polymerase-1 inhibitor, AG14361, restores sensitivity to temozolomide in mismatch repair-deficient cells. Clin. Cancer Res. **10:** 881–889
77 Slama J. T., Aboul-Ela N., Goli D. M., Cheesman B. V., Simmons A. M. and Jacobson M. K. (1995) Specific inhibition of poly(ADP-ribose) glycohydrolase by adenosine diphosphate (hydroxymethyl)pyrrolidinediol. J. Med. Chem. **38:** 389–393
78 Coyle D. L., Kim H., Jacobson E. L. and Jacobson M. K. (2003) Synthesis and characterizaton of potent and selective cell permeable inhibitors of poly(ADP-ribose) glycohydrolase. Med. Sci. Mon. **9 Suppl. 1:** 15
79 Masutani M., Shimokawa T., Igarashi M., Hamada M., Shibata A., Oami S. et al. (2002) Inhibition of poly(ADP-ribose) glycohydrolase activity by cyclic peptide antibiotics containing piperazic acid residues. Proc. Jpn. Acad. **78 Ser. B:** 15–17
80 Kanai Y., Isonishi S. and Terashima Y. (1989) Antibody to poly(ADP-ribose) is an indicator of obstetric complications in pregnant patients with systemic lupus erythematosus. Immunol. Lett. **21:** 217–222
81 Isonishi S. and Kanai Y. (1988) Antibody to poly(ADP-ribose) as a predictor of obstetric complications in autoimmune MRL/Mp-lpr/lpr mice: basis for its application to pregnant patients with systemic lupus erythematosus. Immunol. Lett. **18:** 61–66
82 Yamauchi S. and Kanai Y. (1991) Preferential suppression of antibody to poly(ADP-ribose) in lupus-prone MRL/Mp-lpr/lpr mice by benadrostin. Jikeikai Medical J. **38:** 387–393
83 Miesel R., Kurpisz M. and Kroger H. (1995) Modulation of inflammatory arthritis by inhibition of poly(ADP ribose) polymerase. Inflammation **19:** 379–387
84 Kroger H., Miesel R., Dietrich A., Ohde M., Rajnavolgyi E. and Ockenfels H. (1996) Synergistic effects of thalidomide and poly (ADP-ribose) polymerase inhibition on type II collagen-induced arthritis in mice. Inflammation **20:** 203–215
85 Zingarelli B., Salzman A. L. and Szabo C. (1998) Genetic disruption of poly (ADP-ribose) synthetase inhibits the expression of P-selectin and intercellular adhesion molecule-1 in myocardial ischemia/reperfusion injury. Circ. Res. **83:** 85–94
86 Scott G. S., Hake P., Kean R. B., Virag L., Szabo C. and Hooper D. C. (2001) Role of poly(ADP-ribose) synthetase activation in the development of experimental allergic encephalomyelitis. J. Neuroimmunol. **117:** 78–86
87 Suarez-Pinzon W. L., Mabley J. G., Power R., Szabo C. and Rabinovitch A. (2003) Poly (ADP-ribose) polymerase inhibition prevents spontaneous and recurrent autoimmune diabetes in NOD mice by inducing apoptosis of islet-infiltrating leukocytes. Diabetes **52:** 1683–1688
88 Jawaheer D., Seldin M. F., Amos C. I., Chen W. V., Shigeta R., Monteiro J. et al. (2001) A genomewide screen in multiplex rheumatoid arthritis families suggests genetic overlap with other autoimmune diseases. Am. J. Hum. Genet. **68:** 927–936
89 Pascual M., Lopez-Nevot M. A., Caliz R., Ferrer M. A., Balsa A., Pascual-Salcedo D. et al. (2003) A poly(ADP-ribose) polymerase haplotype spanning the promoter region confers susceptibility to rheumatoid arthritis. Arthritis Rheum. **48**: 638–641
90 Kolb H. and Burkart V. (1999) Nicotinamide in type 1 diabetes. Mechanism of action revisited. Diabetes Care **22 Suppl. 2B:** 16–20
91 Burkart V., Wang Z. Q., Radons J., Heller B., Herceg Z., Stingl L. et al. (1999) Mice lacking the poly(ADP-ribose) polymerase gene are resistant to pancreatic beta-cell destruction and diabetes development induced by streptozotocin. Nat. Med. **5:** 314–319
92 Masutani M., Suzuki H., Kamada N., Watanabe M., Ueda O., Nozaki T. et al. (1999) Poly(ADP-ribose) polymerase gene disruption conferred mice resistant to streptozotocin-induced diabetes. Proc. Natl. Acad. Sci. USA **96:** 2301–2304
93 Pieper A. A., Brat D. J., Krug D. K., Watkins C. C., Gupta A., Blackshaw S. et al. (1999) Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocin-induced diabetes. Proc. Natl. Acad. Sci. USA **96:** 3059–3064
94 Suarez-Pinzon W. L., Mabley J. G., Power R., Szabo C. and Rabinovitch A. (2003) Poly(ADP-ribose) polymerase inhibition prevents spontaneous and recurrent autoimmune diabetes in NOD mice by inducing apoptosis of islet-infiltrating leukocytes. Diabetes **52:** 1683–1688
95 Nistico L., Buzzetti R., Pritchard L. E., Van der Auwera B., Giovannini C., Bosi E. et al. (1996) The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry, Hum. Mol. Genet. **5:** 1075–1080
96 Delrieu O., Dubois-Laforgue D., Timsit J., Tournier-Lasserve E. and Caillat-Zucman S. (2001) A dinucleotide repeat polymorphism at the poly(ADP-ribose) polymerase gene is not associated with predisposition to type 1 diabetes in French Caucasians. J. Autoimmun. **17:** 137–140
97 Tsao B. P., Cantor R. M., Grossman J. M., Shen N., Teophilov N. T. and Wallace D. J. (1999) PARP alleles within the linked chromosomal region are associated with systemic lupus erythematosus. J. Clin. Invest. **103:** 1135–1140
98 Criswell L. A., Moser K. L., Gaffney P. M., Inda S., Ortmann W. A., Lin D. et al. (2000) PARP alleles and SLE: failure to confirm association with disease susceptibility. J. Clin. Invest. **105:** 1501–1502
99 Yamamoto H. and Okamoto H. (1980) Protection by picolinamide, a novel inhibitor of poly (ADP-ribose) synthetase, against both streptozotocin-induced depression of proinsulin synthesis and reduction of NAD content in pancreatic islets. Biochem. Biophys. Res. Commun. **95:** 474–481
100 Yamagami T., Miwa A., Takasawa S., Yamamoto H. and Okamoto H. (1985) Induction of rat pancreatic B-cell tumors by the combined administration of streptozotocin or alloxan and poly(adenosine diphosphate ribose) synthetase inhibitors. Cancer Res. **45:** 1845–1849
101 Anderson M. G., Scoggin K. E., Simbulan-Rosenthal C. M. and Steadman J. A. (2000) Identification of poly(ADP-ribose) polymerase as a transcriptional coactivator of the human T-cell leukemia virus type 1 Tax protein. J. Virol. **74:** 2169–2177

102 Lee D., Kim J. W., Kim K., Joe C. O., Schreiber V., Menissier-De Murcia J. et al. (2002) Functional interaction between human papillomavirus type 18 E2 and poly(ADP -ribose) polymerase 1. Oncogene **21:** 5877–5885
103 Boyonoski A. C., Spronck J. C., Gallacher L. M., Jacobs R. M., Shah G. M., Poirier G. G. et al. (2002) Niacin deficiency decreases bone marrow poly(ADP-ribose) and the latency of ethylnitrosourea-induced carcinogenesis in rats. J. Nutr. **132:** 108–114
104 Boyonoski A. C., Spronck J. C., Jacobs R. M., Shah G. M., Poirier G. G. and Kirkland J. B. (2002) Pharmacological intakes of niacin increase bone marrow poly(ADP-ribose) and the latency of ethylnitrosourea-induced carcinogenesis in rats J. Nutr. **132:** 115–120
105 Albert M. L. and Darnell R. B. (2004) Paraneoplastic neurological degenerations: keys to tumour immunity. Nat. Rev. Cancer **4:** 36–44
106 Darnell R. B. and DeAngelis L. M. (1993) Regression of small-cell lung carcinoma in patients with paraneoplastic neuronal antibodies. Lancet **341:** 21–22
107 Soldatenkov V. A., Chasovskikh S., Potaman V. N., Trofimova I., Smulson M. E. and Dritschilo A. (2002) Transcriptional repression by binding of poly(ADP-ribose) polymerase to promoter sequences. J. Biol. Chem. **277:** 665–670
108 Simbulan-Rosenthal C. M., Haddad B. R., Rosenthal D. S., Weaver Z., Coleman A., Luo R. et al. (1999) Chromosomal aberrations in PARP(–/–) mice: genome stabilization in immortalized cells by reintroduction of poly(ADP-ribose) polymerase cDNA. Proc. Natl. Acad. Sci. USA **96:** 13191–13196
109 Nozaki T., Fujihara H., Kamada N., Ueda O., Takato T., Nakagama H. et al. (2001) Hyperploidy of embryonic fibroblasts derived from *Parp-1* knockout mouse. Proc. Jpn. Acad. **77 Ser. B:** 121–124