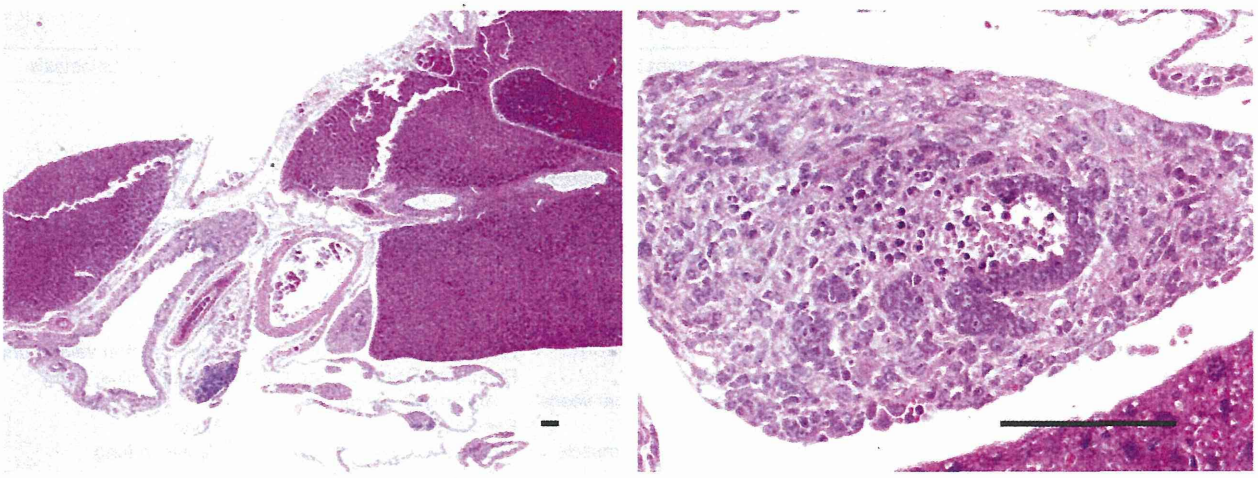

Figure 1 A metastatic lesion developed after grafting with *Parp-1*$^{-/-}$ embryonic stem (ES) cells. A metastastic lesion in the connective tissue around the portal vein of the liver in mice after grafting with *Parp-1*$^{-/-}$ ES cells (left panel). The right panel shows the magnified image. Bars indicate 100 μm.

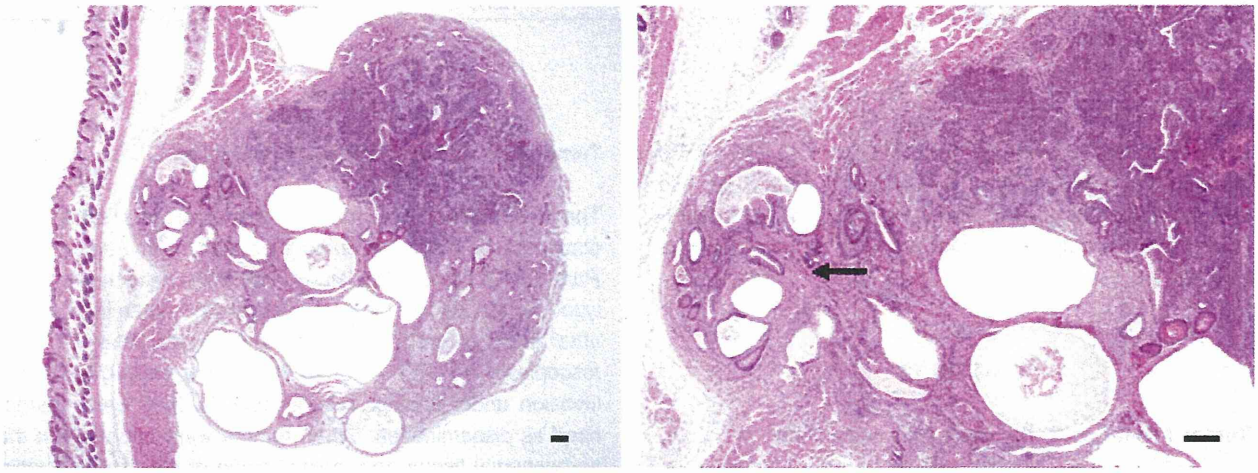

Figure 2 The lesions of invasion developed after grafting with *Parp-1*$^{-/-}$ embryonic stem (ES) cells (left panel). The arrow shows the invasion into the abdominal muscle. The right panel shows the magnified image. Bars indicate 100 μm.

and not distinct cytoplasm borders. The cells frequently formed rosettes within solid sheets (Fig. 3b).

In the three animals grafted with *Parp-1*$^{+/+}$ ES cells and two animals grafted with *Parp-1*$^{-/-}$ ES cells, tumors in the uterus were not observed but disseminated tumors were present.

Induction of TGCs and blood pools in tumors derived from *Parp-1*$^{-/-}$ ES cells

Overall, 6 of 7 tumors in the uterus (86%) derived from *Parp-1*$^{-/-}$ ES cells showed the development of extensive blood pools with giant cells (Fig. 4a). The components of the tumors were very similar among the two genotypes, except for the presence of extensive blood pools. The giant cells possessed megalonuclei and an eosinophilic cytoplasm, suggesting that they were similar to TGCs, as we previously observed in teratocarcinomas derived from *Parp-1*$^{-/-}$ ES cells after subcutaneous injection into nude mice.[10] To examine the properties of these giant cells, an anti-mouse placental lactogen I antibody was produced and checked for immunoreactivity against the TGCs of a mouse embryo placenta at E13.5, and positive staining was confirmed (Fig. 4b). Immunostaining of the tumors revealed that the cytoplasm of the giant cells showed positive staining for this anti-mouse placental lactogen I antibody (Fig. 4c). These giant cells are also positively stained with anti-prolactin antibody (Fig. 4d) as previously described for TGCs appeared in tumors

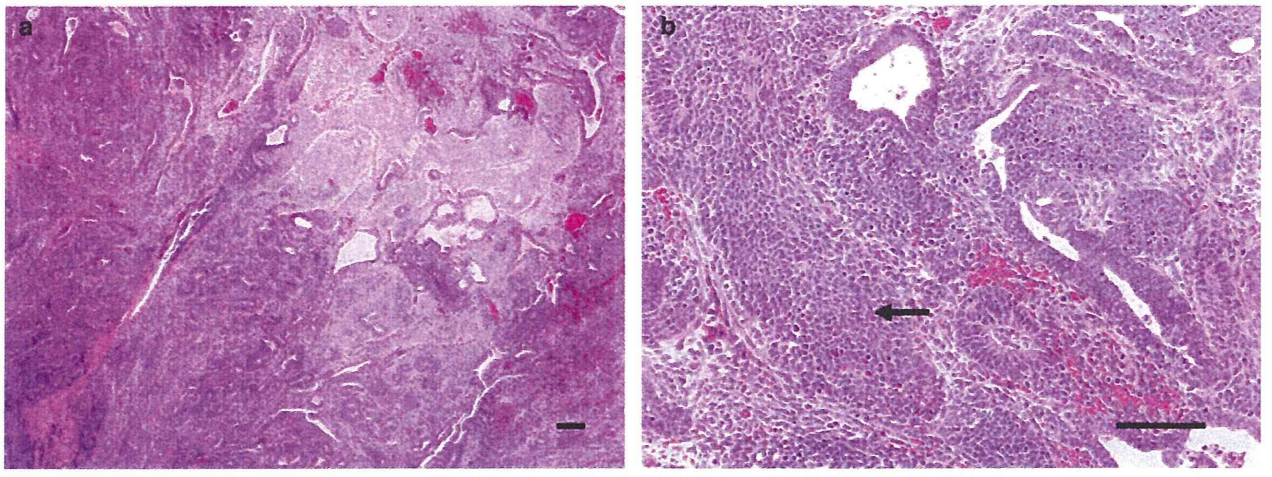

Figure 3 Disseminated lesions developed after grafting with *Parp-1*$^{-/-}$ embryonic stem (ES) cells. (**a**) The disseminated lesion demonstrates various tissue types such as glandular epithelium, but mainly neural tissues. (**b**) Primitive embryonic cells forming a rosette (arrow) and anaplastic cells were observed. Bars indicate 100 μm.

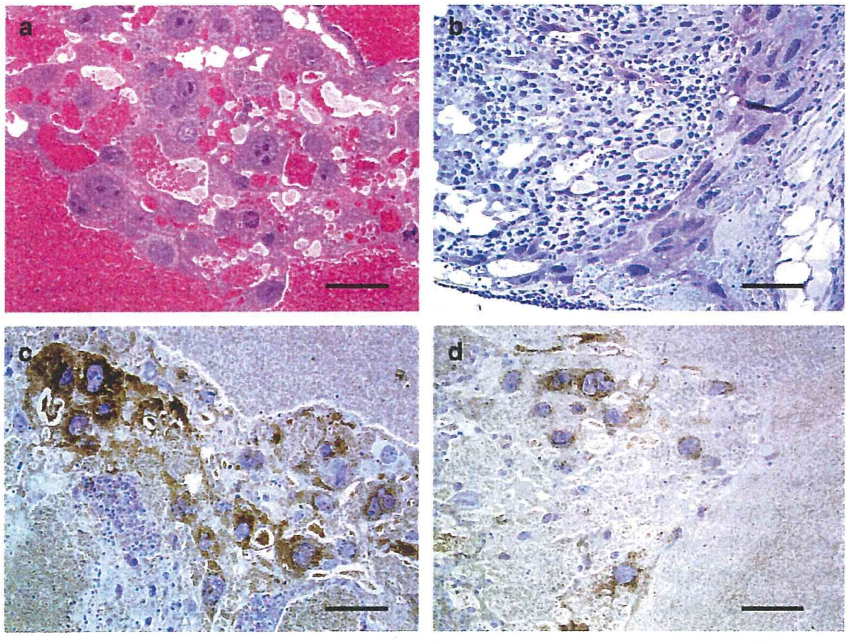

Figure 4 Trophoblast giant cells (TGCs) in the tumors developed after grafting with *Parp-1*$^{-/-}$ ES cells. (**a**) Hematoxylin and eosin staining of a tumor after grafting with *Parp-1*$^{-/-}$ embryonic stem (ES) cells. (**b**) Staining of an embryonic placenta at E13.5 with the anti-mouse placental lactogen I antibody. (**c**) Staining of a tumor with the anti-mouse placental lactogen I antibody. (**d**) Staining of a tumor with the anti-mouse prolactin antibody. The cytoplasmic regions containing the TGCs are positively stained with anti-placental lactogen and anti-prolactin antibodies. Bars indicate 100 μm.

derived from *Parp-1*$^{-/-}$ ES cells after subcutaneous injection.[10] These data support the notion that the developed giant cells in the teratocarcinomas derived from *Parp-1*$^{-/-}$ ES cells were TGCs.

DISCUSSION

Here we investigated whether uterine environment affects tumorigenesis from wild-type and *Parp-1* deficient ES cells compared to subcutaneous environment. The difference we observed between the subcutaneous and uterine environment were the tumor size and the frequencies of metastatic and invasive lesions. *Parp-1* deficiency in ES cells attenuated the growth of teratocarcinomas formed in the fallopian tubules and capsule of the ovary and uterus. Exposure of the grafted ES cells to various hormones, including estrogen, may have affected the growth of ES cells.[14–16] Parp-1 has been suggested to be one of the key coactivator of estrogen receptor α-dependent transcriptional regulation, suggesting a possibility that the defective transduction of estrogen signaling in *Parp-1*$^{-/-}$ ES cells[17] may have attenuated growth of ES cells.

The reduced growth of the tumors derived from *Parp-1*$^{-/-}$ ES cells may also be related to the presence of induced trophoblasts. Trophoblast induction may have attenuated the growth of ES cell or the derived tumor cells more easily in the uterine environment compared to subcutaneous environment. After grafting of ES cells into the horn of the uterus, the ES cells seemed to become disseminated into the surrounding tissues and stroma. However, tumors did not develop in the lumen of the uterus.

Trophoblast giant cells were induced in the tumors derived from *Parp-1*$^{-/-}$ ES cells, but not in those from *Parp-1*$^{+/+}$ ES cells, similar to the case of teratocarcinoma development after subcutaneous grafting of these cells.[10] The TGCs were found in the tumors derived from *Parp-1*$^{-/-}$ ES cells as clusters in teratocarcinomas, suggesting that they were induced during teratocarcinoma formation after injection of the cells. Trophoblasts are known to express vasoregulatory factors, including inducible nitric oxide synthase, endothelial nitric oxide synthase,[18] adrenomedullin,[19] proliferin,[20] immunosuppressive factors, and proteases. Therefore, it is suggested that trophoblasts could be involved in metastasis under certain conditions. Notably, we observed metastastic lesions of the tumors in the liver and lung with *Parp-1*$^{-/-}$ ES cells. The frequency of invasion was also higher for *Parp-1*$^{-/-}$ cells than for *Parp-1*$^{+/+}$ cells. Although TGCs were not detected in the disseminated or invaded regions, there may be a possibility that trophoblast induction would be related to increased metastasis and invasion as described above. To examine whether TGCs and other trophoblasts are colocalized in the metastatic regions, it may be necessary to observe the tumor formation for a longer period. Another possible explanation for the increased frequencies of metastatic and invasive lesions under *Parp-1* deficiency could be that *Parp-1* deficiency may have increased the numbers of cancerous stem cells, which possess malignant phenotypes, through higher genomic instability that may enhance development of cancerous stem cells from ES cells in stressed conditions.[21] It may be also possible that *Parp-1* deficiency leads to resistance to a certain type of necrotic cell death, including reactive oxygen-induced cell death, which involves NAD depletion and Parp-1-dependent apoptosis-inducing factor activation.[22] This property may have promoted the survival of metastasizing *Parp-1*$^{-/-}$ ES cells.

In the present study, *Parp-1* deficiency was suggested to induce invasion and metastatic lesions of tumors that developed from ES cells in the uterus, which could not have been observed in our subcutaneous grafting model of ES cells.[10] The induction model of trophoblasts from *Parp-1*$^{-/-}$ ES cells during teratocarcinoma formation in the uterus and ovary environments may serve as a good model for elucidating the induction mechanism of trophoblast lineage differentiation and the role of trophoblasts during tumor formation. Recently, Parp-1 inhibitors have been in clinical trials as cancer therapies. The present study suggests the possibility that Parp-1 inhibition in germ cell tumors may be associated with a risk for metastatic lesion development accompanying trophoblast induction.

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Review

Poly(ADP-ribosyl)ation in carcinogenesis

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ABSTRACT

Cancer develops through diverse genetic, epigenetic and other changes, so-called 'multi-step carcinogenesis', and each cancer harbors different alterations and properties. Here in this article we review how poly(ADP-ribosyl)ation is involved in multi-step and diverse pathways of carcinogenesis. Involvement of poly- and mono-ADP-ribosylation in carcinogenesis has been studied at molecular and cellular levels, and further by animal models and human genetic approaches. PolyADP-ribosylation acts in DNA damage repair response and maintenance mechanisms of genomic stability. Several DNA repair pathways, including base-excision repair and double strand break repair pathways, involve PARP and PARG functions. These care-taker functions of poly(ADP-ribosyl)ation suggest that polyADP-ribosylation may mainly act in a tumor suppressive manner because genomic instability caused by defective DNA repair response could serve as a driving force for tumor progression, leading to invasion, metastasis and relapse of cancer. On the other hand, the new concept of 'synthetic lethality by PARP inhibition' suggests the significance of PARP activities for survival of cancer cells that harbor defects in DNA repair. Accumulating evidence has revealed that some PARP family molecules are involved in various signaling cascades other than DNA repair, including epigenetic and transcriptional regulations, inflammation/immune response and epithelial-mesenchymal transition, suggesting that poly(ADP-ribosyl)ation both promotes and suppresses carcinogenic processes depending on the conditions.

Expanding understanding of poly(ADP-ribosyl)ation suggests that strategies to achieve cancer prevention targeting poly(ADP-ribosyl)ation for genome protection against life-long exposure to environmental carcinogens and endogenous carcinogenic stimuli.

Contents

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1. Introduction

Cancer development involves conversion of normal cells to malignant cancer cells through the generation of genetic, epigenetic and other changes, so-called 'multi-step carcinogenesis' (Sugimura, 1992). Each cancer shows different combinations of these changes, as recently revealed by whole-genome sequencing and global analysis of epigenetic regulation. During the carcinogenesis period of several months to several tens-of-years, cells suffer environmental stress, and genetic, epigenetic and other changes are induced, with each of these changes affecting further evolution of cancerous cells. These types of changes can occur not only in cancer cells but also in surrounding cells, including 'cancer-associated fibroblasts (CAF)'. 'Oncogene addiction' represents cancer cell states in which cancer cell survival and growth totally depend on the changes that occurred during the carcinogenic processes (Weinstein, 2008). The traits of these accumulated changes are important factors in considering 'individualized cancer therapy' and blocking cancer development at an early phase. Involvement of poly(ADP-ribosyl)ation (PARylation) in the processes of carcinogenesis has been studied at the molecular and cellular levels and further by using animal models and human genetic and epidemiological studies. Multiple proteins functioning in PARylation cascade have been shown to be involved in carcinogenesis (Fig. 1) in various ways and at individual steps. In this review we will focus on recent progress of the studies on the roles of PARylation in carcinogenesis.

2. Maintenance of genomic stability and other molecular functions relating to carcinogenesis

2.1. Involvement in DNA repair

Functional studies have revealed the involvement of PARP-1 mainly in base excision repair (BER) and single strand break (SSB) repair (Dantzer et al., 1999). PARylated PARP-1 recruits the scaffold protein XRCC-1 at single strand breaks (El-Khamisy et al., 2003; Masson et al., 1998) in the intermediate process of BER, after removal of modified bases by glycosylases such as OGG1 (8-hydroxyguanine DNA glycosylase), and facilitates the gap-filling reaction by DNA polymerases. PARP-1 was recently reported to interact with apurinic/apyrimidinic (AP) sites in DNA (Khodyreva et al., 2010) and this association was suggested to enable a rapid activation of PARylation after incision reaction with AP endonuclease 1 and could promote the BER process.

Double strand break (DSB) repair mainly consists of non-homologous end-joining (NHEJ), homologous recombination (HR) repair and single strand annealing repair. The defects in DSB repair will lead to loss of heterozygosity (LOH) and translocation, which could serve as the driver mutations frequently observed during carcinogenesis. PARP-1 binding activity to DSB inhibits NHEJ activity and facilitates homologous recombination (HR) repair in chicken DT40 cells (Hochegger et al., 2006). When Ku70/80 and DNA-PK dependent classical NHEJ is inhibited, back-up NHEJ involving PARP-1 and the Mre11-Rad51-NBS1 (MRN) complex becomes active (Cheng et al., 2011; Mansour et al., 2010). Kruppel-like factor 8 (KLF8), which contains C2H2 zinc fingers is frequently overexpressed in cancers and acts as a transcription factor for cyclinD1. KLF8 also binds PARP-1 in the nuclei and is recruited to DNA damage sites, phosphorylated by DNA-PKcs, and SUMOylated by SUMO E3 ligase PIAS (Lu et al., 2011). PARP-1 is thus implied to support the NHEJ process through interaction with KLF-8, especially in cancer cells (Lu et al., 2011). During the S-phase of cells, PARP-1 level is generally increased and PARP-1 promotes the HR repair process at stalled replication forks (Bryant et al., 2009).

These studies suggest an increased incidence of cancer in a PARP-1 deficient state. In fact, *Parp-1*$^{-/-}$ mice showed a higher susceptibility to carcinogenesis induced by alkylating agents (Piskunova et al., 2009; Tsutsumi et al., 2001). The frequencies of hepatocellular carcinoma development were higher in *Parp-1*$^{-/-}$ than in wild-type mice after treatment with alkylating agents or at advanced ages (Piskunova et al., 2008; Tong et al., 2002). *Parp-1*$^{-/-}$ mice showed higher frequencies of complex

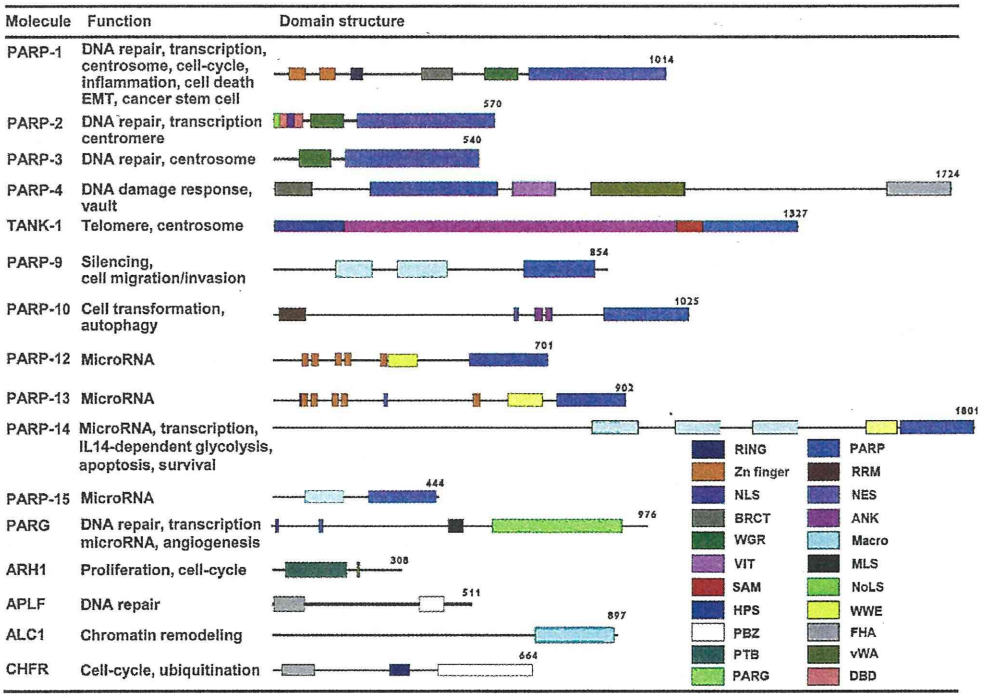

Fig. 1. Domain structure of PARP family proteins and related molecules and their functions. Motifs and domains: RING, RING finger; Zn finger, zinc finger; NLS, nuclear localization signal; BRCT, BRCA-1 C-terminus; WGR, tryptophan- glycine-arginine motif; VIT, vault inter-α-trypsin; SAM, sterile alpha motif; HPS, histidine, proline and serine; PBZ, PAR-binding zinc-finger; PTB, phosphotyrosine binding; PARG, PARG catalytic domain; PARP, PARP catalytic domain; RRM, RNA recognition motif; NES, nuclear export signal; ANK, ankyrin binding motif; Macro, macroH2A domain; MLS, mitochondrial localization signal; NoLS, nucleolar localization signal; WWE, tryptophan-tryptophan-glutamic acid motif; FHA, forkhead-associated domain; DBD, DNA binding domain.

type deletion mutations in the liver after treatment with alkylating agent and advanced ages (Shibata et al., 2009). Accelerated biological aging was further observed in *Parp-1*$^{-/-}$ mice (Piskunova et al., 2008).

PARP-2 interacts with PARP-1 and is involved in BER (Schreiber et al., 2002). PARP-2 knockout mice did not show increased frequency of spontaneous tumors but showed spontaneous development of T-cell lymphomas in the background of *p53*-deficiency with enhanced genomic instability (Nicolas et al., 2010), suggesting that PARP-2 might be essential for particular DNA repair processes, which could not be fully compensated by PARP-1 or other cellular components.

APLF (PALF, aka C2orf13, and XIP1) has tandem PAR binding zinc-finger (PBZ) domains and facilitates SSB and DSB repair through interaction with XRCC1, XRCC4 and PARP-3 (Rulten et al., 2011), suggesting a possibility that its aberration may cause genomic instability and contribute to carcinogenesis. Knockout mice of a PAR binding protein, CHFR (checkpoint with forkhead-associated (FHA) and RING finger domains), also showed an increase of spontaneous development of tumors, supporting the view that the PAR signaling pathway is involved in tumor suppression (Yu et al., 2005b).

2.2. Loss of heterozygosity (LOH) and translocation

In *Parp-1*$^{-/-}$*p53*$^{+/-}$ mice, enhanced tumor development was observed, accompanying LOH of the *p53* gene (Tong et al., 2003). *Parp-1*$^{-/-}$*Ptc1*$^{+/-}$ mice showed a higher frequency of development of medullablastoma and basal cell carcinoma after X-ray irradiation. The patched (Ptc1) gene is a negative regulator of sonic hedgehog signaling and LOH at the *Ptc1* locus was associated with the tumor development in *Parp-1*$^{-/-}$*Ptc1*$^{+/-}$ mice (Tanori et al., 2008). This suggests that LOH induced by X-ray irradiation at the *Ptc1* locus was enhanced by *Parp-1* deficiency, probably through defective DSB repair.

PARP-2 was shown to suppress *IgH/c-Myc* translocation during immunoglobulin class switch recombination (CSR) (Robert et al., 2009). CSR is induced by activation-induced cytidine deaminase-induced DSBs and in this process, PARP activity became increased, although PARP-1 and PARP-2 were not essential for the CSR reaction. PARP-1 located at switch regions may be involved in alternative microhomology-mediated end-joining, and PARP-2 is suggested to act as a translocation suppressor of CSR reaction (Robert et al., 2009).

PARP-4 (VPARP) was found to be an interacting protein of major vault-associated protein (MVP) and regulates vault function. Knockout mice of the *Parp-4* gene were generated, and they showed increased susceptibility to carcinogenesis induced by urethane and an alkylating agent, respectively (Raval-Fernandes et al., 2005). PARP-4 could thus be involved in DNA damage response, but the mechanism of the enhanced carcinogenesis has not been studied yet.

The *Parg*$^{110-/-}$ mice, lacking a full-length form of PARG, were reported to be also susceptible to diethylnitrosamine-induced hepatocellular carcinoma (Min et al., 2010). These mice lacked a nuclear isoform of PARG and had DNA repair defects,

whereas the mitochondrial PARG isoform level increased. γ-Irradiation, MMS and hydrogen peroxide treatment caused higher frequencies of sister chromatid exchanges in *Parg*$^{110-/-}$ cells, suggesting that DNA repair defects might contribute to the augmented susceptibility to tumorigenesis under *Parg* deficiency.

2.3. Repair of DNA lesions induced by environmental carcinogens

Chronic exposure to environmental carcinogens and endogenous DNA damage are essential process in carcinogenesis developing genomic instability. The roles of PARylation in DNA damage response induced by environmental carcinogens are shown in the following recent studies.

2.3.1. Arsenite

Arsenite is an environmental carcinogen and chronic exposure induces cancers in the skin, liver, and lungs, as well as urinary tract. Arsenite causes reactive oxygen species (ROS) and is known to enhance cancer development induced by chemical carcinogens and UV irradiation. Micromolar concentrations of arsenite are found to bind to zinc fingers of PARP-1 instead of zinc ions and disturb the function of PARP-1 in BER (Ding et al., 2009). 8-Hydroxyguanine (8-OHdG) levels actually increased after arsenite treatment and UV irradiation (Ding et al., 2009).

2.3.2. Asbestos

Chronic exposure to asbestos confers a high risk for developing malignant mesothelioma. Asbestos generates ROS and reactive nitrogen species, and this damage is also repaired mainly by BER. In patients with malignant mesothelioma, PARP-1 levels were low in the asbestos-exposed lymphocytes (Tomasetti et al., 2011). This might cause blocking of BER for repair of 8-OHdG lesions and induce DSBs, possibly contributing to developing deletion type mutations.

2.3.3. Cigarette smoke

Lung cancer is one of the diseases caused by cigarette smoke (CS). CS induces oxidative DNA damage, DNA strand breaks and PAR accumulation. *PARP-1* knockdown and *PARG* knockdown both caused delayed repair of DNA strand breaks and sensitized cells to CS (Kovacs et al., 2012), suggesting that PARP-1 and PARG levels may be important determinants of the susceptibility to CS-induced cancers in smokers.

2.3.4. Helicobacter pylori

Chronic infection to *Helicobacter pylori* (*H. pylori*) is a known risk factor for gastric cancer. *H. pylori* infection indirectly causes DNA DSBs and checkpoint activation in the host cells independently of the presence of the cag pathogenicity island (Toller et al., 2011). During persistent infection of *H. pylori*, PARP-1 was activated in host cells through unknown heat- and protease-sensitive factor (Nossa et al., 2009). A polymorphic allele of *PARP-1* at *Val762Ala*, which causes lower catalytic activity, has been reported to be associated with gastric cancer risk with the *cagA* + strain of *H. pylori* in a Han Chinese population (Silva-Fernandes et al., 2012).

2.4. Centrosome and cell cycle regulation

Centrosomes are frequently amplified in tumor cells. PARP-1 is present not only in nuclei but also in centrosomes and is involved in the centrosome regulation through interaction with p53 (Kanai et al., 2003). As PARP inhibitor treatment caused and *Parp-1* deficient cells displayed increased numbers of centrosomes through centrosome deregulation, PARP-1 dysfunction might also cause centrosome amplification and consequent genomic instability. PARP-3 is also localized to daughter centrioles and is involved in centrosome dynamics (Augustin et al., 2003). Various cancer cells possess amplified numbers of centrosomes. Because PARP inhibitor treatment or *Parp-1* deficient cells show increased numbers of centrosomes through centrosome deregulation, PARP-1 dysfunction during carcinogenesis might also cause amplified numbers of centrosomes that lead to genomic instability.

A variant of PARP-3, which is seven amino acid residues longer at the N-terminus, is expressed in humans but not in mice. This longer PARP-3 is involved in centrosome regulation, by binding to NuMA and TANK1. The shorter isoform is present in nuclei and is auto-PARylated by x-irradiation, recruited to DSBs, and is involved in DSB repair (Boehler et al., 2011). PARP-3 is required for mitotic progression and its knockdown causes mitotic arrest and genomic instability through sister chromatid fusion at telomeres and telomere loss (Boehler et al., 2011). Therefore, PARP-3 aberration may lead to genomic instability and promote tumorigenesis.

Tumor cells frequently possess amplified numbers of centrosomes, and therefore the centrosome clustering process, which is regulated by NuMA, may be crucial for supporting mitotic division of cancer cells. Because PARP-3 and TANK1 interact with NuMA (Boehler et al., 2011), the function of PARP-3 and TANK1 is speculated to be important in aneuploid cancer cell survival. This point awaits further elucidation.

Tank1 and *Tank2* knockout mice both show normal development (Chiang et al., 2008). Although human TANK1 negatively regulates telomerase, *Tank1* knockout mice exhibited no alteration in telomere length. This could be explained by the fact that human TRF1 (telomere regulating factor 1) has binding sites for Tank1, whereas mouse TRF1 does not. Tank1 is further

required for resolution of sister chromatids at telomeres in human cells (Dynek and Smith, 2004), thus TANK1 may be important in preventing genomic instability and protecting cells from tumorigenic transformation in humans.

After introduction of DSBs, p53-dependent cell cycle arrest is induced. PARylation of p53 by PARP-1 suppresses its nuclear export and may facilitate the initiation of cell cycle arrest (Kanai et al., 2007). PARP-1 interacts with various factors involved in DNA damage response, including p53. C120rf48 (PARPBP), which is overexpressed in pancreatic cancers, was demonstrated to be an interacting factor for PARP-1 in cancer cells (Piao et al., 2011). It is mainly localized in nuclei and is able to activate PARP-1. Knockdown of *C120rf48* caused apoptosis and sensitization to DNA damaging agents, including adriamycin, UV, and hydrogen peroxide, in pancreatic cancer cells. C120rf48 is required for G1 arrest, suggesting the possibility that this protein interacts with PARP-1 to keep the proper G1 progression in cancer cells.

ADP-ribosyl hydrolase1 (ARH1) cleaves the linkage between monoADP-ribosyl residues and arginine residues of proteins generated by clostridial toxin-related ADP-ribosyl transferases (ARTC). *Arh1*$^{-/-}$ mice showed a higher incidence of spontaneously developed lymphomas and adenocarcinomas, and the frequency of metastases was also increased (Kato et al., 2011). In tumors developed in *Arh1*$^{+/-}$ mice, loss of the wild-type allele, namely LOH was frequently observed, suggesting that the *Arh1* gene therefore may function as a tumor suppressor. *Arh1*$^{-/-}$ fibroblasts showed enhanced growth and loss of G1 phase control. The critical target proteins and further mechanisms for suppression of tumorigenesis have not been elucidated yet. There are five enzymes of the ARTC family in mammals, and their involvements in tumorigenesis has not been fully studied yet.

CHFR binds to poly(ADP-ribose) with a C2H2 type zinc finger and functions as a ubiquitin ligase regulating cell cycle progression, mainly at the M phase. CHFR was shown to bind PARP-1 and degraded PARP-1 at the M-phase and prevented mitotic arrest in cancer cells (Kashima et al., 2012). Notably, this protein is frequently inactivated by mutation or promoter hypermethylation in various types of cancers, suggesting PAR signaling is involved in tumor suppressive functions of CHFR (Ahel et al., 2008).

2.5. Transcriptional regulation

During pre-cancerous stages and transformed states, cells are exposed to diverse stresses, including DNA lesions and aberrant activation of signaling pathways (Jones et al., 2012; Prasad et al., 2010), which may result in activation of PARPs. Activated PARPs PARylate and modify the activities of various stress-associated targets and signaling pathways, leading to gene transcriptional alteration. PARP-1 acts as a coactivator and corepressor of genes important in carcinogenesis, including retinoic acid-inducible retinoic acid receptor β (Pavri et al., 2005) and β-catenin-TCF4 complex (Idogawa et al., 2007). PARP-1 also serves as a coactivator of NF-κB, a major transcription factor involved in inflammation. PARP-1 PARylates p65NF-κB and reduces the interaction between exporter protein Crm1 and enhances its nuclear retention after TLR4 (toll-like receptor 4) stimulation (Zerfaoui et al., 2010). Acetylation of PARP-1 by p300/CREB-binding protein also enhances coactivation of NF-κB-dependent transcription (Hassa et al., 2005), which may support the chronic inflammation observed during carcinogenesis.

ALC1 is a PAR binding protein and a member of the SNF2 ATPase superfamily harboring macrodomains as a PAR binding module. Its ATPase activity and chromatin remodeling activity are activated by PARylated PARP-1 and NAD. ALC1 was recruited to nucleosomes where PAR synthesis occurred (Gottschalk et al., 2009). *ALC1* is classified as an oncogene and its gene amplification is observed in over 50% of human hepatocellular carcinoma (Ahel et al., 2009; Gottschalk et al., 2009). The frequent functional aberration of PAR binding proteins in human cancers suggests that PAR signaling after DNA damage may be an important anti-cancer barrier to prevent carcinogenesis.

2.6. Epigenetic regulation

PARP-1 has been suggested to be involved in epigenetic regulation (Fig. 2). After ERK phosphorylation, PARP-1 activated by phosphorylated ERK2 enhances activation of Elk1, promoting CBP/p300 dependent acetylation and the expression of Elk1-target genes (Cohen-Armon et al., 2007). Under progestin stimulation of breast cancer cells, phosphorylated PARP-1 and CDK2 cooperatively led to displacement of histone H1 from chromatin, regulated the majority of hormone-responsive genes and induced the progestin-dependent cell cycle progression (Wright et al., 2012). Furthermore, PARP-1 dependent PARylation of KDM5B, a lysine-specific demethylase, inhibited its binding to target proteins and kept an active histone hallmark, H3K4me3 in human breast cancer MCF7 cells (Krishnakumar and Kraus, 2010).

The PARP-1/CTCF (CCCTC binding factor) complex is reported to inhibit the activity of DNA methyltransferase 1 (Dnmt1) and protect active chromatin from DNA methylation-dependent silencing (Guastafierro et al., 2008; Reale et al., 2005). On the other hand, epigenetic suppression of particular genes also required PARP-1. *Parp-1* deficiency induced epigenetically silenced genes associated with trophoblast differentiation during tumor formation and culture of ESCs (Nozaki et al., 1999; Ogino et al., 2007). This implies the role of PARP-1 as a barrier for epigenetic activation during carcinogenesis. PARP family proteins are also involved in downregulation of transcription, cooperatively with epigenetic regulator polycomb proteins under DNA damage. PARP-1 and PARP-2 are involved in the recruitment of repressive nucleosome remodeling and deacetylase (NuRD) to DNA damage sites and removes the RNA polymerase II from there, transiently suppressing the transcription (Chou et al., 2010). PARP-3 is also involved in transcriptional silencing (Rouleau et al., 2007).

During very early stages of somatic cell reprogramming, PARP-1 activity was required for demethylation of DNA and activation of a transcription factor, the *Nanog* gene (Doege et al., 2012). This property of PARP-1 suggests that PARP-1 may be

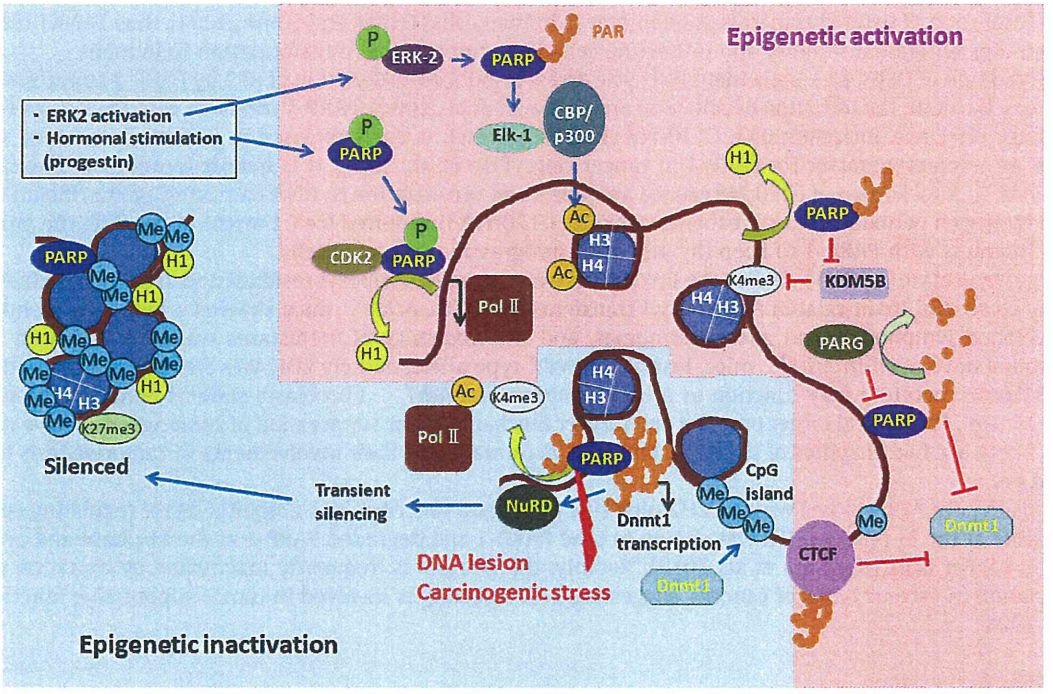

Fig. 2. Involvement of PARP-1 in epigenetic regulation relating to carcinogenesis. DNA is illustrated as brown strings. Epigenetic activation (pink area) and epigenetic suppression (blue area) are shown by interaction with various chromatin regulators and other proteins. PARP-1 could bilaterally regulate the epigenetic factors and convert the hetero/euchromatin. Under stimulation of transcriptional activation, phosphorylated or PARylated PARP-1 leads to epigenetic activation of target sites by interaction with CDK2 or activation of Elk1, which are achieved by histone H1 elimination or histone H3/H4 acetylation. Furthermore, KDM5B and DNMT1 activities are also inhibited by PARylated PARP-1, and this also contributes to epigenetic activation. These processes accelerate open chromatin formation and loading of the RNA Pol II machinery. On the other hand, PARP-1 could induce epigenetic silencing and resulting heterochromatin formation in some conditions; e.g. under DNA damage induction. In cooperation with polycomb proteins, PARP-1 recruits the NuRD complex to damage sites and leads to histone deacetylation and RNA Pol II elimination resulting in epigenetic suppression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

also important in epigenetic regulation during reprogramming into induced pluripotent stem (iPS) cells, some of these processes for iPS cells are expected to be common to the reprogramming processes during carcinogenesis.

2.7. MicroRNA regulation

In cancer cells oncogenic micro RNAs (miRNA) are activated and tumor suppressive miRNAs are inactivated. Therefore the regulation of microRNA might be important for carcinogenesis and maintaining cancer cell homeostasis. PARP family proteins could also regulate gene expression via regulation of miRNA activity. Several PARP family proteins, including TANK1, PARP-12/13/14/15 and PARG, have been reported to co-localize to stress granule components and PARylate the miRNA-binding protein argonaute, and lead to suppression of microRNA-mediated gene knockdown (Leung et al., 2011). This mechanism might contribute to dynamic responses of gene regulation under stressed conditions during carcinogenesis.

2.8. Inflammation

Chronic inflammation is considered as an important process of carcinogenesis. Recently, the concept of senescence-associated secretory phenotype (SASP), which entails an increase in the secretion of pro-inflammatory cytokines, links two distinct carcinogenic stresses, DNA lesions and inflammation related signals at pre-cancerous stages (Davalos et al., 2010). Furthermore, inflammation associated factors could lead to epithelial-mesenchymal transition (EMT), a pathway contributing to cancer stem-cell development (Heldin et al., 2012). Lipopolysaccharide (LPS) stimulation of macrophages activates PARP-1 and PARylates histones to convert them into a transcriptionally active state, and facilitates NF-κB recruitment to nucleosome-occupied promoters, including Il-1β (Martinez-Zamudio and Ha, 2012).

Under interferon-gamma-stimulation, PARP-1 maintains p38 stress kinase activation and protects the interferon-gamma-inducible-protein (IP-10) mRNA from degradation, leading to activation of inflammation responses (Galbis-Martinez et al., 2010). In *ApoE*$^{-/-}$ mice subjected to high fat-diet conditions, PARP-1 suppressed the tissue inhibitor of metalloproteinase (TIMP)-2 expression and activated the matrix metalloproteinase 9 (Hans et al., 2011). PARP-1 co-activated NF-κB mediated inflammation responses by MAPK activation, mRNA stabilization and nuclear transporter regulation. In this situation, the cell migration and EMT induced by inflammation responses are accelerated by PARP-1. Also, under anti-cancer drug treatment, PARP inhibition attenuated inflammatory damage to cells (Mukhopadhyay et al., 2011).