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

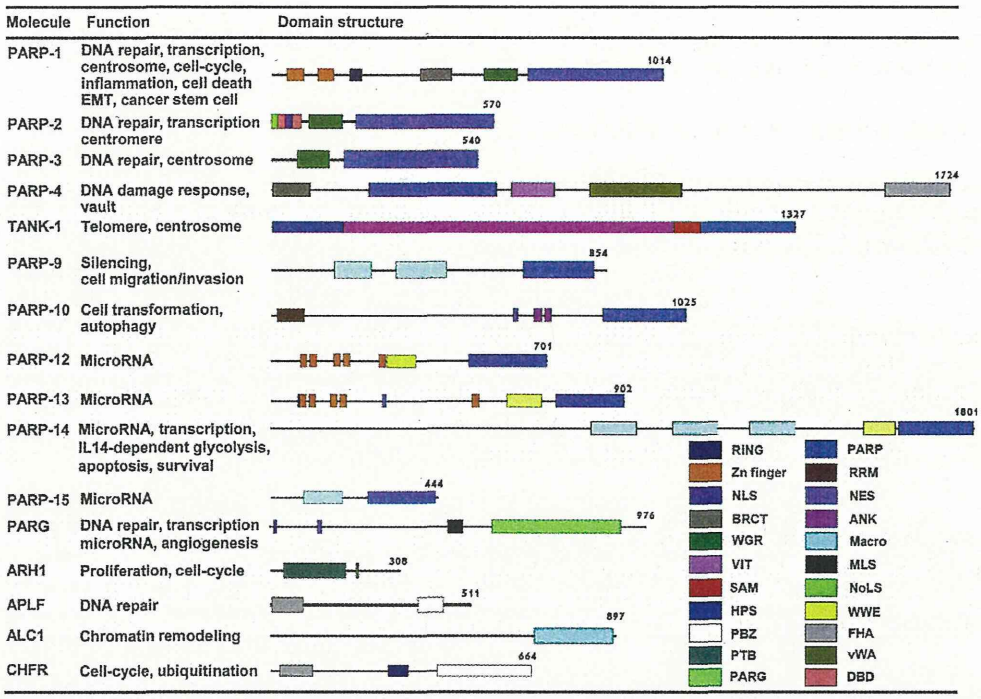

Fig. 1. Domain structure of PARP family proteins and related molecules and their functions. Motifs and domains: RING, RING finger; Zn finger, zinc finger; NLS, nuclear localization signal; BRCT, BRCA-1 C-terminus; WGR, tryptophan- glycine-arginine motif; VIT, vault inter-α-trypsin; SAM, sterile alpha 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

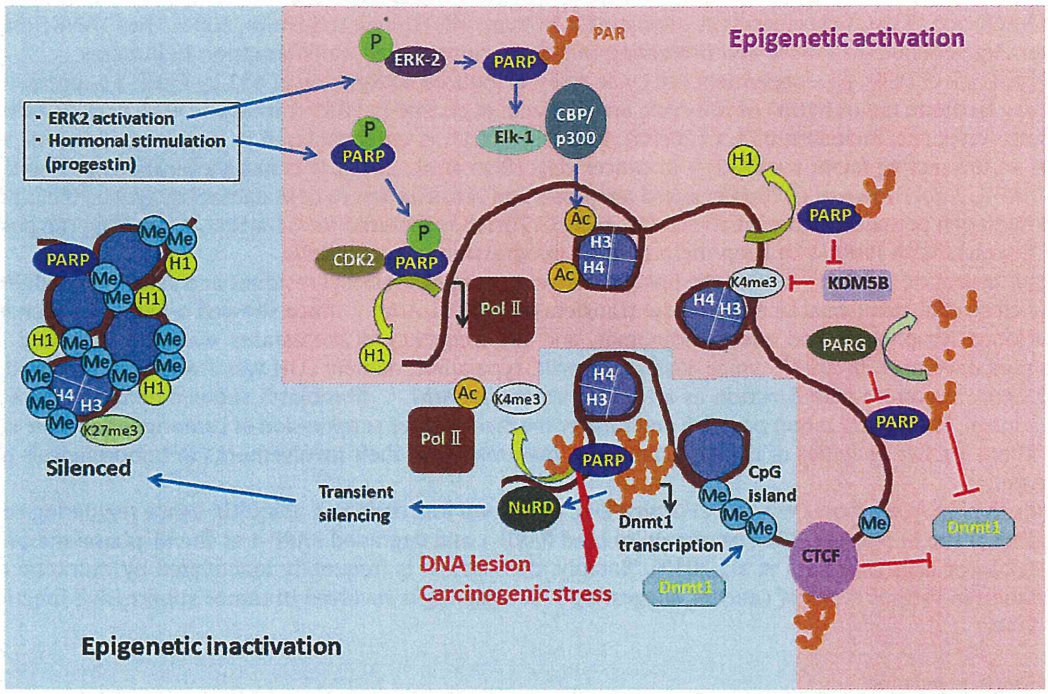

Fig. 2. Involvement of PARP-1 in epigenetic regulation relating to carcinogenesis. DNA is illustrated as brown strings. Epigenetic activation (pink area) and epigenetic suppression (blue area) are shown by interaction with various chromatin regulators and other proteins. PARP-1 could bilaterally regulate 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).

2.9. Cellular transformation

The classical PARP inhibitors were shown to modulate cell transformation after DNA damage. PARP-10 has been demonstrated to mono (ADP-ribosylate) PARP-10 itself and modify core histones (Kleine et al., 2008; Yu et al., 2005a). PARP-10 suppressed EIA-involved cellular transformation possibly through interaction with c-Myc (Yu et al., 2005a). PARP-10 is reported to shuttle between the cytoplasm and nucleus, and involvement in autophagy is suggested (Kleine et al., 2012). However, its function in carcinogenesis awaits further studies.

2.10. Cell death control

Cell death pathways are important for suppressing carcinogenesis. During apoptosis, PARP-1 is cleaved while PARP-1 activity is not essential for induction of apoptosis in most cases. After massive DNA damage and PARP-1 activation, PAR formation causes AIF (apoptosis inducing factor)-dependent cell death induction, in neuronal cells and also in cancer cells (Yu et al., 2006), whereas induced NAD depletion and subsequent ATP depletion also cause a type of necrosis. In these types of cell death, PARP-1 activity is essential and PARP inhibitor was shown to suppress cell death.

Autophagy-associated cell death is also reported to be involved in suppression of cancer. PARP-1 is reported to act in induction of autophagy (Munoz-Gamez et al., 2009; Rodriguez-Vargas et al., 2012). For example, PARP-1 was shown to be involved in induction of autophagy caused by cigarette smoke and when PARP-1 is inhibited, SIRT1 is activated and suppresses autophagy (Hwang et al., 2010). PARP10 is also suggested to be involved in autophagy processes by interacting with poly-ubiquitin receptor p62 (Kleine et al., 2012).

Of note, it has been recently reported that bcl-2 binds to PARP-1 and suppresses its activity and nonapoptotic cell death (Dutta et al., 2012). Therefore, bcl-2 overexpressing B-cell lymphomas and chronic lymphocytic leukemia showed decreased PARP-1 activity, and because cell death is blocked by bcl-2 these tumor cells may show accelerated genomic instability and may develop malignant phenotypes.

3. Tumor cell biology

Tumor malignancy depends highly on migratory and invasive abilities of cancer cells. In some cases, cancer cells gain these abilities during the process of EMT induced by TGF-β signaling factors or inflammation stress through introduction of additional mutations or by causing transcriptional aberration.

3.1. Cell migration, invasion and metastasis regulation

Under stimulation with erythropoietin, produced by cancer during anemia and hypoxia, PARP-1 induced cell migration through transcription of *c-fos*, *Egr-1* in breast cancer cells MDA-MB-435 (Inbar et al., 2012). Indeed, high expression levels of PARP-1, chromatin assembly factor-1 and nestin are regarded as the worst prognosis phenotypes of oral squamous cell carcinomas (Mascolo et al., 2012). It is reported that PARP-1 inhibition caused transcriptional suppression of NF-κB and MMP-2/9 in colon carcinoma cells CT26 (Li et al., 2009) and PARG dysfunction also suppressed metastasis via NF-κB downregulation in colon carcinoma cells (Li et al., 2012).

Furthermore, the PARP family is involved in angiogenesis. In fact, low doses of PARP inhibitor inhibited the migration of human umbilical vein endothelial cells HUVEC (Caldini et al., 2011) and angiogenesis (Pyriochou et al., 2008; Rajesh et al., 2006; Tentori et al., 2007). PARP inhibition down-regulated transcriptional factors, *OCT-1* and *CREB* leading to reduced expression of endothelial cell migration and adhesion associated genes, *SDC4* and *ID-1* (Lacal et al., 2009). *PARG*-knockdown caused downregulation of NF-κB and ERK/p38 activities in colon carcinoma cells and decreased the level of angiogenic factors such as VEGF and inhibited migration and proliferation of co-cultured HUVEC (Pan et al., 2012).

Functional inhibition of PARG also suppressed metastastic properties of colon carcinoma cells in a model of liver metastasis with increased levels of the Akt pathway, and a decreased expression of MMP2/9 and NF-κB pathway (Li et al., 2012).

3.2. Cell survival signaling

PARP-9/14/15 are Bal (B-aggressive lymphoma 1) family proteins (Aguiar et al., 2005), which have macrodomains. PARP-9 was found to be overexpressed in B cell aggressive lymphoma, including diffuse large B-cell lymphoma. PARP-9 contains two macrodomains, which bind both an ADP-ribose monomer and poly(ADP-ribose). PARP-9 was also able to promote tumor cell invasion (Aguiar et al., 2000). PARP-14 recruited histone deacetylase (HDAC)2/3 to IL14 responsive genes and promoted binding of Stat6 and further ADP-ribosylated HDACs to replace them by histone acetyl transferase and activated transcription (Mehrotra et al., 2011), thus showing an IL14-dependent transcriptional switch property. PARP-14 activated IL14-dependent glycolysis in B cells and also suppressed apoptosis. PARP-14 thus supported IL-14-induced survival during Myc-induced B cell lymphomagenesis (Cho et al., 2011). PARP-14 was also highly expressed in multiple myeloma and its high expression was associated with the poor diagnosis. PARP-14 showed binding to JNK1 and inhibited its activity and

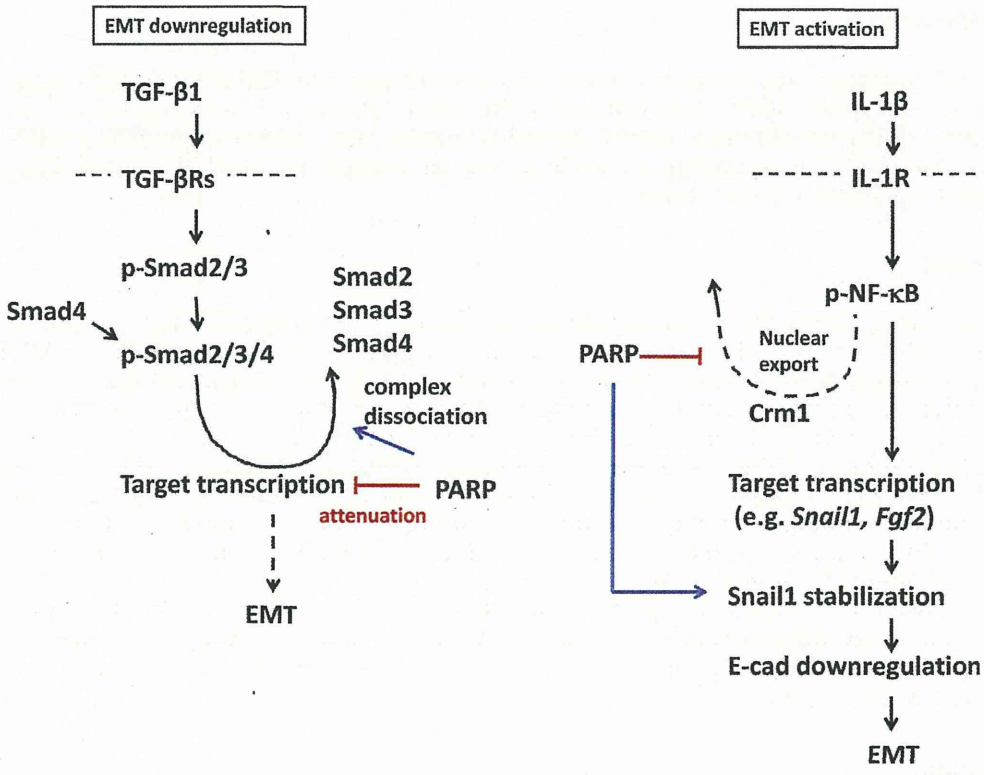

Fig. 3. The model for EMT suppression and activation by PARP-1. PARP-1 suppresses the TGF-β dependent EMT by Smad degradation but accelerates the Snail 1 dependent EMT by E-cad suppression via Snail l stabilization. The bilateral opposing characteristics of PARP-1 in EMT induction are consistent with its buffering roles for maintaining cellular homeostasis.

enhanced JNK-2-dependent survival signaling (Barbarulo et al., 2012), which counteract JNK1-mediated apoptosis. Bal family PARPs thus widely support survival signaling and also promote tumor malignancy through their macro domain function.

3.3. EMT and cancer stem cell maintenance

In the EMT pathway, E-cadherin (E-cad) is the main target to be suppressed by the TGF-β signaling pathway (Brandl et al., 2010; Nieto, 2002). In TGF-β/Smad regulation, PARP-1 dependent dissociation of Smad complexes from DNA by PARylating Smad3/4 attenuated Smad-specific gene responses and TGF-β-induced EMT (Huang et al., 2011; Lonn et al., 2010) as shown in Fig. 3 (left panel). On the other hand, under conditions of Snail/E-cad suppression, PARP-1 binding to integrin-linked kinase responsive element maintained Snail 1 transcription and resulted in downregulation of E-cad transcription (McPhee et al., 2008), resulting in EMT activation (Fig. 3, right panel). Furthermore, NF-κB and PARP-1 activated fibronectin gene transcription, which is important for cancer cell maintenance and migration ability (Stanisavljevic et al., 2011). Taken together, it is suggested that PARP-1 suppresses the TGF-β dependent EMT by Smad degradation but accelerates the Snail 1 dependent EMT by E-cad suppression via Snail l stabilization (Fig. 3).

The involvement of PARPs in cancer stem cell (CSC) maintenance has been also suggested; PARP inhibitor showed anti-CSC activity in basal-like breast cancer cell lines (Shimo et al., 2012). This could be related to the coactivator function of PARP-1 for NF-κB because constitutive NF-κB activation correlated to proliferation of basal-like subtype breast cancer cells (Yamaguchi et al., 2009). Furthermore, PARP inhibitor blocked development of cancerous stem cells by suppressing senescence induction process of differentiating ESCs (Fujimori et al., 2012). Further studies are required to elucidate the roles of PARP family molecules in CSC regulation.

4. Animal models and genetic studies in human cancers

4.1. Animal models

With classical types of PARP inhibitors, the roles of PARP in carcinogenesis were previously investigated using various carcinogens in animal models. Using streptozotocin, the incidence of insulinoma in the pancreas increased by the initial combinational treatment with benzamide or 3-aminobenzamide (Yamagami et al., 1985), suggesting the involvement of PARylation in the suppression of tumorigenesis. Potent PARP inhibitors are now in clinical trials for cancer therapy. By single

treatment or in combination with carcinogens, the effects of PARP inhibitors on carcinogenesis are expected to be evaluated in the future to consider long-term carcinogenic risks.

On the other hand, it is reported that PARP-1 acts as an enhancer of tumorigenesis in some experimental models. After 7,12-dimethylbenz(a)anthracene plus 12-O-tetradecanoylphorbol-13-acetate treatment, PARP-1 inhibition results in a delay in skin tumor formation. This could be related to compromised activation of AP-1, NF-κB and HIF-1α by PARP inhibition (Martin-Oliva et al., 2006). PARP-1 downregulation also abolished tumorigenicity of melanoma (Tentori et al., 2008) and suppression of PARG promoted tumorigenicity (Molloy-Simard et al., 2012). The susceptibility of knockout mice models suggests that multiple ADP-ribosylation-related proteins are involved in carcinogenesis (Table 1).

4.2. Genetic studies in human cancers

Genetic studies on the association of polymorphism of the *PARP-1* gene with the cancer have been carried out as summarized in Table 2. The *Ala/Ala* allele at *VAl762Ala* single nucleotide polymorphism (SNP) was shown to lead to lower PARP-1 activity (Wang et al., 2007) and this *Ala* allele was associated with the risk of prostate cancers in Caucasians (Lockett et al., 2004), for esophageal (Hao et al., 2004), and lung cancers (Zhang et al., 2005) in Chinese populations. *Val762Ala* polymorphism was also associated with the risk of cervical carcinoma in Chinese populations (Ye et al., 2012). However, recent meta-analysis showed that association of the *Val762Ala* polymorphism with an increased risk of cancer was found in Asians, whereas a decreased risk of cancer, such as glioma, was detected among Caucasians (Yu et al., 2012). No significant association was detected between *Val762Ala* polymorphism and cancer in an overall population, suggesting that the polymorphism could be affected by genes that may be different among ethnic populations. Investigation of breast cancers from French patients revealed the genetic variants and SNPs of *PARP-1* at 10%, in which one SNP (*Ala284Ala*) was likely associated with loss of estrogen- and progesterone-receptor expression (Cao et al., 2007).

When microRNA binding site polymorphisms in DNA repair genes were analyzed, SNP rs8679 at the 3'-untranslated region of the *PARP-1* gene was shown to be associated with an increased breast cancer risk. One of the candidate target miRNAs is *miR145*, and a further validation study is awaited (Teo et al., 2012). It is also notable that increased risk of melanoma with SNP rs321925, present in the intron of the *PARP-1* gene near the SNP *Val762*, was observed in Caucasian populations (Zhang et al., 2011), although no association with *Val762Ala* was observed. The biological significance is yet to be clarified.

Mutations of the *PARP-1* gene were quite rare in cancers, in contrast to the high mutation frequency in the DNA polymerase β, which is also involved in base excision repair (BER) (Chan et al., 2006), suggesting PARP-1 function may be required for cancer cell survival.

PARP-2 was reported to be involved in the risk of therapy-related acute myeloid leukemia, a secondary malignant leukemia developed after chemotherapy (Cahan and Graubert, 2010). The mutations and SNP association of other PARP family proteins, PARG, ARTCs, and ARHs have not been extensively studied yet.

On the other hand, the expression changes of PARP family proteins have been reported and from the early step of carcinogenesis. *PARP-1* expression became augmented, including in colorectal cancers (Nosho et al., 2006), and endometrial cancers (Postawski et al., 2011). In the lung cancers, PARP-1 expression is higher in small cell lung cancers (SCLC). In fact, PARP inhibitors reduced growth and augmented the effect of cytotoxic agents in SCLC (Byers et al., 2012). *PARP-1* expression was

Table 1
The susceptibility to carcinogenesis in mouse models.

Molecule	Spontaneous/induced	Outcome	Tissue or tumor type	References
Parp-1$^{-/-}$ (ex2)	Spontaneous (18–24 months)	Increased	Hepatocellular carcinoma	Tong et al. (2002)
Parp-1$^{-/-}$ (ex1)	Spontaneous (7 months)	No change	Various tissues	Tsutsumi et al. (2001)
	Spontaneous (15 months)	No change	Various tissues	Ogawa et al., 2006
Parp-1$^{-/-}$ (ex2)	Spontaneous	Increased	Uterine, lungs, hepatocellular carcinoma	Piskunova et al. (2009)
Parp-1$^{-/-}$ (ex2)	Spontaneous	Increased	Breast	Tong et al. (2007)
Parp-1$^{-/-}$ (ex1)	BHP	Increased	Hemangioma, hemangiosarcoma	Tsutsumi et al. (2001)
Parp-1$^{-/-}$ (ex1)	Azoxymethane	Increased	Colon, liver (nodule)	Nozaki et al. (2003)
Parp-1$^{-/-}$ (ex2)	Diethylnitrosamine	Increased	Hepatocellular carcinoma	Tong et al. (2002)
Parp-1$^{-/-}$ (ex1)	4-Nitroquinoline 1-oxide	No change	Oral cavity, esophagus	Gunji et al. (2006)
Parp-1$^{-/-}$ (ex1)	IQ	No change	Liver, forestomach	Ogawa et al. (2006)
Parp-1$^{-/-}$ (ex2)	*p53*$^{-/-}$	Increased	Colon, breast, brain	Tong et al. (2001, 2007)
Parp-1$^{-/-}$ (ex4)	*p53*$^{-/-}$	Decreased	Thymic lymphoma	Conde et al. (2001)
Parp-1$^{-/-}$ (ex2)	*Ku80*$^{-/-}$	Increased	Liver	Tong et al. (2002)
Parp-1$^{-/-}$ (ex2)	*SCID*	Increased	Thymus	Morrison et al. (1997)
Parp-1$^{-/-}$ (ex2)	*Ptc*$^{-/-}$	Increased	Medulloblastoma, basal cell carcinoma	Tanori et al. (2008)
Parp-1$^{-/-}$ (ex2)	*Wrn*$^{Dhel/Dhel}$	Increased	Various tissues	Lebel et al., 2003
Parp-2$^{-/-}$ *p53*$^{-/-}$		Increased	T-cell lymphoma	Nicolas et al. (2010)
Parp-4$^{-/-}$	Diethylnitrosamine	Increased	Colon	Raval-Fernandes et al. (2005)
Parp-4$^{-/-}$	Urethane	Increased	Lungs	Raval-Fernandes et al. (2005)
Parg$^{-/-}$ (110 kD)	Diethylnitrosamine	Increased	Liver	Min et al. (2010)
Arh1$^{-/-}$	Spontaneous	Increased	Lymphoma, adenocarcinoma	Kato et al. (2011)

Table 2
Polymorphisms in the *PARP-1* gene and its association with cancer risk.

SNP	Combination	Studied population	Risk	Cancer-type	References
Val762Ala	–	Caucasian	Increased	Prostate	Lockett et al. (2004)
	–	Han Chinese	Increased	Esophageal	Hao et al. (2004)
	–	Han Chinese	Increased	Lung	Zhang et al. (2005)
	Cag^{+}*H. pylori* infrction	Caucasian	Increased	Gastric	Silva-Fernandes et al. (2012)
	–	Chinese	Increased	Cervical	Ye et al. (2012)
	–	Caucasian	No difference	Breast	Cao et al. (2007)
	–	Japanese	No difference	Lung	Sakiyama et al., 2005
	–	Caucasian	Decreased	Glioma	Liu et al., 2009
	–	Han Chinese	No difference	Bladder	Wang et al., 2010
	–	Japanese	No difference	Soft-tissue	Nakayama et al., 2008
	XRCC1Arg399Gln	Han Chinese	Increased	Lung	Zhang et al. (2005)
	–	Caucasian	No difference	Melanoma	Zhang et al. (2011)
rs321925 (intron)	–	Caucasian	Increased	Melanoma	Zhang et al. (2011)
Lys940Arg	–	Japanese	No difference	Lung	Sakiyama et al., 2005
rs8679 (37-UTR)	–	Caucasian	Increased	Breast cancer	Teo et al., 2012

also associated with human papillomavirus positivity in precancerous high-grade squamous intraepithelial lesions (Hassumi-Fukasawa et al., 2012).

5. Cancer biomarkers and diagnosis

Cancer biomarkers are important for diagnosis and monitoring therapeutic effects and to determine the therapeutic strategies for individual cancers. There are various tumor biomarkers for different cancers, including CA-125, and α-fetoprotein. The amount of PARylated proteins in peripheral blood leukocytes was proposed to be a biomarker for head and neck, breast, and cervical cancers, because their levels decreased to less than half in the patients of these cancers (Lakadong et al., 2010). The underlying mechanism awaits further investigation.

6. Cancer prevention

When rats were fed a niacin (nicotinic acid)-deficient diet, NAD levels decreased in bone marrow and showed increased incidence of tumor development after treatment with ethylnitrosourea (Boyonoski et al., 2002). Non-lymphocytic leukemia was frequently observed among these tumors (Bartleman et al., 2008), which is a common secondary cancer after chemotherapy, suggesting that niacin supplementation may be useful for prevention of secondary cancer development. In skin keratinocytes, decreased levels of niacin led to a decrease in NAD levels, under which resulting lowered PARP activity enhanced the sensitivity to UV-damage (Benavente et al., 2009). A myristylnicotinate derivative of niacin is being evaluated for prevention of skin cancers in clinical trials (Tashtoush et al., 2007).

In clinical trials for ovarian, breast and prostate cancer patients, PARP inhibitors showed effectiveness to carriers of a *BRCA1* or *BRCA2* mutation with few side-effects (Audeh et al., 2010; Fong et al., 2009; Tutt et al., 2010). PARP inhibitors targeted the tumor cells with *BRCA1/2* mutations and specifically induced cancer cell death (Bryant et al., 2005; Farmer et al., 2005). This concept has been applied to several clinical strategies as 'synthetic lethality'. PARP inhibition in BRCA-deficient cells led to the persistence of DNA lesions, normally repaired by HR, resulting in cell-death induction. In *BRCA2* mutated breast carcinoma cells, the anti-tumor effect of PARP inhibitor was enhanced by additional inhibition of nicotinamide phosphoribosyltransferase (NAMPT), which mediates nicotinamide mononucleotide (NMN) production from nicotinamide (Bajrami et al., 2012). Under deficiency of *PTEN*, which is required for transcription of a key factor in HR repair, Rad51, PARP inhibitor also decreased the cancer cell survival (Mendes-Pereira et al., 2009). Furthermore, in androgen receptor (AR)-dependent prostate cancers, PARP-1 modulated the AR-dependent transcription as a coactivator and promoted tumor growth (Schiewer et al., 2012).

Therefore, further investigation is necessary to establish whether prophylactic treatment with PARP inhibitors in one allele mutation carriers of *BRCA* or *PTEN* can efficiently remove the biallelic mutated precancerous cell populations and be used as a chemopreventive strategy in cancer. Further animal studies need to be carried out to assess the side effects of PARP inhibition on genomic stability during long term treatment.

7. Perspectives

As we reviewed in this article, PARylation is involved in carcinogenesis in many ways (Fig. 4), however, a fine control of PARylation at each stage will enable intervention in carcinogenesis. For this purpose, understanding the PARylation related proteins and the function of enzymes that use NAD, including SIRT family proteins, might be particularly important. By identifying the environmental carcinogens, the strategies for minimizing exposure to carcinogens may become possible. However,

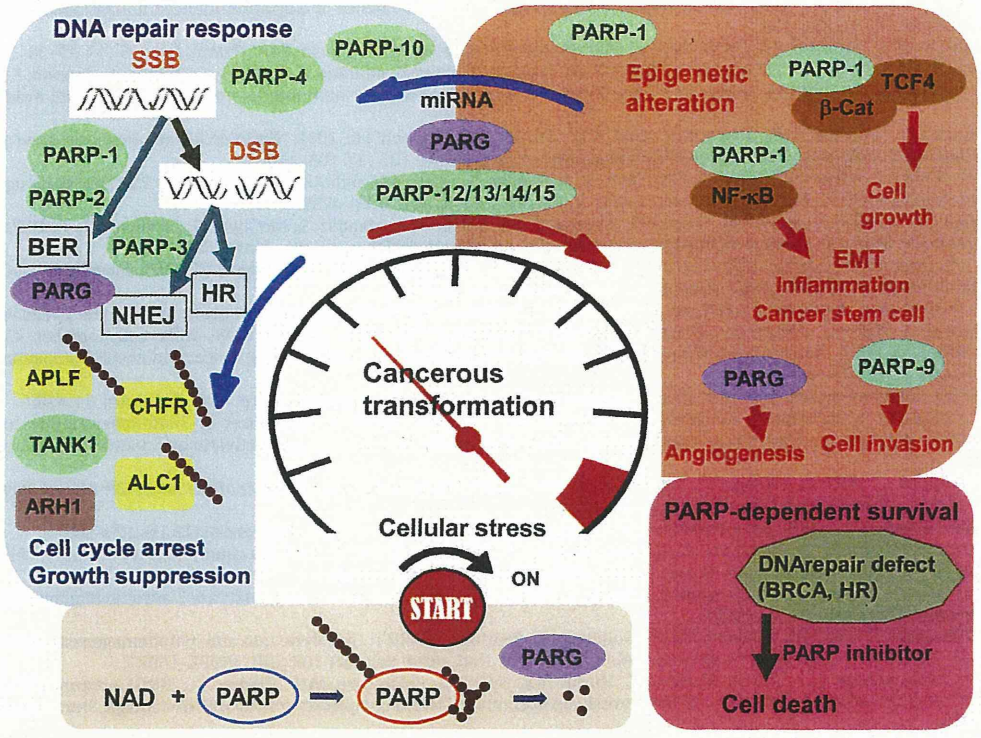

Fig. 4. Involvement of PARylation in cancer development. PARylation suppresses carcinogenesis through DNA repair and other DNA damage responses and downregulates cancer-associated pathways and these responses might serve as cancer barriers at various stages. On the other hand, PARPs support carcinogenesis through involvement in transcription, epigenetic regulation, EMT, cell invasion and signaling cascades. If the state of oncogene addiction is present, where cancer cells survive in a PARP-dependent manner, PARP inhibitor may be an effective therapeutic agent.

'aging' is one of the strongest inescapable risk factors for cancer. Because of this and other inescapable carcinogenic risk factors, various preventive and cancer suppressive strategies should be further developed. As observed in the TANK1, TANK2, and PARP-3 examples, some of the functions of PARP family proteins are considerably different among species, even between humans and mice. Therefore we also have to be careful in applying the results of animal models to human carcinogenesis.

Establishing strategies for prevention and complete cure of cancer may be difficult in the near future. Therefore, development of new strategies for delaying the onset of cancer development and delaying the progression of cancer might be also important.

Acknowledgements

We appreciate the suggestions provided by Yoshitaka Hippo, Hitoshi Nakagama, and Takashi Sugimura. This work was supported in part by the Third Term Comprehensive 10-Year Strategy for Cancer Control (10103833) from the Ministry of Health, Labor and Welfare of Japan.

References

Aguiar, R.C., Takeyama, K., He, C., Kreinbrink, K., Shipp, M.A., 2005. B-aggressive lymphoma family proteins have unique domains that modulate transcription and exhibit poly(ADP-ribose) polymerase activity. J. Biol. Chem. 280 (40), 33756–33765.

Aguiar, R.C., Yakushijin, Y., Kharbanda, S., Salgia, R., Fletcher, J.A., Shipp, M.A., 2000. BAL is a novel risk-related gene in diffuse large B-cell lymphomas that enhances cellular migration. Blood 96 (13), 4328–4334.

Ahel, D., Horejsi, Z., Wiechens, N., Polo, S.E., Garcia-Wilson, E., Ahel, I., Flynn, H., Skehel, M., West, S.C., Jackson, S.P., Owen-Hughes, T., Boulton, S.J., 2009. Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. Science 325 (5945), 1240–1243.

Ahel, I., Ahel, D., Matsusaka, T., Clark, A.J., Pines, J., Boulton, S.J., West, S.C., 2008. Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins. Nature 451 (7174), 81–85.

Audeh, M.W., Carmichael, J., Penson, R.T., Friedlander, M., Powell, B., Bell-McGuinn, K.M., Scott, C., Weitzel, J.N., Oaknin, A., Loman, N., Lu, K., Schmutzler, R.K., Matulonis, U., Wickens, M., Tutt, A., 2010. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet 376 (9737), 245–251.

Augustin, A., Spenlehauer, C., Dumond, H., Menissier-De Murcia, J., Piel, M., Schmit, A.C., Apiou, F., Vonesch, J.L., Kock, M., Bornens, M., De Murcia, G., 2003. PARP-3 localizes preferentially to the daughter centriole and interferes with the G1/S cell cycle progression. J. Cell Sci. 116 (Pt 8), 1551–1562.

Bajrami, I., Kigozi, A., Van Weverwijk, A., Brough, R., Frankum, J., Lord, C.J., Ashworth, A., 2012. Synthetic lethality of PARP and NAMPT inhibition in triple-negative breast cancer cells. EMBO Mol. Med. 4 (10), 1087–1096.

Barbarulo, A., Iansante, V., Chaidos, A., Naresh, K., Rahemtulla, A., Franzoso, G., Karadimitris, A., Haskard, D.O., Papa, S., Bubici, C., 2012. Poly(ADP-ribose) polymerase family member 14 (PARP14) is a novel effector of the JNK2-dependent pro-survival signal in multiple myeloma. Oncogene. http://doi.org.dx//10.1038/onc.2012.448. [Epub. ahead of print], in press.

Bartleman, A.P., Jacobs, R., Kirkland, J.B., 2008. Niacin supplementation decreases the incidence of alkylation-induced nonlymphocytic leukemia in Long-Evans rats. Nutr. Cancer 60 (2), 251–258.
Benavente, C.A., Jacobson, M.K., Jacobson, E.L., 2009. NAD in skin: therapeutic approaches for niacin. Curr. Pharm. Des. 15 (1), 29–38.
Boehler, C., Gauthier, L.R., Mortusewicz, O., Biard, D.S., Saliou, J.M., Bresson, A., Sanglier-Cianferani, S., Smith, S., Schreiber, V., Boussin, F., Dantzer, F., 2011. Poly(ADP-ribose) polymerase 3 (PARP3), a newcomer in cellular response to DNA damage and mitotic progression. Proc. Natl. Acad. Sci. USA 108 (7), 2783–2788.
Boyonoski, A.C., Spronck, J.C., Gallacher, L.M., Jacobs, R.M., Shah, G.M., Poirier, G.G., Kirkland, J.B., 2002. Niacin deficiency decreases bone marrow poly(ADP-ribose) and the latency of ethylnitrosourea-induced carcinogenesis in rats. J. Nutr. 132 (1), 108–114.
Brandl, M., Seidler, B., Haller, F., Adamski, J., Schmid, R.M., Saur, D., Schneider, G., 2010. IKK(alpha) controls canonical TGF(ss)-SMAD signaling to regulate genes expressing SNAIL and SLUG during EMT in panc1 cells. J. Cell Sci. 123 (Pt 24), 4231–4239.
Bryant, H.E., Petermann, E., Schultz, N., Jemth, A.S., Loseva, O., Issaeva, N., Johansson, F., Fernandez, S., McGlynn, P., Helleday, T., 2009. PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination. EMBO J. 28 (17), 2601–2615.
Bryant, H.E., Schultz, N., Thomas, H.D., Parker, K.M., Flower, D., Lopez, E., Kyle, S., Meuth, M., Curtin, N.J., Helleday, T., 2005. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434 (7035), 913–917.
Byers, L.A., Wang, J., Nilsson, M.B., Fujimoto, J., Saintigny, P., Yordy, J., Giri, U., Peyton, M., Fan, Y.H., Diao, L., Masrorpour, F., Shen, L., Liu, W., Duchemann, B., Tumula, P., Bhardwaj, V., Welsh, J., Weber, S., Glisson, B.S., Kalhor, N., Wistuba, I.I., Girard, L., Lippman, S.M., Mills, G.B., Coombes, K.R., Weinstein, J.N., Minna, J.D., Heymach, J.V., 2012. Proteomic profiling identifies dysregulated pathways in small cell lung cancer and novel therapeutic targets including PARP1. Cancer Discov. 2 (9), 798–811.
Cahan, P., Graubert, T.A., 2010. Integrated genomics of susceptibility to alkylator-induced leukemia in mice. BMC Genomics 11, 638.
Caldini, R., Fanti, E., Magnelli, L., Barletta, E., Tanganelli, E., Zampieri, M., Chevanne, M., 2011. Low doses of 3-aminobenzamide, a poly(ADP-ribose) polymerase inhibitor, stimulate angiogenesis by regulating expression of urokinase type plasminogen activator and matrix metalloprotease 2. Vasc. Cell 3 (1), 12.
Cao, W.H., Wang, X., Frappart, L., Rigal, D., Wang, Z.Q., Shen, Y., Tong, W.M., 2007. Analysis of genetic variants of the poly(ADP-ribose) polymerase-1 gene in breast cancer in French patients. Mutat. Res. 632 (1–2), 20–28.
Chan, K.K., Zhang, Q.M., Dianov, G.L., 2006. Base excision repair fidelity in normal and cancer cells. Mutagenesis 21 (3), 173–178.
Cheng, Q., Barboule, N., Frit, P., Gomez, D., Bombarde, O., Couderc, B., Ren, G.S., Salles, B., Calsou, P., 2011. Ku counteracts mobilization of PARP1 and MRN in chromatin damaged with DNA double-strand breaks. Nucleic Acids Res. 39 (22), 9605–9619.
Chiang, Y.J., Hsiao, S.J., Yver, D., Cushman, S.W., Tessarollo, L., Smith, S., Hodes, R.J., 2008. Tankyrase 1 and tankyrase 2 are essential but redundant for mouse embryonic development. PLoS One 3 (7), e2639.
Cho, S.H., Ahn, A.K., Bhargava, P., Lee, C.H., Eischen, C.M., McGuinness, O., Boothby, M., 2011. Glycolytic rate and lymphomagenesis depend on PARP14, an ADP ribosyltransferase of the B aggressive lymphoma (BAL) family. Proc. Natl. Acad. Sci. USA 108 (38), 15972–15977.
Chou, D.M., Adamson, B., Dephoure, N.E., Tan, X., Nottke, A.C., Hurov, K.E., Gygi, S.P., Colaiacovo, M.P., Elledge, S.J., 2010. A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. Proc. Natl. Acad. Sci. USA 107 (43), 18475–18480.
Cohen-Armon, M., Visochek, L., Rozensal, D., Kalal, A., Geistrikh, I., Klein, R., Bendetz-Nezer, S., Yao, Z., Seger, R., 2007. DNA-independent PARP-1 activation by phosphorylated ERK2 increases Elk1 activity: a link to histone acetylation. Mol. Cell. 25 (2), 297–308.
Conde, C., Mark, M., Oliver, F.J., Huber, A., de Murcia, G., Menissier-de Murcia, J., 2001. Loss of poly(ADP-ribose) polymerase-1 causes increased tumour latency in p53-deficient mice. EMBO J. 20 (13), 3535–3543.
Dantzer, F., Schreiber, V., Niedergang, C., Trucco, C., Flatter, E., De La Rubia, G., Oliver, J., Rolli, V., Menissier-de Murcia, J., de Murcia, G., 1999. Involvement of poly(ADP-ribose) polymerase in base excision repair. Biochimie 81 (1–2), 69–75.
Davalos, A.R., Coppe, J.P., Campisi, J., Desprez, P.Y., 2010. Senescent cells as a source of inflammatory factors for tumor progression. Cancer Metastasis Rev. 29 (2), 273–283.
Ding, W., Liu, W., Cooper, K.L., Qin, X.J., de Souza Bergo, P.L., Hudson, L.G., Liu, K.J., 2009. Inhibition of poly(ADP-ribose) polymerase-1 by arsenite interferes with repair of oxidative DNA damage. J. Biol. Chem. 284 (11), 6809–6817.
Doege, C.A., Inoue, K., Yamashita, T., Rhee, D.B., Travis, S., Fujita, R., Guarnieri, P., Bhagat, G., Vanti, W.B., Shih, A., Levine, R.L., Nik, S., Chen, E.I., Abeliovich, A., 2012. Early-stage epigenetic modification during somatic cell reprogramming by Parp1 and Tet2. Nature 488 (7413), 652–655.
Dutta, C., Day, T., Kopp, N., van Bodegom, D., Davids, M.S., Ryan, J., Bird, L., Kommajosyula, N., Weigert, O., Yoda, A., Fung, H., Brown, J.R., Shapiro, G.I., Letai, A., Weinstock, D.M., 2012. BCL2 suppresses PARP1 function and nonapoptotic cell death. Cancer Res. 72 (16), 4193–4203.
Dynek, J.N., Smith, S., 2004. Resolution of sister telomere association is required for progression through mitosis. Science 304 (5667), 97–100.
El-Khamisy, S.F., Masutani, M., Suzuki, H., Caldecott, K.W., 2003. A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. Nucleic Acids Res. 31 (19), 5526–5533.
Farmer, H., McCabe, N., Lord, C.J., Tutt, A.N., Johnson, D.A., Richardson, T.B., Santarosa, M., Dillon, K.J., Hickson, I., Knights, C., Martin, N.M., Jackson, S.P., Smith, G.C., Ashworth, A., 2005. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434 (7035), 917–921.
Fong, P.C., Boss, D.S., Yap, T.A., Tutt, A., Wu, P., Mergui-Roelvink, M., Mortimer, P., Swaisland, H., Lau, A., O'Connor, M.J., Ashworth, A., Carmichael, J., Kaye, S.B., Schellens, J.H., de Bono, J.S., 2009. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N. Engl. J. Med. 361 (2), 123–134.
Fujimori, H., Shikanai, M., Teraoka, H., Masutani, M., Yoshioka, K., 2012. Induction of cancerous stem cells during embryonic stem cell differentiation. J. Biol. Chem. 287 (44), 36777–36791.
Galbis-Martinez, M., Saenz, L., Ramirez, P., Parrilla, P., Yelamos, J., 2010. Poly(ADP-ribose) polymerase-1 modulates interferon-gamma-inducible protein (IP)-10 expression in murine embryonic fibroblasts by stabilizing IP-10 mRNA. Mol. Immunol. 47 (7–8), 1492–1499.
Gottschalk, A.J., Timinszky, G., Kong, S.E., Jin, J., Cai, Y., Swanson, S.K., Washburn, M.P., Florens, L., Ladurner, A.G., Conaway, J.W., Conaway, R.C., 2009. Poly(ADP-ribosyl)ation directs recruitment and activation of an ATP-dependent chromatin remodeler. Proc. Natl. Acad. Sci. USA 106 (33), 13770–13774.
Guastafierro, T., Cecchinelli, B., Zampieri, M., Reale, A., Riggio, G., Sthandier, O., Zupi, G., Calabrese, L., Caiafa, P., 2008. CCCTC-binding factor activates PARP-1 affecting DNA methylation machinery. J. Biol. Chem. 283 (32), 21873–21880.
Gunji, A., Uemura, A., Tsutsumi, M., Nozaki, T., Kusuoka, O., Omura, K., Suzuki, H., Nakagama, H., Sugimura, T., Masutani, M., 2006. Parp-1 deficiency does not increase the frequency of tumors in the oral cavity and esophagus of ICR/129Sv mice by 4-nitroquinoline 1-oxide, a carcinogen producing bulky adducts. Cancer Lett. 241 (1), 87–92.
Hans, C.P., Feng, Y., Naura, A.S., Troxclair, D., Zerfaoui, M., Siddiqui, D., Jihang, J., Kim, H., Kaye, A.D., Matrougui, K., Lazartigues, E., Boulares, A.H., 2011. Opposing roles of PARP-1 in MMP-9 and TIMP-2 expression and mast cell degranulation in dyslipidemic dilated cardiomyopathy. Cardiovasc. Pathol. 20 (2), e57–68.
Hao, B., Wang, H., Zhou, K., Li, Y., Chen, X., Zhou, G., Zhu, Y., Miao, X., Tan, W., Wei, Q., Lin, D., He, F., 2004. Identification of genetic variants in base excision repair pathway and their associations with risk of esophageal squamous cell carcinoma. Cancer Res. 64 (12), 4378–4384.
Hassa, P.O., Haenni, S.S., Buerki, C., Meier, N.I., Lane, W.S., Owen, H., Gersbach, M., Imhof, R., Hottiger, M.O., 2005. Acetylation of poly(ADP-ribose) polymerase-1 by p300/CREB-binding protein regulates coactivation of NF-kappaB-dependent transcription. J. Biol. Chem. 280 (49), 40450–40464.
Hassumi-Fukasawa, M.K., Miranda-Camargo, F.A., Zanetti, B.R., Galano, D.F., Ribeiro-Silva, A., Soares, E.G., 2012. Expression of BAG-1 and PARP-1 in precursor lesions and invasive cervical cancer associated with human papillomavirus (HPV). Pathol. Oncol. Res. 18 (4), 929–937.
Heldin, C.H., Vanlandewijck, M., Moustakas, A., 2012. Regulation of EMT by TGFbeta in cancer. FEBS Lett. 586 (14), 1959–1970.
Hochegger, H., Dejsuphong, D., Fukushima, T., Morrison, C., Sonoda, E., Schreiber, V., Zhao, G.Y., Saberi, A., Masutani, M., Adachi, N., Koyama, H., de Murcia, G., Takeda, S., 2006. Parp-1 protects homologous recombination from interference by Ku and Ligase IV in vertebrate cells. EMBO J. 25 (6), 1305–1314.

Huang, D., Wang, Y., Wang, L., Zhang, F., Deng, S., Wang, R., Zhang, Y., Huang, K., 2011. Poly(ADP-ribose) polymerase 1 is indispensable for transforming growth factor-beta Induced Smad3 activation in vascular smooth muscle cell. PLoS One 6 (10), e27123.
Hwang, J.W., Chung, S., Sundar, I.K., Yao, H., Arunachalam, G., McBurney, M.W., Rahman, I., 2010. Cigarette smoke-induced autophagy is regulated by SIRT1-PARP-1-dependent mechanism: implication in pathogenesis of COPD. Arch. Biochem. Biophys. 500 (2), 203–209.
Idogawa, M., Masutani, M., Shitashige, M., Honda, K., Tokino, T., Shinomura, Y., Imai, K., Hirohashi, S., Yamada, T., 2007. Ku70 and poly(ADP-ribose) polymerase-1 competitively regulate beta-catenin and T-cell factor-4-mediated gene transactivation: possible linkage of DNA damage recognition and Wnt signaling. Cancer Res. 67 (3), 911–918.
Inbar, D., Cohen-Armon, M., Neumann, D., 2012. Erythropoietin-driven signalling and cell migration mediated by polyADP-ribosylation. Br. J. Cancer 107 (8), 1317–1326.
Jones, D.T., Gronych, J., Lichter, P., Witt, O., Pfister, S.M., 2012. MAPK pathway activation in pilocytic astrocytoma. Cell. Mol. Life Sci. 69 (11), 1799–1811.
Kanai, M., Tong, W.M., Sugihara, E., Wang, Z.Q., Fukasawa, K., Miwa, M., 2003. Involvement of poly(ADP-Ribose) polymerase 1 and poly(ADP-Ribosyl)ation in regulation of centrosome function. Mol. Cell. Biol. 23 (7), 2451–2462.
Kanai, Y., Akatsu, H., Iizuka, H., Morimoto, C., 2007. Could serum antibody to poly(ADP-ribose) and/or histone H1 be marker for senile dementia of Alzheimer type? Ann. NY Acad. Sci. 1109, 338–344.
Kashima, L., Idogawa, M., Mita, H., Shitashige, M., Yamada, T., Ogi, K., Suzuki, H., Toyota, M., Ariga, H., Sasaki, Y., Tokino, T., 2012. CHFR protein regulates mitotic checkpoint by targeting PARP-1 protein for ubiquitination and degradation. J. Biol. Chem. 287 (16), 12975–12984.
Kato, J., Zhu, J., Liu, C., Stylianou, M., Hoffmann, V., Lizak, M.J., Glasgow, C.G., Moss, J., 2011. ADP-ribosylarginine hydrolase regulates cell proliferation and tumorigenesis. Cancer Res. 71 (15), 5327–5335.
Khodyreva, S.N., Prasad, R., Ilina, E.S., Sukhanova, M.V., Kutuzov, M.M., Liu, Y., Hou, E.W., Wilson, S.H., Lavrik, O.I., 2010. Apurinic/apyrimidinic (AP) site recognition by the 5'-dRP/AP lyase in poly(ADP-ribose) polymerase-1 (PARP-1). Proc. Natl. Acad. Sci. USA 107 (51), 22090–22095.
Kleine, H., Herrmann, A., Lamark, T., Forst, A.H., Verheugd, P., Luscher-Firzlaff, J., Lippok, B., Feijs, K.L., Herzog, N., Kremmer, E., Johansen, T., Muller-Newen, G., Luscher, B., 2012. Dynamic subcellular localization of the mono-ADP-ribosyltransferase ARTD10 and interaction with the ubiquitin receptor p62. Cell Commun. Signal 10 (1), 28.
Kleine, H., Poreba, E., Lesniewicz, K., Hassa, P.O., Hottiger, M.O., Litchfield, D.W., Shilton, B.H., Luscher, B., 2008. Substrate-assisted catalysis by PARP10 limits its activity to mono-ADP-ribosylation. Mol. Cell. 32 (1), 57–69.
Kovacs, K., Erdelyi, K., Hegedus, C., Lakatos, P., Regdon, Z., Bai, P., Hasko, G., Szabo, E., Virag, L., 2012. Poly(ADP-ribosyl)ation is a survival mechanism in cigarette smoke-induced and hydrogen peroxide-mediated cell death. Free Radic. Biol. Med. 53 (9), 1680–1688.
Krishnakumar, R., Kraus, W.L., 2010. PARP-1 regulates chromatin structure and transcription through a KDM5B-dependent pathway. Mol. Cell. 39 (5), 736–749.
Lacal, P.M., Tentori, L., Muzi, A., Ruffini, F., Dorio, A.S., Xu, W., Arcelli, D., Zhang, J., Graziani, G., 2009. Pharmacological inhibition of poly(ADP-ribose) polymerase activity down-regulates the expression of syndecan-4 and Id-1 in endothelial cells. Int. J. Oncol. 34 (3), 861–872.
Lakadong, R.O., Kataki, A.C., Sharan, R.N., 2010. ADP-ribose polymer – a novel and general biomarker of human cancers of head & neck, breast, and cervix. Mol. Cancer 9, 286.
Leung, A.K., Vyas, S., Rood, J.E., Bhutkar, A., Sharp, P.A., Chang, P., 2011. Poly(ADP-ribose) regulates stress responses and microRNA activity in the cytoplasm. Mol. Cell. 42 (4), 489–499.
Li, M., Threadgill, M.D., Wang, Y., Cai, L., Lin, X., 2009. Poly(ADP-ribose) polymerase inhibition down-regulates expression of metastasis-related genes in CT26 colon carcinoma cells. Pathobiology 76 (3), 108–116.
Li, Q., Li, M., Wang, Y.L., Fauzee, N.J., Yang, Y., Pan, J., Yang, L., Lazar, A., 2012. RNA interference of PARG could inhibit the metastatic potency of colon carcinoma cells via PI3-kinase/Akt pathway. Cell. Physiol. Biochem. 29 (3–4), 361–372.
Liu, Y., Scheurer, M.E., El-Zein, R., Cao, Y., Do, K.A., Gilbert, M., Aldape, K.D., Wei, Q., Etzel, C., Bondy, M.L., 2009. Association and interactions between DNA repair gene polymorphisms and adult glioma. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 18 (1), 204–214.
Lockett, K.L., Hall, M.C., Xu, J., Zheng, S.L., Berwick, M., Chuang, S.C., Clark, P.E., Cramer, S.D., Lohman, K., Hu, J.J., 2004. The ADPRT V762A genetic variant contributes to prostate cancer susceptibility and deficient enzyme function. Cancer Res. 64 (17), 6344–6348.
Lonn, P., van der Heide, L.P., Dahl, M., Hellman, U., Heldin, C.H., Moustakas, A., 2010. PARP-1 attenuates Smad-mediated transcription. Mol. Cell. 40 (4), 521–532.
Lu, H., Wang, X., Li, T., Urvalek, A.M., Yu, L., Li, J., Zhu, J., Lin, Q., Peng, X., Zhao, J., 2011. Identification of poly (ADP-ribose) polymerase-1 (PARP-1) as a novel Kruppel-like factor 8-interacting and -regulating protein. J. Biol. Chem. 286 (23), 20335–20344.
Mansour, W.Y., Rhein, T., Dahm-Daphi, J., 2010. The alternative end-joining pathway for repair of DNA double-strand breaks requires PARP1 but is not dependent upon microhomologies. Nucleic Acids Res. 38 (18), 6065–6077.
Martin-Oliva, D., Aguilar-Quesada, R., O'Valle, F., Munoz-Gamez, J.A., Martinez-Romero, R., Garcia Del Moral, R., Ruiz de Almodovar, J.M., Villuendas, R., Piris, M.A., Oliver, F.J., 2006. Inhibition of poly(ADP-ribose) polymerase modulates tumor-related gene expression, including hypoxia-inducible factor-1 activation, during skin carcinogenesis. Cancer Res. 66 (11), 5744–5756.
Martinez-Zamudio, R., Ha, H.C., 2012. Histone ADP-ribosylation facilitates gene transcription by directly remodeling nucleosomes. Mol. Cell. Biol. 32 (13), 2490–2502.
Mascolo, M., Ilardi, G., Romano, M.F., Celetti, A., Siano, M., Romano, S., Luise, C., Merolla, F., Rocco, A., Vecchione, M.L., De Rosa, G., Staibano, S., 2012. Overexpression of chromatin assembly factor-1 p60, poly(ADP-ribose) polymerase 1 and nestin predicts metastasizing behaviour of oral cancer. Histopathology. 61 (6), 1089–1105.
Masson, M., Niedergang, C., Schreiber, V., Muller, S., Menissier-de Murcia, J., de Murcia, G., 1998. XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. Mol. Cell. Biol. 18 (6), 3563–3571.
McPhee, T.R., McDonald, P.C., Oloumi, A., Dedhar, S., 2008. Integrin-linked kinase regulates E-cadherin expression through PARP-1. Dev. Dyn. 237 (10), 2737–2747.
Mehrotra, P., Riley, J.P., Patel, R., Li, F., Voss, L., Goenka, S., 2011. PARP-14 functions as a transcriptional switch for Stat6-dependent gene activation. J. Biol. Chem. 286 (3), 1767–1776.
Mendes-Pereira, A.M., Martin, S.A., Brough, R., McCarthy, A., Taylor, J.R., Kim, J.S., Waldman, T., Lord, C.J., Ashworth, A., 2009. Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. EMBO Mol. Med. 1 (6–7), 315–322.
Min, W., Cortes, U., Herceg, Z., Tong, W.M., Wang, Z.Q., 2010. Deletion of the nuclear isoform of poly(ADP-ribose) glycohydrolase (PARG) reveals its function in DNA repair, genomic stability and tumorigenesis. Carcinogenesis 31 (12), 2058–2065.
Molloy-Simard, V., St-Laurent, J.F., Vigneault, F., Gaudreault, M., Dargis, N., Guerin, M.C., Leclerc, S., Morcos, M., Black, D., Molgat, Y., Bergeron, D., de Launoit, Y., Boudreau, F., Desnoyers, S., Guerin, S., 2012. Altered Expression of the Poly(ADP-Ribosyl)ation Enzymes in Uveal Melanoma and Regulation of PARG Gene Expression by the Transcription Factor ERM. Invest. Ophthalmol. Vis. Sci. 53 (10), 6219–6231.
Morrison, C., Smith, G.C., Stingl, L., Jackson, S.P., Wagner, E.F., Wang, Z.Q., 1997. Genetic interaction between PARP and DNA-PK in V(D)J recombination and tumorigenesis. Nat. Genet. 17 (4), 479–482.
Mukhopadhyay, P., Horvath, B., Kechrid, M., Tanchian, G., Rajesh, M., Naura, A.S., Boulares, A.H., Pacher, P., 2011. Poly(ADP-ribose) polymerase-1 is a key mediator of cisplatin-induced kidney inflammation and injury. Free Radic. Biol. Med. 51 (9), 1774–1788.
Munoz-Gamez, J.A., Rodriguez-Vargas, J.M., Quiles-Perez, R., Aguilar-Quesada, R., Martin-Oliva, D., de Murcia, G., Menissier de Murcia, J., Almendros, A., Ruiz de Almodovar, M., Oliver, F.J., 2009. PARP-1 is involved in autophagy induced by DNA damage. Autophagy 5 (1), 61–74.

Nakayama, R., Sato, Y., Masutani, M., Ogino, H., Nakatani, F., Chuman, H., Beppu, Y., Morioka, H., Yabe, H., Hirose, H., Sugimura, H., Sakamoto, H., Ohta, T., Toyama, Y., Yoshida, T., Kawai, A., 008. Association of a missense single nucleotide polymorphism, Cys1367Arg of the WRN gene, with the risk of bone and soft tissue sarcomas in Japan. Cancer Sci 99 (2), 333–339.
Nicolas, L., Martinez, C., Baro, C., Rodriguez, M., Baroja-Mazo, A., Sole, F., Flores, J.M., Ampurdanes, C., Dantzer, F., Martin-Caballero, J., Aparicio, P., Yelamos, J., 2010. Loss of poly(ADP-ribose) polymerase-2 leads to rapid development of spontaneous T-cell lymphomas in p53-deficient mice. Oncogene 29 (19), 2877–2883.
Nieto, M.A., 2002. The snail superfamily of zinc-finger transcription factors. Nat. Rev. Mol. Cell Biol. 3 (3), 155–166.
Nosho, K., Yamamoto, H., Mikami, M., Taniguchi, H., Takahashi, T., Adachi, Y., Imamura, A., Imai, K., Shinomura, Y., 2006. Overexpression of poly(ADP-ribose) polymerase-1 (PARP-1) in the early stage of colorectal carcinogenesis. Eur. J. Cancer 42 (14), 2374–2381.
Nossa, C.W., Jain, P., Tamilselvam, B., Gupta, V.R., Chen, L.F., Schreiber, V., Desnoyers, S., Blanke, S.R., 2009. Activation of the abundant nuclear factor poly(ADP-ribose) polymerase-1 by Helicobacter pylori. Proc. Natl. Acad. Sci. USA 106 (47), 19998–20003.
Nozaki, T., Fujihara, H., Watanabe, M., Tsutsumi, M., Nakamoto, K., Kusuoka, O., Kamada, N., Suzuki, H., Nakagama, H., Sugimura, T., Masutani, M., 2003. Parp-1 deficiency implicated in colon and liver tumorigenesis induced by azoxymethane. Cancer Sci. 94 (6), 497–500.
Nozaki, T., Masutani, M., Watanabe, M., Ochiya, T., Hasegawa, F., Nakagama, H., Suzuki, H., Sugimura, T., 1999. Syncytiotrophoblastic giant cells in teratocarcinoma-like tumors derived from Parp-disrupted mouse embryonic stem cells. Proc. Natl. Acad. Sci. USA 96 (23), 13345–13350.
Ogawa, K., Masutani, M., Kato, K., Tang, M., Kamada, N., Suzuki, H., Nakagama, H., Sugimura, T., Shirai, T., 2006. Parp-1 deficiency does not enhance liver carcinogenesis induced by 2-amino-3-methylimidazo[4,5-f]quinoline in mice. Cancer Lett. 236 (1), 32–38.
Ogino, H., Nozaki, T., Gunji, A., Maeda, M., Suzuki, H., Ohta, T., Murakami, Y., Nakagama, H., Sugimura, T., Masutani, M., 2007. Loss of Parp-1 affects gene expression profile in a genome-wide manner in ES cells and liver cells. BMC Genomics 8, 41.
Pan, J., Fauzee, N.J., Wang, Y.L., Sheng, Y.T., Tang, Y., Wang, J.Q., Wu, W.Q., Yan, J.X., Xu, J., 2012. Effect of silencing PARG in human colon carcinoma LoVo cells on the ability of HUVEC migration and proliferation. Cancer Gene Ther. 19 (10), 715–722.
Pavri, R., Lewis, B., Kim, T.K., Dilworth, F.J., Erdjument-Bromage, H., Tempst, P., de Murcia, G., Evans, R., Chambon, P., Reinberg, D., 2005. PARP-1 determines specificity in a retinoid signaling pathway via direct modulation of mediator. Mol. Cell. 18 (1), 83–96.
Piao, L., Nakagawa, H., Ueda, K., Chung, S., Kashiwaya, K., Eguchi, H., Ohigashi, H., Ishikawa, O., Daigo, Y., Matsuda, K., Nakamura, Y., 2011. C12orf48, termed PARP-1 binding protein, enhances poly(ADP-ribose) polymerase-1 (PARP-1) activity and protects pancreatic cancer cells from DNA damage. Genes Chromosomes Cancer 50 (1), 13–24.
Piskunova, T.S., Yurova, M.N., Ovsyannikov, A.I., Semenchenko, A.V., Zabezhinski, M.A., Popovich, I.G., Wang, Z.Q., Anisimov, V.N., 2008. Deficiency in poly(ADP-ribose) polymerase-1 (PARP-1) accelerates aging and spontaneous carcinogenesis in mice. Curr. Gerontol. Geriatr. Res. 754190.
Piskunova, T.S., Zabezhinskii, M.A., Popovich, I.G., Tyndyk, M.L., Iurova, M.N., Anisimov, V.N., 2009. Diethylnitrosamine-induced carcinogenesis in PARP-1(−/−) and PARP-1(+/+) mice. Vopr. Onkol. 55 (5), 608–611.
Postawski, K., Monist, M., Keith, G., 2011. PARP-1 activity in normal and cancerous human endometrium and its relationship with quantity of abasic sites (AP). Ginekol. Pol. 82 (1), 16–21.
Prasad, S., Ravindran, J., Aggarwal, B.B., 2010. NF-kappaB and cancer: how intimate is this relationship. Mol. Cell. Biochem. 336 (1–2), 25–37.
Pyriochou, A., Olah, G., Deitch, E.A., Szabo, C., Papapetropoulos, A., 2008. Inhibition of angiogenesis by the poly(ADP-ribose) polymerase inhibitor PJ-34. Int. J. Mol. Med. 22 (1), 113–118.
Rajesh, M., Mukhopadhyay, P., Godlewski, G., Batkai, S., Hasko, G., Liaudet, L., Pacher, P., 2006. Poly(ADP-ribose)polymerase inhibition decreases angiogenesis. Biochem. Biophys. Res. Commun. 350 (4), 1056–1062.
Raval-Fernandes, S., Kickhoefer, V.A., Kitchen, C., Rome, L.H., 2005. Increased susceptibility of vault poly(ADP-ribose) polymerase-deficient mice to carcinogen-induced tumorigenesis. Cancer Res. 65 (19), 8846–8852.
Reale, A., Matteis, G.D., Galleazzi, G., Zampieri, M., Caiafa, P., 2005. Modulation of DNMT1 activity by ADP-ribose polymers. Oncogene 24 (1), 13–19.
Robert, I., Dantzer, F., Reina-San-Martin, B., 2009. Parp1 facilitates alternative NHEJ, whereas Parp2 suppresses IgH/c-myc translocations during immunoglobulin class switch recombination. J. Exp. Med. 206 (5), 1047–1056.
Rodriguez-Vargas, J.M., Ruiz-Magana, M.J., Ruiz-Ruiz, C., Majuelos-Melguizo, J., Peralta-Leal, A., Rodriguez, M.I., Munoz-Gamez, J.A., de Almodovar, M.R., Siles, E., Rivas, A.L., Jaattela, M., Oliver, F.J., 2012. ROS-induced DNA damage and PARP-1 are required for optimal induction of starvation-induced autophagy. Cell Res. 22 (7), 1181–1198.
Rouleau, M., McDonald, D., Gagne, P., Ouellet, M.E., Droit, A., Hunter, J.M., Dutertre, S., Prigent, C., Hendzel, M.J., Poirier, G.G., 2007. PARP-3 associates with polycomb group bodies and with components of the DNA damage repair machinery. J. Cell. Biochem. 100 (2), 385–401.
Rulten, S.L., Fisher, A.E., Robert, I., Zuma, M.C., Rouleau, M., Ju, L., Poirier, G., Reina-San-Martin, B., Caldecott, K.W., 2011. PARP-3 and APLF function together to accelerate nonhomologous end-joining. Mol. Cell. 41 (1), 33–45.
Schiewer, M.J., Goodwin, J.F., Han, S., Brenner, J.C., Augello, M.A., Dean, J.L., Liu, F., Planck, J.L., Ravindranathan, P., Chinnaiyan, A.M., McCue, P., Gomella, L.G., Raj, G.V., Dicker, A.P., Brody, J.R., Pascal, J.M., Centenera, M.M., Butler, L.M., Tilley, W.D., Feng, F.Y., Knudsen, K.E., 2012. Dual roles of PARP-1 promote cancer growth and progression. Cancer Discov. 2 (12), 1134–1149.
Sakiyama, T., Kohno, T., Mimaki, S., Ohta, T., Yanagitani, N., Sobue, T., Kunitoh, H., Saito, R., Shimizu, K., Hirama, C., Kimura, J., Maeno, G., Hirose, H., Eguchi, T., Saito, D., Ohki, M., Yokota, J., 2005. Association of amino acid substitution polymorphisms in DNA repair genes TP53, POLI, REV1 and LIG4 with lung cancer risk. International journal of cancer. Journal international du cancer 114 (5), 730–737.
Schreiber, V., Ame, J.C., Dolle, P., Schultz, I., Rinaldi, B., Fraulob, V., Menissier-de Murcia, J., de Murcia, G., 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 (25), 23028–23036.
Shibata, A., Maeda, D., Ogino, H., Tsutsumi, M., Nohmi, T., Nakagama, H., Sugimura, T., Teraoka, H., Masutani, M., 2009. Role of Parp-1 in suppressing spontaneous deletion mutation in the liver and brain of mice at adolescence and advanced age. Mutat. Res. 664 (1–2), 20–27.
Shimo, T., Kurebayashi, J., Kanomata, N., Yamashita, T., Kozuka, Y., Moriya, T., Sonoo, H., 2012. Antitumor and anticancer stem cell activity of a poly ADP-ribose polymerase inhibitor olaparib in breast cancer cells. Breast Cancer, [epub ahead of print].
Silva-Fernandes, I.J., da Silva, T.A., Agnez-Lima, L.F., Ferreira, M.V., Rabenhorst, S.H., 2012. Helicobacter pylori genotype and polymorphisms in DNA repair enzymes: where do they correlate in gastric cancer? J. Surg. Oncol. 106 (4), 448–455.
Stanisavljevic, J., Porta-de-la-Riva, M., Batlle, R., de Herreros, A.G., Baulida, J., 2011. The p65 subunit of NF-kappaB and PARP1 assist Snail1 in activating fibronectin transcription. J. Cell Sci. 124 (Pt 24), 4161–4171.
Sugimura, T., 1992. Multistep carcinogenesis: a 1992 perspective. Science 258 (5082), 603–607.
Tanori, M., Mancuso, M., Pasquali, E., Leonardi, S., Rebessi, S., Di Majo, V., Guilly, M.N., Giangaspero, F., Covelli, V., Pazzaglia, S., Saran, A., 2008. PARP-1 cooperates with Ptc1 to suppress medulloblastoma and basal cell carcinoma. Carcinogenesis 29 (10), 1911–1919.
Tashtoush, B.M., Kim, H., Jacobson, E.L., Jacobson, M.K., 2007. Formulation compatibility of myristyl nicotinate with drugs used to treat dermatological conditions involving an impaired skin barrier. Drug Dev. Ind. Pharm. 33 (11), 1176–1182.
Tentori, L., Lacal, P.M., Muzi, A., Dorio, A.S., Leonetti, C., Scarsella, M., Ruffini, F., Xu, W., Min, W., Stoppacciaro, A., Colarossi, C., Wang, Z.Q., Zhang, J., Graziani, G., 2007. Poly(ADP-ribose) polymerase (PARP) inhibition or PARP-1 gene deletion reduces angiogenesis. Eur. J. Cancer 43 (14), 2124–2133.
Tentori, L., Muzi, A., Dorio, A.S., Bultrini, S., Mazzon, E., Lacal, P.M., Shah, G.M., Zhang, J., Navarra, P., Nocentini, G., Cuzzocrea, S., Graziani, G., 2008. Stable depletion of poly (ADP-ribose) polymerase-1 reduces in vivo melanoma growth and increases chemosensitivity. Eur. J. Cancer 44 (9), 1302–1314.
Teo, M.T., Landi, D., Taylor, C.F., Elliott, F., Vaslin, L., Cox, D.G., Hall, J., Landi, S., Bishop, D.T., Kiltie, A.E., 2012. The role of microRNA-binding site polymorphisms in DNA repair genes as risk factors for bladder cancer and breast cancer and their impact on radiotherapy outcomes. Carcinogenesis 33 (3), 581–586.

Toller, I.M., Neelsen, K.J., Steger, M., Hartung, M.L., Hottiger, M.O., Stucki, M., Kalali, B., Gerhard, M., Sartori, A.A., Lopes, M., Muller, A., 2011. Carcinogenic bacterial pathogen Helicobacter pylori triggers DNA double-strand breaks and a DNA damage response in its host cells. Proc. Natl. Acad. Sci. USA 108 (36), 14944–14949.

Tomasetti, M., Amati, M., Nocchi, L., Saccucci, F., Strafella, E., Staffolani, S., Tarquini, L.M., Carbonari, D., Alleva, R., Borghi, B., Neuzil, J., Bracci, M., Santarelli, L., 2011. Asbestos exposure affects poly(ADP-ribose) polymerase-1 activity: role in asbestos-induced carcinogenesis. Mutagenesis 26 (5), 585–591.

Tong, W.M., Cortes, U., Hande, M.P., Ohgaki, H., Cavalli, L.R., Lansdorp, P.M., Haddad, B.R., Wang, Z.Q., 2002. Synergistic role of Ku80 and poly(ADP-ribose) polymerase in suppressing chromosomal aberrations and liver cancer formation. Cancer Res. 62 (23), 6990–6996.

Tong, W.M., Hande, M.P., Lansdorp, P.M., 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 (12), 4046–4054.

Tong, W.M., Ohgaki, H., Huang, H., Granier, C., Kleihues, P., 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 (1), 343–352.

Tong, W.M., Yang, Y.G., Cao, W.H., Galendo, D., Frappart, L., Shen, Y., Wang, Z.Q., 2007. Poly(ADP-ribose) polymerase-1 plays a role in suppressing mammary tumourigenesis in mice. Oncogene 26 (26), 3857–3867.

Tsutsumi, M., Masutani, M., Nozaki, T., Kusuoka, O., Tsujiuchi, T., Nakagama, H., Suzuki, H., Konishi, Y., Sugimura, T., 2001. Increased susceptibility of poly(ADP-ribose) polymerase-1 knockout mice to nitrosamine carcinogenicity. Carcinogenesis 22 (1), 1–3.

Tutt, A., Robson, M., Garber, J.E., Domchek, S.M., Audeh, M.W., Weitzel, J.N., Friedlander, M., Arun, B., Loman, N., Schmutzler, R.K., Wardley, A., Mitchell, G., Earl, H., Wickens, M., Carmichael, J., 2010. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet 376 (9737), 235–244.

Wang, M., Qin, C., Zhu, J., Yuan, L., Fu, G., Zhang, Z., Yin, C., 2010. Genetic variants of XRCC1, APE1, and ADPRT genes and risk of bladder cancer. DNA and cell biology 29 (6), 303–311.

Wang, X.G., Wang, Z.Q., Tong, W.M., Shen, Y., 2007. PARP1 Val762Ala polymorphism reduces enzymatic activity. Biochem. Biophys. Res. Commun. 354 (1), 122–126.

Weinstein, B., 2008. Relevance of the concept of oncogene addiction to hormonal carcinogenesis and molecular targeting in cancer prevention and therapy. Adv. Exp. Med. Biol. 617, 3–13.

Wright, R.H., Castellano, G., Bonet, J., Le Dily, F., Font-Mateu, J., Ballare, C., Nacht, A.S., Soronellas, D., Oliva, B., Beato, M., 2012. CDK2-dependent activation of PARP-1 is required for hormonal gene regulation in breast cancer cells. Genes Dev. 26 (17), 1972–1983.

Yamagami, T., Miwa, A., Takasawa, S., Yamamoto, H., 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 (4), 1845–1849.

Yamaguchi, N., Ito, T., Azuma, S., Ito, E., Honma, R., Yanagisawa, Y., Nishikawa, A., Kawamura, M., Imai, J., Watanabe, S., Semba, K., Inoue, J., 2009. Constitutive activation of nuclear factor-kappaB is preferentially involved in the proliferation of basal-like subtype breast cancer cell lines. Cancer Sci. 100 (9), 1668–1674.

Ye, F., Cheng, Q., Hu, Y., Zhang, J., Chen, H., 2012. PARP-1 Val762Ala polymorphism is associated with risk of cervical carcinoma. PLoS One 7 (5), e37446.

Yu, H., Ma, H., Yin, M., Wei, Q., 2012. Association between PARP-1 V762A polymorphism and cancer susceptibility: a meta-analysis. Genet. Epidemiol. 36 (1), 56–65.

Yu, M., Schreek, S., Cerni, C., Schamberger, C., Lesniewicz, K., Poreba, E., Vervoorts, J., Walsemann, G., Grotzinger, J., Kremmer, E., Mehraein, Y., Mertsching, J., Kraft, R., Austen, M., Luscher-Firzlaff, J., Luscher, B., 2005a. PARP-10, a novel Myc-interacting protein with poly(ADP-ribose) polymerase activity, inhibits transformation. Oncogene 24 (12), 1982–1993.

Yu, S.W., Andrabi, S.A., Wang, H., Kim, N.S., Poirier, G.G., Dawson, T.M., Dawson, V.L., 2006. Apoptosis-inducing factor mediates poly(ADP-ribose) (PAR) polymer-induced cell death. Proc. Natl. Acad. Sci. USA 103 (48), 18314–18319.

Yu, X., Minter-Dykhouse, K., Malureanu, L., Zhao, W.M., Zhang, D., Merkle, C.J., Ward, I.M., Saya, H., Fang, G., van Deursen, J., Chen, J., 2005b. Chfr is required for tumor suppression and Aurora A regulation. Nat. Genet. 37 (4), 401–406.

Zerfaoui, M., Errami, Y., Naura, A.S., Suzuki, Y., Kim, H., Ju, J., Liu, T., Hans, C.P., Kim, J.G., Abd Elmageed, Z.Y., Koochekpour, S., Catling, A., Boulares, A.H., 2010. Poly(ADP-ribose) polymerase-1 is a determining factor in Crm1-mediated nuclear export and retention of p65 NF-kappa B upon TLR4 stimulation. J. Immunol. 185 (3), 1894–1902.

Zhang, M., Qureshi, A.A., Guo, Q., Han, J., 2011. Genetic variation in DNA repair pathway genes and melanoma risk. DNA Repair (Amst) 10 (1), 111–116.

Zhang, X., Miao, X., Liang, G., Hao, B., Wang, Y., Tan, W., Li, Y., Guo, Y., He, F., Wei, Q., Lin, D., 2005. Polymorphisms in DNA base excision repair genes ADPRT and XRCC1 and risk of lung cancer. Cancer Res. 65 (3), 722–726.

Biochemical and Biophysical Research Communications 436 (2013) 313–318

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The *H19* induction triggers trophoblast lineage commitment in mouse ES cells

Hiroaki Fujimori [a,b], Hiroaki Mukai [a,c], Yasufumi Murakami [c], Myriam Hemberger [d], Yoshitaka Hippo [e], Mitsuko Masutani [a,b,*]

[a] Division of Genome Stability Research, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
[b] Biochemistry Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
[c] Department of Biological Science and Technology, Faculty of Industrial Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan
[d] Epigenetics Programme, The Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT, UK
[e] Division of Cancer Development System, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

ARTICLE INFO

Article history:
Received 3 May 2013
Available online 4 June 2013

Keywords:
H19
Trophoblast
ES cell
Cdx2
Oct3/4
PARP-1

ABSTRACT

Trophoblast lineage differentiation is properly regulated to support embryogenesis. Besides normal developmental process, during germ cell tumor formation or development of other reproductive system diseases, unregulated trophoblast differentiation is also observed and affects the pathogenesis of the diseases. During normal embryogenesis, cell fate of late-stage blastcyst is regulated by a reciprocal repression of the key transcriptional factors; Oct3/4 dominancy inhibits *Cdx2* expression in inner cell mass (ICM) and leads them to epiblast/primitive ectoderm but Cdx2 dominancy in trophectoderm (TE) leads them to trophoblast lineage. In contrast during early blastcyst stage, the *Cdx2* expression is restricted in TE and not present in ICM, although Oct3/4 signaling does not inhibit the *Cdx2* expression in ICM, implying that some factors could be inactivated leading to the suppressed *Cdx2* expression in ICM of early blastcyst.

ES cells (ESCs), which are derived from ICM, could be a unique model to study trophoblast differentiation in an ectopic context. We previously showed that poly(ADP-ribose) polymerase-1 (*Parp-1*) deficient ESCs highly expressed non-coding RNA *H19* and could differentiate into trophoblast lineage. The expression of *H19* is known to start at pre-blastcyst stage during mouse development, and the gene shows high expression only in trophoectoderm (TE) at blastcyst stage. However, its role in trophoblast differentiation has not been clarified yet. Thus, we hypothesized that the *H19* activation may act as a trigger for induction of trophoblast differentiation cascade in mouse ESCs. To investigate this issue, we asked whether a forced *H19* expression drives ESCs into trophoblast lineage or not. We demonstrated that the *H19* induction leads to trophoblast lineage commitment through induction of the *Cdx2* expression.

We also showed that the expression of *Cdx2* is induced in ESCs by forced *H19* expression even under a high level of Oct3/4, which could act as a suppressor for *Cdx2* expression. It is thus suggested that the *H19* induction promotes trophoblast lineage commitment against the repression pressure by Oct3/4 in differentiating ESCs. Taken together, this study suggests that the *H19* expression is able to function as a cascade activator of trophoblast lineage commitment possibly by overriding the Oct3/4 action in ESCs.

1. Introduction

Trophoblast lineage differentiation is properly regulated during embryogenesis. Besides normal developmental process, during development of germ cell tumors or other reproductive system disorders, unregulated trophoblast differentiation is also observed and malignancy of the tumors and pathogenic properties of the diseases are affected by the presence of trophoblasts [1]. The regulation mechanism of trophoblast differentiation during normal embryonal development and pathogenesis of the diseases, including tumorigenesis has not been fully elucidated.

Abbreviations: ESCs, embryonic stem cells; ICM, inner cell mass; PE, primitive ectoderm; TE, trophectoderm; Parp-1, poly(ADP-ribose) polymerase 1; Plf1, proliferin 1; Dnmt1, DNA methyltransferase 1; Gapdh, glyceraldehyde 3-phosphate dehydrogenase; Tc, tetracycline; Zeo, zeocin; LIF, leukemia inhibitory factor; *Cdx2*, caudal-type homeobox protein 2; Fgf5, fibroblast growth factor 5; Tpbpa, trophoblast specific protein α; Igf2, insulin-like growth factor 2.

* Corresponding author at: Division of Genome Stability Research, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Fax: +81 3 3543 9305.
E-mail address: mmasutan@ncc.go.jp (M. Masutani).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.05.100

Trophoblast differentiation is initiated with *Tead4* and *Klf5* activation in the outer layer of morulae, which specifies them to trophoblast lineage and induces genes for maintenance of trophoectoderm (TE) commitment in blastcyst stage [2]. However, one of the most significant regulations to define the TE and epiblasts has been suggested as a reciprocal expression pattern of *Cdx2* (caudal-type homeobox protein 2) in TE and Oct3/4 in epiblasts, which is established during the period of blastcyst stage. Previous reports demonstrated the repressive interaction between Oct3/4 and Cdx2 [2]. Furthermore, perturbed expression of the two genes induces the activation of TE-related genes in inner cell mass (ICM) cells/ES cells (ESCs) or that of ICM-related genes in TE cells during differentiation [3,4]. Accumulating *in vivo* studies show that; while Oct3/4 signaling does not inhibit *Cdx2* expression in ICM of early blastcyst, *Cdx2* expression is restricted in TE, implying that some factors could be inactivated or absent, that leads to suppression of the *Cdx2* expression in ICM [5].

We previously observed poly (ADP-ribose) polymerase-1 (*Parp-1*) deficient ESCs could differentiate to trophoblast lineage, while normal ESCs could not [6–8]. Mouse ESCs have been established from the ICM of blastocysts [9,10], which starts differentiation during mouse embryogenesis into the epiblast and primitive ectoderm (PE) on embryonic day 4.5; however, generally they do not give rise to trophectoderm (TE) derivatives [11,12]. The placenta of *Parp-1*$^{-/-}$ mouse also shows an increase in trophoblast giant cell number and a decrease in spongiotrophoblast number [8]. Parp-1 is involved in the regulation of transcription [13,14] and chromatin remodeling [15] through poly(ADP-ribosylation) of proteins and interaction with proteins. The absence of Parp-1 alters transcription of particular genes and induces trophoblast differentiation. *Parp-1*$^{-/-}$ ESCs shows early and enhanced expression of extraembryonic/trophoblast differentiation-associated gene, the *H19*, a non-coding RNA gene, and a homeobox transcription factor *Cdx2* gene upon ESC differentiation, and later a trophoblast specific gene, *Plf1* (proliferin 1) [16] is induced in *Parp-1*$^{-/-}$ ESCs. *Dnmt1* (DNA methyltransferase 1) deficient ESCs also differentiate into trophoblast lineage [17], accompanying induction of the *H19* expression [18].

The *H19* gene expression is initially activated at 2-cell stage, but from blastcyst stage, it is highly restricted in TE including primary trophoblasts and ectoplacental cone, although its role in trophoblast differentiation has not been clarified yet [19]. We hypothesized that the *H19* expression may induce trophoblast differentiation cascade and investigated here using ectopic context model of ESCs in this study. Our study showed that the *H19* gene expression initiates trophoblast differentiation commitment in mouse ESCs.

2. Materials and methods

2.1. Cell culture

Wild-type J1 ESC clones and *Parp-1*$^{-/-}$ 210–58 and 226–47 ESC clones were used in this study [6]. These ES clones were cultivated as previously described [16]. Briefly, ES cells were cultured in the Dulbecco's Modified Eagle's Medium (Invitrogen) containing 20% fetal bovine serum (Thermo Fisher Scientific), non-essential amino acids (Invitrogen) and leukemia inhibitory factor (LIF), ESGRO (Millipore) on gelatin-coated dishes (AGC Techno Glass). For differentiation, ESCs were transferred to the differentiation condition 1 day after vector transfection and were cultured for 3 days. ZHTc6 ESCs were cultured as previously described [4]. Culture medium was supplemented with tetracycline (Tc, 40 ng/mL, Sigma) and zeocin (Zeo, 100 μg/mL, Invitrogen). For selection of Oct3/4 positive cells, ZHTc6 ESCs were inoculated in medium including Zeo, at least for 2 weeks. Exogenous *Oct3/4* induction in ZHTc6 ESCs was achieved by Tc withdrawal for 2 days in LIF containing condition.

2.2. Forced expression of the H19 in mouse ESCs

A 3170 bp fragment of the mouse *H19* (−252 to +2918 base from the transcription initiation site) was purified by digestion of cosmid 5-10-A [20]. The *Cyp7a1* enhancer/promoter in the pCyp7a1-GFP-Neo vector [21] was replaced by the *EF-1a* promoter fragment of the pEF/myc/nuc vector (Invitrogen) to yield pEF-GFP-Neo. Then, the *H19* fragment was inserted under the *EF-1a* promoter sequence. The resulting plasmid pEF-*H19*/GFP-Neo (Fig. 3A) was linearized with XbaI (Takara Bio) and then transfected into the ESC line J1 by using Lipofectamine 2000 (Invitrogen) according to the manufacture's protocol in serum-free condition. For establishment of stable cell-lines, transfected clones were selected by growth in the presence of 175 μg/mL G418 (Invitrogen). Following 9 days of selection, 24 colonies were collected. From them, ten ESC lines that proliferated with piled-up morphology were selected, and one clone showing a strong *H19* expression was used in the present study. For transient expression experiments, non-linearized *H19* vector (pEF-H19/GFP-Neo) or control vector (pEF-GFP-Neo) was transfected into control ESC clone or ZHTc6 ESCs as described above.

2.3. Northern blot analysis

RNAs of *H19* (2.3 kbps) and *Gapdh* (1.6 kbps) were probed as previously described [16]. The membrane was exposed to a Fuji Imaging Plate (Fuji Film), and the radioactivity was analyzed using BAS-2500 Bio-imaging analyzer (Fuji Film).

3. Results

3.1. H19 overexpression enhanced expression of trophoblast marker genes after LIF withdrawal in ESCs

Previously we reported that the *H19* gene expression is increased in undifferentiated ES cells and also further augmented in the differentiation condition under LIF removal by semi-quantified RT-PCR [16]. When analyzed by quantitative RT-PCR, we found that under undifferentiated condition in the presence of LIF, the increased expression level of the *H19* was found to be 6–10 folds in *Parp-1*$^{-/-}$ ESCs (Fig. 1A). Northern blot analysis also confirmed that the expression of full-length form of the 2.3 kb *H19* RNA is upregulated in *Parp-1*$^{-/-}$ ESCs in the presence of LIF and further augmented after LIF withdrawal (Fig. 1B). We thus hypothesized that the functional *H19* may act as a trigger and induce trophoblast differentiation cascade in mouse ESCs.

To investigate this issue, we asked whether a forced upregulation of *H19* expression promotes the commitment of ESCs to trophoblast lineage or not. As shown in Fig. 1C, we transfected wild-type J1 ESCs with an *H19* overexpression vector to obtain ESCs constitutively overexpressing the *H19* transgene. Real-time RT-PCR analysis confirmed the establishment of cells expressing the *H19* transgene (Fig. 1D) and enhanced expression of *Cdx2* in the ESCs (Fig. 1E, ESCs). Four days after differentiation condition induced by withdrawal of LIF, the *H19*-transduced clone showed an increased expression of the trophoblast marker genes, *Cdx2* and *Plf1*, but not the *Oct3/4* gene (Fig. 1E and F), compared to the control vector-transduced clone. The *Cdx2* is a marker gene for TE at an early stage of trophoblast differentiation, and induction of *Cdx2* is known to be sufficient for differentiation of ESCs into the TE [3]. The *Plf1* is a late stage marker of terminally differentiated

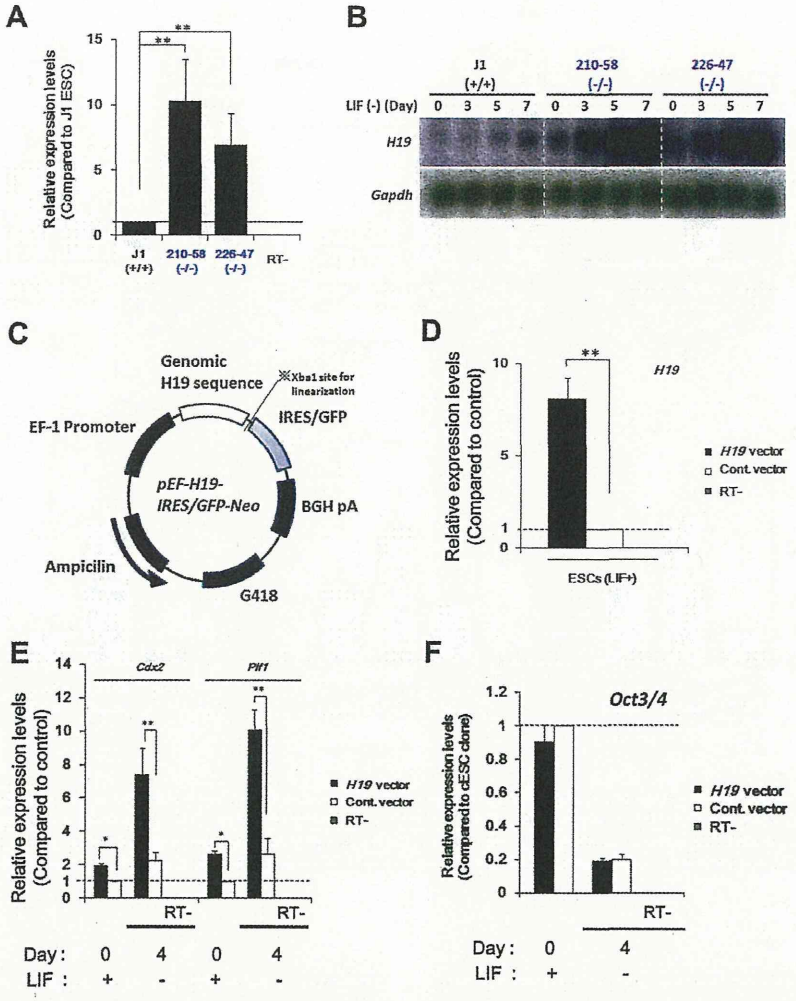

Fig. 1. Stable overexpression of *H19* activated expression of trophoblast related genes. (A and B) Upregulation of *H19* expression in *Parp-1*$^{-/-}$ ESCs. The expression level was analyzed by real-time PCR (A) and northern blot (B). The expression level was normalized using the level of *Gapdh* (**$P < 0.01$). RT- means without reverse transcription. (C) The vector construct for constitutive *H19* overexpression. (D) The *H19* overexpression in the *H19* vector-transfected clone. (E) Enhancement of trophoblast marker gene expression in the *H19*-overexpressing ESC clones. The ESC clone showed enhanced expression of trophoblast marker genes (*Cdx2* and *Plf1*), and these expression levels were higher at day 4 (D4) than in the control ESC clone (E). LIF withdrawal induced differentiation in the *H19* vector- or control vector-transfected ESC clones at day 4 (D4), but *Oct3/4* expression levels did not change under *H19* overexpression (F). Expression levels were analyzed by real-time PCR and were normalized using the expression level of *Gapdh* (*$P < 0.05$ and **$P < 0.01$, respectively).

trophoblast giant cells [22,23]. The *Oct3/4* level was not affected in the *H19*-transduced clone, confirming that *Oct3/4* is not regulated by the *H19* (Fig. 1F). Taken together, a forced expression of the *H19* triggered commitment to trophoblast lineage in ESCs under differentiation condition by LIF withdrawal.

To exclude potential bias through the cloning process in the above experiments, which used a stable clone, we transiently overexpressed the *H19* in a wild-type ESC clone, and cultured under differentiation conditions after LIF withdrawal (Fig. 2A and B). Although there was no difference in the expression level of *Fgf5*, a marker of PE, three days after differentiation induction (Fig. 2D), expression of the trophoblast marker genes, *Cdx2*, *Tpbpa* (trophoblast specific protein α) and *Plf1* were enhanced in the *H19*-transduced population (Fig. 2D). These results confirmed that under differentiation pressure by LIF withdrawal in ESCs, forced expression of the *H19* acts as a trigger for induction of expression of the *Cdx2*, a main transcription factor required for commitment to trophoblast lineage.

3.2. Exogenous induction of the H19 leads to the expression of a trophoblast key regulator gene Cdx2, even under Oct3/4 overexpression

In the present study, we demonstrated that expression of the *Cdx2* is activated in undifferentiated ESCs by a forced expression of the *H19*, which could trigger trophoblast lineage commitment. It has been suggested that Oct3/4 could repress the *Cdx2* induction during ICM differentiation [2]. This led us to clarify whether the *H19* could override Oct3/4 suppressive pressure on *Cdx2* transcription or not. ZHTc6 ESC clone was employed in this experiment, in which exogenous *Oct3/4* is regulated under the control of the Tc responsive element (Tet-OFF system) [4] (Fig. 3A). Two days after Tc withdrawal in the presence of LIF, increased expression of *Oct3/4* slightly upregulated the *Fgf5* (fibroblast growth factor 5) but did not repress the *Cdx2* in ZHTc6 ESCs (Fig. 3B and C, block 3), which assured the Oct3/4 accumulation by Tc withdrawal and indicated that Oct3/4 upregulation is not sufficient to suppress

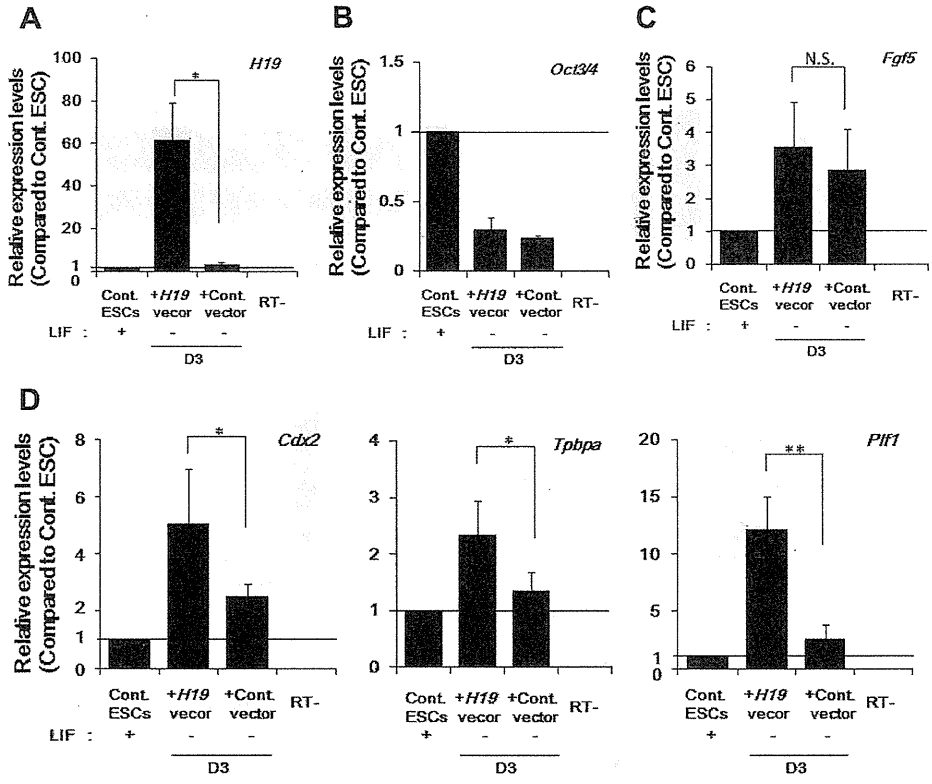

Fig. 2. The transient expression of exogenous *H19* enhanced trophoblast lineage differentiation during ESC differentiation. (A and B) Control ESC clones transfected with *H19* or control vector were induced to differentiate by LIF withdrawal. (C and D) Three days after differentiation (D3), trophoblast marker genes, *Cdx2*, *Tpbpa* and *Plf1*, were upregulated in *H19* vector-transfected ESCs (D), whereas no difference was observed in the levels of *Fgf5* between the *H19* vector- and control vector-transfected ESCs (C). Expression levels were analyzed by real-time PCR and were normalized using the expression level of *Gapdh* (*$P < 0.05$ and **$P < 0.01$, respectively). RT- means without reverse transcription.

the *Cdx2* at this period. The *Oct3/4* overexpression system successfully worked; the *Fgf5* expression is further augmented on day 6, which corresponds to 4 days after control vector transfection, indicating that the PE differentiation was further enhanced (Fig. 3B–D, block 5). On the other hand, expression of the *Nanog* (an epiblast marker) and *T* (a mesoderm marker) was not significantly altered (Fig. 3B and C, blocks 5 and 6) indicating that the PE differentiation is the main differentiation direction in this system. Of note, two days after Tc withdrawal, the *Oct3/4* forced expression repressed the *H19* expression prior to *Cdx2* suppression in the control vector transfected cells (Fig. 3C, the upper panel, block 3). Six days after Tc withdrawal, the *Oct3/4* forced expression reduced the *Cdx2* expression accompanying the attenuated level of *H19* expression (Fig. 3D, block 5). Importantly, forced expression of the *H19* to Oct3/4 overexpressing cells led to increased expression of *Cdx2* expression (Fig. 3D, block 6). Taken together, these results suggest that the *H19* expression is able to enhance the *Cdx2* expression even under the negative pressure of Oct3/4 on *Cdx2* in differentiating ESCs.

4. Discussion

In this study we demonstrated that the *H19* induction triggers trophoblast lineage commitment independently on Oct3/4 level in mouse ESCs cells. We showed that the expression of *Cdx2*, a key molecule for control of trophoblast differentiation, is induced by the *H19* expression. Our study also supported the notion that the *H19* upregulation in *Parp-1*$^{-/-}$ ESCs, which we previously observed, could activate the cascade of trophoblast lineage commitment by contributing to induction of the *Cdx2* expression (Fig. 4). Furthermore, during germ cell tumor formation, perturbed expression of the *H19* and trophoblast differentiation could be observed and affects malignancy of the tumors in some cases [1], suggesting that the trophoblast differentiation during tumorigenesis may be also initiated by the *H19* expression.

The role of non-coding RNA *H19* in trophoblast lineage commitment and differentiation regulation has not been fully elucidated. The *H19* expression depends on the DNA methylation state of the imprinting control region (ICR) located between the *H19* and *Igf2* (insulin-like growth factor 2) genes. A recent report demonstrated that *Dnmt1* deficient ESCs differentiated into trophoblast lineage [17]. *Dnmt1* deficiency induced DNA demethylation of the ICR and led to high level of the *H19* expression [18]. Although mouse ESCs do not generally differentiate into trophoblast lineage, it is suggested that the barrier for trophoblast lineage commitment in ESCs might be just dependent on epigenetic silencing.

The *H19* locus is paternally imprinted, and expressed only from the maternal allele [24]. The transcriptional product from the *H19* locus is a capped and polyadenylated non-coding RNA of 2.3 kb. This RNA is co-regulated with eight other imprinting genes co-organizing an imprinting gene network and is spliced and generates microRNA-675 but does not encode any protein [24]. In mouse models, the *H19* deficiency increases fetal weight and increases the frequency of teratoma formation [25]. Furthermore, the engineered expression of the *H19* could lead to complete parthenogenesis development [24]. These reports suggest the *H19* function as an

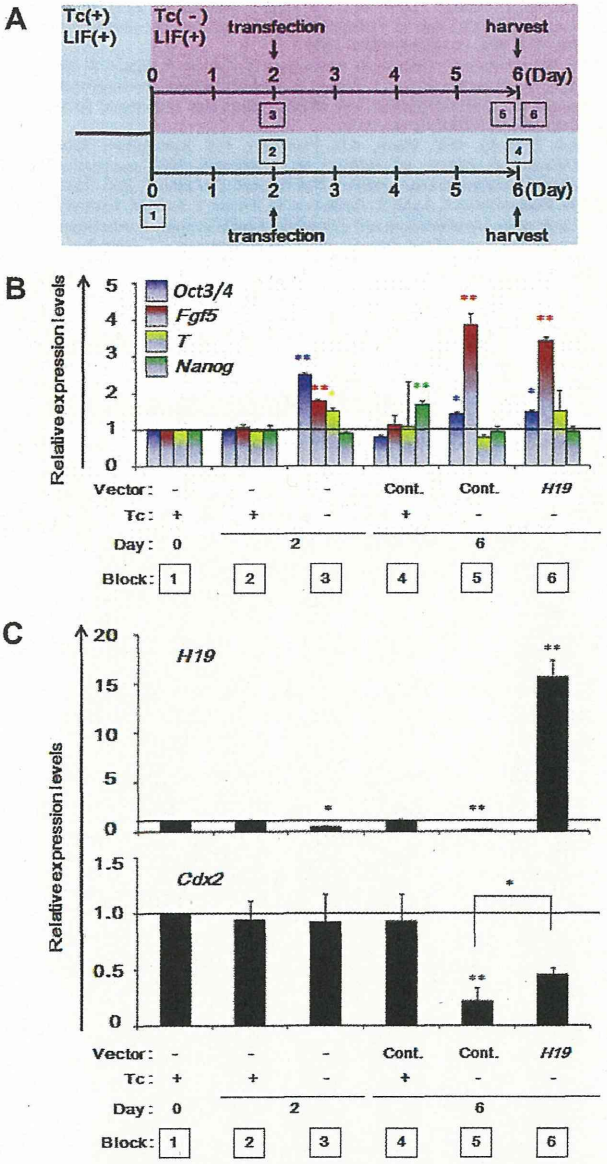

Fig. 3. The effect of *H19* overexpression in the presence of Tc-dependent Oct3/4 accumulation. (A) Experimental procedure. Each squared number indicates the RNA extraction points of the data shown in B and C. (B and C) Enhanced *Cdx2* transcription by *H19* overexpression in Oct3/4 accumulation conditions. Two days after Tc withdrawal in the presence of LIF, corresponding to the time when *Fgf5* transcription was increased (B), *H19* or control vectors were transfected into ZHTc6 ESCs (see A). Four days after transfection (the day 6 after Tc withdrawal), *H19* overexpression caused the augmented expression of *Cdx2* (C, block 5 and 6). The relative expression ratios of each gene (to the level at day 0) were shown after normalization to *Gapdh*. Unlinked asterisks indicate a significant difference in the expression level compared to that of ESCs (block 1) (*$P < 0.05$ and **$P < 0.01$, respectively).

important regulator of cell growth and differentiation. Further mechanistic role of the *H19* function in trophoblast lineage regulation should be elucidated.

In this study we also showed that forced expression of the *H19* even under a high level of Oct3/4 led to trophoblast lineage possibly acting as a cascade activator of trophoblast lineage by overriding the Oct3/4 action on the *Cdx2* in ESCs. Oct3/4 and Cdx2 are known to interact and regulate their levels each other. The main reported pathway of trophoblast lineage commitment is Oct3/4 down-regulation that leads to the *Cdx2* induction, which acts as a

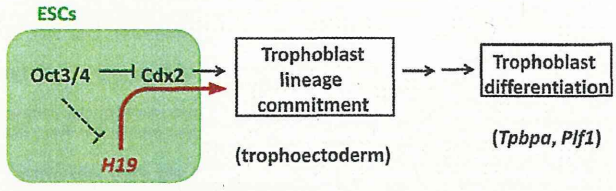

Fig. 4. A model; the *H19* upregulation could drive trophoblast lineage commitment in mouse ESCs. ESCs do not differentiate into trophoblast lineage because of the suppression of the *Cdx2* expression by Oct3/4. Forced expression of the *H19* is able to induce the *Cdx2* transcription (red letters and an arrow), which is critical for trophoblast lineage commitment of ESCs. Endogenous expression of the *H19* is downregulated by Oct3/4 (dotted lines) in ESCs. Forced expression of the *H19* drives *Cdx2* transcription even under the negative pressure by a high level of Oct3/4. This ectopic context model of ESCs suggests that *H19* upregulation might therefore contribute to induction of trophoblast lineage during tumorigenesis and pathogenesis of other reproductive system diseases. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

key transcription factor to induce Eomes, Mash-2, mSna, and Hand1 cascade. Our study suggested that the *H19* overexpression is able to promote trophoblast lineage commitment even under the suppressive pressure by Oct3/4. It is of question whether this Oct3/4 independent trophoblast lineage commitment by the *H19* could be involved in the differentiation control of trophoblast lineage in normal development and aberrant induction of trophoblast lineage during tumorigenesis and other pathogenesis of the reproductive system diseases.

Acknowledgments

This work was supported by Grants-in-Aid for Third Term Comprehensive 10-Year Strategy for Cancer Control (10103833), a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan (19-9), a Grant-in-Aid for Scientific Research on Priority Areas from the ministry of Education, Science, Sports, and Culture of Japan (22300343), a Grant-in-Aid for the National Cancer Center Research and Development Fund (23-RI-14).

We appreciate the suggestions and help provided by H. Ogino, M. Maeda, T. Ochiya, H. Nakagama, and T. Sugimura. We thank H. Sasaki for kindly providing us with 5-10-A cosmid, and T. Sugimoto and H. Niwa for kind donation of ZHTc6 ESCs.

References

[1] S. Furukawa, M. Haruta, Y. Arai, S. Honda, J. Ohshima, W. Sugawara, Y. Kageyama, Y. Higashi, K. Nishida, Y. Tsunematsu, H. Nakadate, M. Ishii, Y. Kaneko, Yolk sac tumor but not seminoma or teratoma is associated with abnormal epigenetic reprogramming pathway and shows frequent hypermethylation of various tumor suppressor genes, Cancer Sci. 100 (2009) 698–708.

[2] P.L. Pfeffer, D.J. Pearton, Trophoblast development, Reproduction 143 (2012) 231–246.

[3] H. Niwa, Y. Toyooka, D. Shimosato, D. Strumpf, K. Takahashi, R. Yagi, J. Rossant, Interaction between Oct3/4 and *Cdx2* determines trophectoderm differentiation, Cell 123 (2005) 917–929.

[4] H. Niwa, J. Miyazaki, A.G. Smith, Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells, Nat. Genet. 24 (2000) 372–376.

[5] A. Ralston, B.J. Cox, N. Nishioka, H. Sasaki, E. Chea, P. Rugg-Gunn, G. Guo, P. Robson, J.S. Draper, J. Rossant, Gata3 regulates trophoblast development downstream of Tead4 and in parallel to *Cdx2*, Development 137 (2010) 395–403.

[6] T. Nozaki, M. Masutani, M. Watanabe, T. Ochiya, F. Hasegawa, H. Nakagama, H. Suzuki, T. Sugimura, Syncytiotrophoblastic giant cells in teratocarcinoma-like tumors derived from Parp-disrupted mouse embryonic stem cells, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 13345–13350.

[7] M. Masutani, T. Nozaki, M. Watanabe, T. Ochiya, F. Hasegawa, H. Nakagama, H. Suzuki, T. Sugimura, Involvement of poly(ADP-ribose) polymerase in trophoblastic cell differentiation during tumorigenesis, Mutat. Res. 477 (2001) 111–117.

[8] M. Hemberger, T. Nozaki, E. Winterhager, H. Yamamoto, H. Nakagama, K. Kamada, H. Suzuki, T. Ohta, M. Ohki, M. Masutani, J.C. Cross, Parp1-deficiency induces differentiation of ES cells into trophoblast derivatives, Dev. Biol. 257 (2003) 371–381.
[9] M.J. Evans, M.H. Kaufman, Establishment in culture of pluripotential cells from mouse embryos, Nature 292 (1981) 154–156.
[10] G.R. Martin, Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 7634–7638.
[11] R.S. Beddington, E.J. Robertson, Axis development and early asymmetry in mammals, Cell 96 (1999) 195–209.
[12] P. Murray, D. Edgar, The regulation of embryonic stem cell differentiation by leukaemia inhibitory factor (LIF), Differentiation 68 (2001) 227–234.
[13] W. Yu, V. Ginjala, V. Pant, I. Chernukhin, J. Whitehead, F. Docquier, D. Farrar, G. Tavoosidana, R. Mukhopadhyay, C. Kanduri, M. Oshimura, A.P. Feinberg, V. Lobanenkov, E. Klenova, R. Ohlsson, Poly(ADP-ribosyl)ation regulates CTCF-dependent chromatin insulation, Nat. Genet. 36 (2004) 1105–1110.
[14] T. Zhang, J.G. Berrocal, J. Yao, M.E. Dumond, R. Krishnakumar, D.D. Ruhl, K.W. Ryu, M.J. Gamble, W.L. Kraus, Regulation of poly(ADP-ribose) polymerase-1-dependent gene expression through promoter-directed recruitment of a nuclear NAD+ synthase, J. Biol. Chem. 287 (2012) 12405–12416.
[15] R. Krishnakumar, M.J. Gamble, K.M. Frizzell, J.G. Berrocal, M. Kininis, W.L. Kraus, Reciprocal binding of PARP-1 and histone H1 at promoters specifies transcriptional outcomes, Science 319 (2008) 819–821.
[16] H. Ogino, T. Nozaki, A. Gunji, M. Maeda, H. Suzuki, T. Ohta, Y. Murakami, H. Nakagama, T. Sugimura, M. Masutani, Loss of Parp-1 affects gene expression profile in a genome-wide manner in ES cells and liver cells, BMC Genomics 8 (2007) 41.
[17] R.K. Ng, W. Dean, C. Dawson, D. Lucifero, Z. Madeja, W. Reik, M. Hemberger, Epigenetic restriction of embryonic cell lineage fate by methylation of Elf5, Nat. Cell Biol. 10 (2008) 1280–1290.
[18] D. Biniszkiewicz, J. Gribnau, B. Ramsahoye, F. Gaudet, K. Eggan, D. Humpherys, M.A. Mastrangelo, Z. Jun, J. Walter, R. Jaenish, Dnmt1 overexpression causes genomic hypermethylation, loss of imprinting, and embryonic lethality, Mol. Cell. Biol. 22 (2002) 2124–2135.
[19] A.S. Doherty, M.R. Mann, K.D. Tremblay, M.S. Bartolomei, R.M. Schultz, Differential effects of culture on imprinted *H19* expression in the preimplantation mouse embryo, Biol. Reprod. 62 (2000) 1526–1535.
[20] W. Purbowasito, C. Suda, T. Yokomine, M. Zubair, T. Sado, K. Tsutsui, H. Sasaki, Large-scale identification and mapping of nuclear matrix-attachment regions in the distal imprinted domain of mouse chromosome 7, DNA Res. 11 (2004) 391–407.
[21] K. Asahina, H. Fujimori, K. Shimizu-Saito, Y. Kumashiro, K. Okamura, Y. Tanaka, K. Teramota, S. Arii, H. Teraoka, Expression of the liver-specific gene Cyp7a1 reveals hepatic differentiation in embryoid bodies derived from mouse embryonic stem cells, Genes Cells 9 (2004) 1297–1308.
[22] S.J. Lee, F. Talamantes, E. Wilder, D.I.H. Linzer, D. Nathans, Trophoblastic giant-cells of the mouse placenta as the site of proliferin synthesis, Endocrinology 122 (1988) 1761–1768.
[23] S. Tanaka, T. Kunath, A.K. Hadjantonakis, A. Nagy, J. Rossant, Promotion of trophoblast stem cell proliferation by FGF4, Science 282 (1998) 2072–2075.
[24] A. Gabory, H. Jammes, L. Dandolo, The *H19* locus: role of an imprinted non-coding RNA in growth and development, Bioessays 32 (2010) 473–480.
[25] P.A. Leighton, J.R. Saam, R.S. Ingram, C.L. Stewart, S.M. Tilghman, An enhancer deletion affects both *H19* and Igf2 expression, Genes Dev. 9 (1995) 2079–2089.