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Review Article

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p53-dependent cell cycle checkpoint after DNA damage and its relevance to PARP1

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Abstract

The poly(ADP-ribose) polymerase (PARP) inhibitors, including 3-aminobenzamide (3-AB), suppress G1 arrest after DNA damage following gamma-irradiation, suggesting that PARP1, a major PARP family protein, is involved in the induction of G1 arrest. Furthermore, p53 stabilization following gamma-irradiation is not inhibited, but the p53-responsive transient increases of *WAF1/CIP1/p21* and *MDM2* mRNA have been shown to be suppressed by 3-AB. Therefore, it is suggested that PARP1 participates as a downstream mediator of p53 dependent signal-transduction pathway through the modulation of *WAF1/CIP1/p21* and *MDM2* mRNA expression. In this review, we discuss p53 cell cycle checkpoint after DNA damage, and its relevance to PARP1. Moreover, the role of PARP1 as a sensor of DNA damage will be proposed. Regulation of p53 and PARP1 activities is an attractive and promising target for the development of clinical treatments for particular diseases. Therefore, it is anticipated that the clinical application of drugs that specifically regulate PARP1 activity will develop in the near future.

p53 and G1 checkpoint in cancer

During the development of cancer, multiple abnormalities occur in the genes that are directly related to the regulation of cell cycle progression [1]. Mutations in the retinoblastoma (RB) protein, a cell cycle regulatory protein with tumor-suppressive functions, have been reported to occur in various types of cancers [2]. Further, cyclin D gets activated by chromosomal translocation and/or amplification in many cancers [3]. Additionally, p16/MTS1, which inhibits the cyclin D dependent kinase activity, was identified as a novel tumor suppressor gene that gets inactivated in melanoma, colon cancer, breast cancer, etc. [4,5]. Mutations in the p53 tumor suppressor gene are involved in approximately 50 % of the human cancers [6,7]. Among 75 % of these cancers, missense mutations with amino acid substitutions were detected [8]. According to Tsuchida *et al.* p53 mutations occur in approximately 90 % of the cell cultures obtained from human oral squamous cell carcinoma [9].

p53 induces the transcription of p53 target genes that exhibit various functions involved in the regulation of DNA damage, aging, cancer, gene activation, hypoxia stress, etc. As diversities exist in the p53 target genes, in addition to the conventional functions such as apoptosis, cell cycle arrest, DNA repair, and p53 activity suppression, other functions including cellular development, immunity, epigenetic regulation, and undifferentiated cell state maintenance were reported [10]. An important p53 function during cell cycle arrest (following p53 stabilization after the DNA damage) is the induction of cyclin-dependent kinase inhibitor 1 (WAF1/CIP1/p21). WAF1/CIP1/p21 is a transcription regulatory factor that inhibits the cyclin dependent kinase (CDK) and delays or terminates the cell cycle at G1 phase [9,11-13]. The G1 phase arrest checkpoint mechanism regulates the normal cell cycle and is believed to be strongly involved in carcinogenesis subsequent to DNA damage. In cells with *p53* mutations or deficiency, the G1 phase arrest does not occur, and progression to the S phase occurs even after DNA damage owing to the abnormal transcriptional regulation by p53 [11,14]. Therefore, it is hypothesized that DNA damage accumulation causes mutated cells to progress into a cancer.

DNA damage after exposure to ionizing radiation

Regarding the resistance to cancer treatment using radiation or chemotherapy, cell cycle arrest in the G1 and G2 phases after DNA damage is considered as an important factor. It is known that the expression levels of cancer genes such as the rat sarcoma oncogene homolog (*RAS*), myelocytomatosis viral oncogene homolog (*MYC*), and rapidly accelerated fibrosarcoma (*RAF*) vary with the radio-sensitivity of cells [15-17], and therefore the cellular response to cell cycle arrest after DNA damage is considered as an important factor and one of mechanisms that determine the radio-sensitivity. Gamma rays produce excited and ionic molecules in cells. Although all intracellular molecules are targeted, DNA damage is broadly classified into direct and indirect effects [18]. The direct effect is caused by direct contact of the ionizing radiation energy with the DNA. The indirect effect is caused by the interaction of free radical species with the DNA. As water molecules (H_2O) are the most frequently available intracellular molecules, radicals —including hydroxyl radical (HO·), hydrogen atom (H·), and hydrated electron (e–aq)— are generated from H_2O. Therefore, H_2O is considered as the molecule that causes the strongest indirect effect. The damage owing to these direct and indirect ionizing radiation effects occurs at the base or sugar–phosphodiester framework

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that constitutes the DNA. Although the detection of actual damage to the bases present in cells is difficult owing to their instability, the damage to the sugar–phosphodiester framework is detectable as it mainly appears in the form of DNA strand breaks (DSBs). DSBs mainly occur as phosphodiester bond cleavages and, to some extent, as the decomposition of deoxyribose rings.

p53 stabilization after DNA damage

After DNA damage by gamma rays, cell cycle arrest mainly occurs in the G1 and G2 phases. DNA repair is believed to occur prior to the progression of cell cycle into the S or M phase. Kastan et al. suggested the aforementioned fact that the p53 gene product is the key molecule for G1 phase arrest [19]. They investigated the responses of various cells against gamma rays and reported that p53-deficient cells exhibit G2 phase arrest but not G1 phase arrest after gamma-irradiation. Furthermore, in cells treated with ultraviolet (UV) rays, 4-nitro, 4-nitrosoquinoline-1-oxide, or DNA damaging factors—including gamma rays and actinomycin D—an increase in the p53 level was observed owing to the post-translational mechanism that prolongs the p53 half-life [20,21]. Previous reports demonstrated that p53 recognizes the DNA strand broken ends and binds to these strands [22,23]. It is considered that p53 with an extended half-life gets accumulated in cells, activates or suppresses the transcription of gene targets that contain a p53 binding sequence, and induces G1 phase arrest.

Transcriptional response induced by p53 accumulation

p53 forms a tetramer and functions as a transcription factor by binding to its target genes using the p53 consensus binding sequence (a sequence comprising a 10mer sequence composed of RRRCWWGYYY [R: A/G, Y: T/C, W: A/T] is repeated twice in tandem separated by a spacer of 0-13 bp) [24,25]. p53 activates the transcription of genes, such as the growth arrest and DNA-damage-inducible protein (*GADD45*), mouse double minute 2 homolog (*MDM2*), *WAF1/CIP1/p21*, epidermal growth factor receptor (*EGFR)*, and muscle creatine kinase (*CK*) [26-28]. Furthermore, it is considered that p53 binds to the large T antigen, which is an oncogene product of DNA-type cancer virus SV40 [2,29], E1B of adenovirus [30], and E6 of papilloma virus. These virus-derived oncoproteins inhibit p53 transcriptional activity and cause cell transformation [31]. Moreover, the oncogene product MDM2 was reported to directly bind to p53 [32,33]. In naturally transformed cells, *MDM2* is abnormally amplified [34], and this aberrant MDM2 occurs in approximately 30 % and 15 % of human osteosarcoma and breast cancer, respectively. It is believed that MDM2 ubiquitinates, degrades, and binds to p53, and that it participates in a negative feedback regulation of p53 to inhibit its transcription regulatory activity [35,36]. A low molecular weight compound that binds to MDM2/MDMX and suppresses the degradation of wild-type p53 by inhibiting the interaction between MDM2 and p53 has attracted attention as a molecular target drug, and clinical trials have been conducted in this regard [37].

G1 checkpoint after DNA damage

The information regarding the G1 phase arrest mechanism after DNA damage is described in this section. After DNA damage, the stabilization and intracellular accumulation of p53 takes place, therefore, its transcriptional activation ability increases. As a transcription regulation factor, p53 induces the expression of a protein that suppresses the activity of G1 cyclin and CDK complex enzyme [38]. This protein was identified as WAF1/CIP1/p21 and found to be similar to the senescent cell derived inhibitor 1 (*SDI1*) gene product whose expression increases with cellular aging [39]. Furthermore, it is considered that via the inhibition of RB phosphorylation by the CDK complex and the activity of transcriptional regulatory factors (such as E2F, which is regulated by RB), WAF1/CIP1/p21 hinders the transcription of the gene necessary for G1/S transition, and therefore causes G1 phase arrest. The process from DNA damage to the increase of p53 level is elucidated in the subsequent sentences. It was reported that in cell cultures derived from B lymphocytes of ataxia telangiectasia patients, an increase of p53 level after DNA damage was not observed [19]. Additionally, it was reported that the causative gene product of ataxia is involved in the signaling pathway leading from DNA damage to p53 elevation.

The G1 phase arrest after DNA damage and apoptosis are known to be closely associated. For instance, in B lymphocytes exposed to gamma irradiation, the G1 phase arrest and apoptosis are induced in the presence and absence of growth factor, respectively [38]. Similar to the G1 phase arrest, apoptosis might be accompanied by p53 stabilization [38]. Therefore, the quantitative information of DNA damage might act as the determining factor in the process of cell decision to opt either for G1 phase arrest or apoptosis after gamma irradiation. In other words, there is a possibility that cells opt for G1 phase arrest when DNA damage is considerably low, and apoptosis when damage is high. The important question is, by what sensor is the quantitative information of DNA damage perceived, and through which signal is this quantitative information transmitted to the key molecule, p53?

G2 checkpoint after DNA damage

Unlike the G1 phase arrest mechanism G2 phase arrest is observed in cells with *p53* mutation. For a long time, genetic analyses have been performed in budding yeast, and six genes (*RAD9, RAD17, RAD24, MEC3, MEC1, and MEC2*) have been identified to be essential for G2 phase arrest [40,41]. Moreover, among the aforementioned genes, *MEC1* and *MEC2* are indispensable in the step in which the completion of DNA replication occurs. The human homologue of budding yeast *RAD24* was identified and found to be identical to a mammalian cell factor that promotes the ADP-ribosylation reaction of bacterial mono (ADP-ribose) transferase and is called 14-3-3 protein [42]. Since the 14-3-3 protein binds to the middle T antigen of polyoma virus [43], it is considered to be involved in DNA damage signaling between the cell membrane and the nucleus. However, as previously described, the analysis of the G2 phase arrest mechanism in mammalian cells has not progressed as much as in yeast cells. Consequently, whether the sensor of DNA damage in G2 phase arrest is distinct to that of the G1 phase arrest remains unknown, as well as the mechanisms through which the quantitative information of DNA damage is transmitted.

Poly(ADP-ribose) synthase 1 (PARP1) is involved in the physiological responses to DSBs in the nuclei of highly evolved eukaryotes, such as mammals. PARP1 specifically recognizes DSBs and promptly synthesizes poly (ADP-ribose) chains using β-nicotinamide adenine dinucleotide (β-NAD) as the substrate. It is known that PARP1 constitutively exists in the nucleus of particular cells in the ratio of approximately 1 per 10 kb DNA [44]. PARP1 activity occurs in most eukaryotic cellular nuclei, including slime molds, animals and plants [45,46]. As an exception, PARP1 activity is not observed in mature granulocytes (leukocytes with rod-shaped or segmented nuclei) in mammals [47]. Most PARP1 is present in the chromatin of the nucleus [48,49]. Immunohistological observations have shown that it is found in the periphery of nucleus (heterochromatin region) in some types of cells [47]. Analyses of protein and gene levels indicate that PARP1 exhibits three functional domains that are well conserved throughout various species [50]. PARP1 binds to a DNA nick through its Zn finger

that spreads over 30 bases on the periphery of the nick portion [51,52]. Furthermore, the initial PARP1 activation occurs by its binding to the ends of either single- or double-strand DNA breaks [53]. PARP1 catalyzes the poly ADP-ribosylation of proteins containing glutamic and aspartic acid residues. Histone H1 and H2B, high-mobility group (HMG) proteins, DNA polymerase α and β, and topoisomerase I and II are well-known acceptor proteins of poly (ADP-ribose). It was reported that poly ADP-ribosylation inhibits the enzyme activity of acceptor proteins and causes histones to lose affinity toward the DNA. Moreover, when PARP1 loses its affinity toward the DNA, its ability to synthesize poly (ADP-ribose) is suppressed owing to the auto-poly-ADP-ribosylation reaction [54-56]. Studies regarding the physiological functions of PARP1 were performed in the presence of a PARP inhibitor, and the involvement of PARP1 in DNA repair was identified by enhancing cytotoxicity through various DNA damaging treatments such as exposure to gamma rays [57,58]. Since PARP1 specifically recognizes DNA broken ends, it is believed to participate in DNA repair, by removing the damaged DNA portion after breakage. It was reported that DNA repair against alkylating agents is inhibited exclusively in mutant cells with low PARP1 expression or dominant negative mutants that contain a high number of DNA binding sites [59]. Furthermore, in an experiment, the DNA repair was delayed when PARP1 anti-sense RNA was ectopically expressed in cells to inhibit PARP1 function [60]. Moreover, in the cell-free DNA repair system, DNA repair is temporarily interrupted depending on the PARP1 efficiency in the presence of NAD [61]. Additionally, other reports have indicated that PARP1 highly promotes DNA ligase activity on the chromatin DNA [62]. The previously proposed histone-shuttling model indicates that the loss of affinity of poly-ADP-ribosylated histones for DNA causes the structure surrounding DNA broken ends to further loosen, and thereby promotes DNA repair [63]. PARP1 is necessary for DNA replication as approximately 10 Kb DNA replication intermediates were accumulated in cells upon treatment with a PARP1 inhibitor [64]. Additionally, owing to the facts such as an increase in the sister chromosome conversion frequency [65], loss of amplified *c-myc* in HL-60 cells [66], the loss of external cancer gene in NIH 3T3 cells [67] suggested that PARP1 is involved in DNA recombination. Regarding gene transcription, experiments using PARP1 anti-sense RNA expression plasmid demonstrated that the induction of major histocompatibility complex (MHC) class II gene expression by gamma interferon (γ-IFN) is in turn induced by PARP1 inhibition [68]. Moreover, it was reported that the transcription from the HIV long terminal repeat induced during DNA damage by UV can be suppressed by a PARP inhibitor [69]. Therefore, the involvement of PARP1 in various physiological conditions that cause DSBs is well established. We found that PARP inhibitors, including 3-aminobenzamide (3-AB), suppressed G1 arrest after dNA damage following gamma irradiation, suggesting that PARP1 is critical for the induction of G1 arrest. Furthermore, we found that p53 stabilization was not inhibited by gamma-irradiation, and that the p53-responsive transient increases of *WAF1/CIP1/p21* and *MDM2* mRNA were suppressed by 3-AB. Therefore, it is suggested that PARP1 participates in p53 dependent G1 arrest signal-transduction pathway through the modulation of *WAF1/CIP1/p21* and *MDM2* mRNA expression [70,71].

The role of PARP1 as a sensor of DNA damage

As PARP1 is constitutively expressed in the nucleus, it specifically recognizes DSBs and synthesizes the poly (ADP-ribose) strands corresponding to the number of DSBs using NAD as substrate. Hence, it is considered as a promising sensor candidate that monitors DNA damage. A prompt signal for DSBs and an accurate transmission of the vast information regarding DSBs are considered as necessary capabilities of a sensor molecule. After gamma-irradiation, PARP1, present in the ratio of approximately one per several Kb of DNA, rapidly synthesizes poly (ADP-ribose) in a dose dependent manner by consuming NAD. For example, 2 Gy gamma-irradiation produces approximately 1,000 single-strand breaks in a cell. Considering that 3×10^{-18} moles of NAD molecules per cell are consumed within 30 min after 2 Gy gamma irradiation, it was calculated that approximately 10^5 molecules of poly (ADP-ribose) with an average chain length of 20 molecules are produced per cell through DNA strand breakage. Similarly, approximately 5×10^6 molecules of poly (ADP-ribose) may be synthesized after 100 Gy irradiation. Contrarily, as p53 recognizes DSBs, another theory suggests that p53 might act as the direct signal for DSBs [23]. However, an immunoprecipitation experiment indicated that approximately 10^4 p53 molecules per cell were present under normal conditions, and thus it is unlikely that p53 could directly and efficiently transmit the vast information regarding $\geq 10^4$ DSBs. Therefore, it is considered that poly (ADP-ribose) might be a more effective signaling candidate to transmit the vast information on DSBs. Due to their capacity to bind DNA broken ends, p53 and PARP1 are considered potential signaling molecules for the detection of DSBs in G1 phase arrest. Several p53 isoforms and family members were identified, and multiple reports have indicated that some of the isoforms interact with p53 and transactivate specific target gene groups [72-74]. Moreover, the possibility that the cooperation between the wild-type p53 and some p53 isoforms may transmit the vast information regarding DSBs needs to be explored. If PARP1 transmits quantitative information regarding DSBs, the decision as to whether the direct poly-ADP-ribosylation or the interaction between PARP1 and the other information transmission factors is selected and the information transmission factor needs to be investigated in order to completely understand the mechanism. Previously, using the western blot method we revealed the presence of intracellular proteins that non-covalently interact with PARP1 [75]. In the future, extensive research will be essential to understand the sensor molecules that sense and transmit the vast information regarding DSBs.

Modification of p53 and PARP1 activities and their application in disease treatments

As mutant p53 stabilizes and accumulates in cancer cells, cancer patients often exhibit p53 antibody positivity; therefore, p53 antibody is used as a tumor marker for diagnosis [76]. Drugs that can convert this mutant p53 into wild-type might be useful as therapeutic agents for cancer [77,78]. Anticancer drugs that activate the p53 pathway without causing DNA damage are being developed [79,80]. The development of protective agents that increase the radiation resistance of normal tissues by the regulation of cell death is currently under progress. As p53 regulators selectively protect normal tissues that exhibit proper p53 function against cell death caused by DNA damage, and do not protect cancer cells that exhibit abnormal p53, it might be applied to overcome the dose limitation of radiation therapy and to reduce the side-effects of anticancer drugs [81,82]. The denaturation and deactivation of p53 is known to occur owing to the dissociation of zinc ions that coordinate with the zinc ion binding site in p53 and the substitution of metal ions other than zinc [83]. Additionally, compounds that target the zinc binding site in p53 have been studied. However, drugs that inhibit p53 function increase the risk of carcinogenesis promotion. Therefore, efforts are being undertaken to prevent p53-dependant cell death by inducing the expression of WAF1/CIP1/p21 and suppressing the expression of the p53-upregulated modulator of apoptosis, which promotes cell

death [84]. It is anticipated that these p53 target drug discoveries will encourage the development of novel radiation protection agents. Similarly, because PARP1 participates not only in DNA repair but also in the p53-dependent cell-cycle check-point after DNA damage, we believe that cell death control via the regulation of PARP1 activity might be useful for future applications in cancer therapy. Although PARP inhibitors that block PARP family proteins have started to be employed in clinical settings, we predict the development and clinical application of drugs that will specifically regulate PARP1 activity.

Conflicts of interest

The authors have declared that no conflicts of interest exist.

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