Special Lecture Note

Poly(ADP-ribose) Polymerase and Cancer: In Relation to the Lectures Presented by Dr Gilbert de Murcia

Mitsuko Masutani1 and Masanao Miwa2

1Biochemistry Division, National Cancer Center Research Institute, Tokyo and 2Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan

Received July 25, 2002; accepted August 20, 2002

BRIEF INTRODUCTION TO POLY(ADP-RIbose) POLYMERASE

Throughout the process of carcinogenesis, the cellular responses to DNA damage play an important role in converting normal cells into a malignant state. Poly(ADP-ribose) synthesis is one such cellular DNA damage response, as illustrated in Fig. 1. Poly(ADP-ribose) polymerase-1 (PARP-1) polyADP-ribosylates PARP-1 itself and nuclear proteins, using nicotinamide adenine dinucleotide (NAD) as a substrate. The role of PARP-1 in DNA strand breaks (1,2) and base excision repair (3) has been reported. In addition, the role of PARP-1 in the induction of necrotic cell death through cellular NAD depletion after excessive DNA damage has been described (4).

PARP-1 is also involved in transcriptional control (5) and cellular differentiation (6). PARP-1 deficiency has been shown to be a risk factor for carcinogenesis in mice after treatment with alkylating agents (7) or in the combination of a deficiency of DNA-PK (8) or p53 (9).

A correlation between PARP-1 gene overexpression and low genomic instability has been reported in human breast carcinomas (10). The peripheral blood lymphocytes from laryngeal cancer showed significantly lower levels of poly(ADP-ribose) formation compared with that in healthy controls (11).

PARP-1 comprises an N-terminal DNA binding domain, an automodification domain and a C-terminal catalytic domain and various cellular proteins interact with PARP-1. The N-terminal DNA binding domain contains two zinc finger motifs. Transcription enhancer factor-1 (TEF-1), retinoid X receptor α, DNA polymerase α, X-ray repair cross-complementing factor-1 (XRCC1) and PARP-1 itself interact with PARP-1 in this domain (1). The automodification domain contains a BRCT motif, one of the protein–protein interaction modules. This motif is originally found in the C-terminus of BRCA1 (breast cancer susceptibility protein 1) and is present in various proteins related to DNA repair, recombination and cell-cycle checkpoint control. POU-homeodomain-containing octamer transcription factor-1 (Oct-1), Yin Yang (YY)1 and ubiquitin-conjugating enzyme 9 (ubc9) could interact with this BRCT motif in PARP-1 (1).

Recently, several PARP family proteins have been identified. Tankyrase was found as an interacting protein of telomere regulatory factor 1 (TRF-1) and is involved in telomere regulation (12). Vault PARP (VPARP) is a component in the vault complex, which acts as a nuclear-cytoplasmic transporter (13). PARP-2 (14,15), PARP-3 (15) and 2,3,7,8-tetrachlorodibenzo-p-dioxin inducible PARP (TiPARP) (16) have also been identified. Therefore, poly(ADP-ribose) metabolism could be related to a variety of cell regulatory functions.

THE LECTURES BY DR GILBERT DE MURCIA

Dr Gilbert de Murcia, one of the leading scientists in poly(ADP-ribose) research, visited Japan from July 15 to 26, 2001, as a lecturer in the lectureship program of the Foundation for Promotion of Cancer Research. Before Dr de Murcia’s contribution, since 1984, the lectureship program had invited a total of 56 distinguished scientists in various fields related to cancer research from outside Japan. Dr de Murcia’s visit was an important opportunity to consider the relationship between PARP and cancer. Dr de Murcia has been Research Director of the Centre National de la Recherche Scientifique (CNRS), Strasbourg, Ecole Supérieure de Biotechnologie de Strasbourg.
since 1990. He graduated from the Université Louis Pasteur, Strasbourg, in Biophysics in 1972 and received his PhD at CNRS in 1983. On the basis of his early research on the structural modification of chromatin by small molecules, he started to concentrate on the biochemistry of polyADP-ribosylation. With molecular and biological approaches, he and his wife Dr Josiane Ménissier-de Murcia, being his colleague and also a distinguished scientist in the field of poly(ADP-ribose), who visited with him, clarified the involvement of poly(ADP-ribose) polymerase-1 (PARP-1) in DNA repair (2,3) and inflammatory responses (17) and elucidated the role of PARP-2 (18) and PARP-3. Two official lectures were presented during Dr de Murcia’s stay. The first lecture was given on July 19 with the title ‘PolyADP-ribosylation reactions in the network of DNA damage surveillance and inflammatory cell injury’ at the International Lecture Hall in the National Cancer Center, Tokyo. The audience included basic and clinical researchers on cancer in various fields. The second lecture was given on July 24 with the title ‘Structure and function of novel poly(ADP-ribose) polymerase homologues’ at the Institute of Medical Sciences, University of Tsukuba, Tsukuba City and impressed both students and young and senior scientists. Dr Ménissier-de Murcia volunteered to give a seminar under the title ‘Interplay of poly(ADP-ribose) polymerase-1 (PARP-1) with ATM and p53’ at the National Cancer Center Research Institute, Tokyo, and also in the University of Tsukuba.
Dr de Murcia demonstrated in his lectures how PARP-1 recognizes DNA strand breaks using N-terminal DNA binding domains, containing two zinc fingers. These zinc fingers I and II have CX2-CX29/30-HX3-C motifs in common and are involved in the activation of the enzyme and its binding to single-strand DNA interruption (2). Interestingly, a similar zinc finger is present in the mammalian DNA ligase III, which is an essential component of base-excision repair (BER). He elegantly showed, using an electron micrograph technique, that PARP-1 binds to single strand breaks and causes bending of DNA by an angle of 100°, such as the number of enzymes involved in BER like uracil DNA glycosylase, AP endonuclease 1, XRCC1 and DNA polymerase β. Dr de Murcia showed that PARP-1 facilitates efficient base-excision repair through this DNA binding property and also by its capacity for protein–protein interaction. PARP-1 is present in the base-excision repair complex and interacts with XRCC1, a central adaptor molecule, DNA polymerase β and DNA ligase III and may regulate the action of repair enzymes and local chromatin remodeling. In the embryonic fibroblasts derived from PARP-1–/– mice, he showed that DNA repair after treatment with alkylating agents is markedly delayed (1,19). Dr de Murcia first succeeded in demonstrating that PARP-1–/– mice and cells are hypersensitive to γ-irradiation and alkylating agents and PARP-1 is involved in DNA damage recovery (19,20). This was further confirmed by Masutani et al. (21) and Wang et al. (22). He also showed that PARP-1 is one of the crucial regulators of inflammatory response as the key coactivator of nuclear factor κB (NF-κB) (17). NF-κB is a cellular redox sensor and regulates the expression of the genes involved in inflammatory responses, including the inducible nitric oxide synthase (iNOS) gene. It also functions in the regulation of DNA damage response and apoptosis. PARP-1–/– mice exhibited reduced levels of NF-κB activation and became resistant to lipopolysaccharide-induced septic shock through impaired iNOS gene expression and marked reduction of nitric oxide production was observed (17).

Dr de Murcia and Dr Ménissier-de Murcia demonstrated that embryonic fibroblasts from PARP-1–/– p53–/– mice, harboring the disrupted exon 4 of PARP-1 gene, show a reduced level of iNOS expression and markedly delayed cell proliferation compared with PARP-1+/+ p53–/– counterparts (24). In vivo tumorigenesis in PARP-1–/– p53–/– mice was also delayed (24). This is in contrast with the increased susceptibility to tumorigenesis observed in PARP-1–/– p53+/+ mice harboring an exon 2 disruption of the PARP-1 gene reported by Tong et al. (9). Some modifying effects from one genetic background of mice and/or the gene-targeting site in the PARP-1 gene may cause the difference of susceptibility to tumorigenesis between PARP-1–/– mice harboring exon 2 and exon 4 disruption. This point needs to be investigated further to understand the physiological role of PARP-1. Dr Ménissier-de Murcia also showed the functional synergy between PARP-1 and ATM (ataxia-telangiectasia mutated protein) in cell proliferation during embryogenesis. PARP-1–/– ATM–/– mice die at embryonic day 8.0, accompanying the extensive apoptosis in the cells of embryonic tissue but not in those of the extraembryonic tissues (23). The embryonic cells during this period lack cell-cycle arrest and are hypersensitive to DNA damage.

Dr de Murcia also presented novel and exciting properties of PARP-2 and PARP-3, as breaking information. PARP-2 contains a DNA-binding domain rich in basic amino acid residues and is activated after DNA damage (25). PARP-2–/– mice, which he developed by disrupting exon 9, showed an increased sensitivity to ionizing radiation. Dr de Murcia also reported that DNA repair after N-methyl-N-nitrosourea treatment is delayed in embryonic fibroblasts derived from PARP-2–/– mice (25). By immunofluorescence staining, he showed that PARP-2 localizes preferentially during mitosis to centromeres, whereas PARP-1 is distributed in whole chromosomes and nucleolus as well as centrosomes, as previously reported by Miwa’s group (26). Dr de Murcia found that PARP-2 could recognize aberrant structures in DNA duplexes, including cruciform, DNA loop and DNA flap sites, as PARP-1 also does (1). PARP-1–/– PARP-2–/– mice became lethal and this suggests the compensative function of PARP-1 and PARP-2. PARP-3...
contains a short N-terminal domain. He demonstrated that PARP-3 specifically localizes to the daughter centrioles throughout the cell cycle. Dr de Murcia predicted that PARP-1, PARP-2 and PARP-3 possess crucial roles in the regulation of mitotic apparatus from their distinct subcellular localization and properties.

Dr de Murcia and Dr Ménissier-de Murcia held scientific discussions with many young and senior researchers during their stay in Tokyo and Tsukuba and very much motivated them (Fig. 2). Dr de Murcia also visited the Medical School of Kyoto University, gave an informal seminar and met with various scientists, including Professor Shunichi Takeda and Professor Kunihiro Ueda.

**Remarks**

More than 15 members of the PARP family of genes have now been demonstrated to be present in the mammalian genome. PARP family proteins and poly(ADP-ribose) glycohydrolase (PARG), which degrades poly(ADP-ribose) to ADP-ribose, could be involved in a variety of cell regulatory functions including DNA damage response and transcriptional regulation and might be related to carcinogenesis and the biology of cancer in many respects. Dr de Murcia recently opened a unique web site, ‘The PARP Link’ (http://parlink.u-strasbg.fr), with Dr Patrick Stiegl, in which varied useful information on the study of poly(ADP-ribose) is available. For the elucidation of the relation between cancer and poly(ADP-ribose) metabolism, the accumulation of evidence from basic molecular biological studies and transgenic and knockout animal models will be important. We expect that international communication and exchange of research will further promote the development of these fields of study.

**Acknowledgements**

The Second Term Comprehensive 10-Year Strategy for Cancer Control is conducted and supported by the Ministry of Health, Labor and Welfare of Japan. The lecturership program of the Foundation for Promotion of Cancer Research, one of the projects of ‘the 10-Year Strategy for Cancer Control’, has been subsidized by the Japan Motorcycle Racing Organization through its promotion funds from Autocrine. We greatly appreciate Dr de Murcia’s visit with Dr Ménissier-de Murcia and his distinguished lectures in Japan in spite of his busy schedule. We thank Mr Masataka Kohda, President and Dr Michiya Ohtaka, Executive Director of the Foundation for Promotion of Cancer Research for their support of the lecturership program. We are also grateful to Dr Hitoshi Nakagama, Chief of Biochemistry Division, National Cancer Center Research Institute and Dr Takashi Sugimura, President Emeritus, National Cancer Center for their valuable advice.

**References**


