

Princess Takamatsu Symposium on DNA Repair and Human Cancers

Lawrence A. Loeb^{1,2} and Susumu Nishimura³

Abstract

The 40th International Symposium of the Princess Takamatsu Cancer Research Fund, entitled “DNA Repair and Human Cancers,” was held on November 10–12, 2009 at Hotel Grand Palace, Tokyo, Japan. The meeting focused on the role of DNA repair in preventing mutations by endogenous and exogenous DNA damage and increasing the efficacy of chemotherapeutic agents by interfering with DNA repair. The 14 presentations by the speakers from the United States, four from the United Kingdom, one each from Italy, The Netherlands, and France, and 13 from Japan, covered most aspects of DNA repair, spanning DNA damage, molecular structures of repair enzymes, and clinical studies on inhibition of DNA repair processes. Extensive time was reserved for discussions with the active participation of the 150 invited Japanese scientists. The choice of a symposium on DNA repair in human cancers resulted in part from the excellent basic and clinical studies that have been carried out for many years in Japan, and the general lack of recognition versus the importance of DNA repair in understanding carcinogenesis. *Cancer Res*; 70(11); 4269–73. ©2010 AACR.

Background

The importance of DNA repair in preventing human cancers was first catapulted into prominence by studies on rare genetic diseases that exhibit an exceptionally high incidence of specific malignancies. The affected individuals harbor mutations in DNA repair genes that function in the removal of specific DNA lesions. The classic example is xeroderma pigmentosum (XP), in which exposure to sunlight increases the incidence of skin cancers more than 1,000 fold, and the mutated genes encode proteins that are defective in removing UV-induced lesions. The high incidence of cancer in many inherited human DNA repair diseases, and the extensive studies on cancers induced by exposure to DNA-damaging environmental agents, highlight the importance of DNA repair in preventing mutations and reducing cancer incidence.

Discounting surprises, it is likely that we have identified all major DNA repair pathways in humans. However, we still lack a detailed knowledge of the molecular mechanisms of many DNA repair processes, the functional redundancy of different DNA repair pathways, and the cellular mechanisms for regulation of DNA repair processes. Importantly, we lack rapid assays for quantifying DNA repair in human cells and

for screening agents that target specific DNA repair processes. In short, considering the centrality of DNA repair in cancer prevention, it is apparent that there is insufficient research that focuses on DNA repair and associated processes for the maintenance of genetic stability. Despite these limitations, we should soon be in a position to ask if we can stratify individuals on the basis of susceptibility to specific DNA damaging agents, and assess the most effective approaches to diminishing DNA repair in order to enhance killing of tumor cells by chemotherapy and radiotherapy.

The first Princess Takamatsu International Symposium was initiated in 1970 by the late Princess Kikuko Takamatsu's strong wish to reduce the incidence of cancer throughout the world. Princess Takamatsu helped to organize these symposia, obtained funding, and hosted dinners for the conferees at her residence. These annual symposia and other activities of the Princess Takamatsu Cancer Research Fund have been continued and supported by recurrent private donations in admiration of Princess Takamatsu's wishes to conquer cancer. The current patron, H.I.H. Prince Tomohito of Mikasa, was unable to attend and partake in the elegant traditions that characterize these meetings because of illness. The meeting this year was held in a friendly and cooperative atmosphere with intensive discussions and comments among the participants.

Takashi Sugimura (National Cancer Center, Tokyo, Japan; Advisor of the Symposium) opened the meeting by summarizing the themes and identifying recent participants in the Princess Takamatsu International Symposia. He considered the extensive contribution of the Japanese National Cancer Center Research Institute to defining the role of poly (ADP-ribose) polymerase (PARP) in cellular metabolism, the identification of carcinogenic heterocyclic amines produced by cooking, and studies on anticancer activities of naturally occurring toxins. Sugimura's approach to cancer

Authors' Affiliations: Departments of ¹Pathology and ²Biochemistry, University of Washington, Seattle, Washington and ³Laboratory Animal Resource Center, University of Tsukuba, Tsukuba, Ibaraki, Japan

Note: L.A. Loeb was the International Organizer of the Symposium and S. Nishimura was the Chairman of the Organizing Committee of the Symposium.

Corresponding Author: Lawrence A. Loeb, Department of Pathology, University of Washington, Box 357705, Seattle, WA 98195-7705. Phone: 206-543-6015; Fax: 206-543-3967; E-mail: laloeb@u.washington.edu.

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research has been to gain insights from the differences in biological processes among organisms and apply these insights to designing therapies for human cancer.

Mutations and Cancer

Lawrence Loeb (University of Washington, Seattle, WA) in his introduction, considered the evidence for, and the implication of, a mutator phenotype in human cancers. His conjecture was that the low mutation rates in normal cells are insufficient to account for the large numbers of mutations found in human cancers. The initial mutator phenotype hypothesis, presented in 1974, was based on mutations in DNA polymerases that render them error-prone, and mutations in DNA repair enzymes that diminish the removal of potentially mutagenic DNA lesions. With the increasing number of genes that encode products required for the maintenance of genetic stability, the mutator phenotype hypothesis has been broadened to include genes involved in multiple types of DNA transactions. Mutations in these genes can result in an exponential increase in mutations throughout the genome, among which are mutations in oncogenes, tumor suppressor genes, and additional genetic stability genes. Recent support for a mutator phenotype in cancer comes from two sources: measurements of random mutations in introns of human tumors and from DNA sequencing of human tumors. The frequency of random mutations in normal human tissues is less than 1 in 10^9 base pairs. There is a more than 100-fold increase in the frequency of random mutations in many of the tumors that have been analyzed. The Human Cancer Genome Sequence was formulated to identify clonal mutations commonly present in tumors and thus provide new targets for chemotherapy. Instead, these studies increasingly have documented the enormous number of mutations that are present throughout the cancer genome and thus provide strong evidence that cancers exhibit a mutator phenotype. Most recent studies indicate that cells from diverse human tumors have thousands of different clonal mutations. Can one alter mutation rates in tumor cells? A decreased rate of mutagenesis in tumors could prolong the life span of cancer patients (treatment by delay).

DNA Damage

Each normal cell in our body is believed to be exposed to as many as 50,000 DNA damaging events per day. A major source of DNA damage is oxygen free radicals, and one of the most prevalent DNA adducts resultant from oxygen-mediated damage is 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). This oxygen-modified base was discovered by Susumu Nishimura and Hiroshi Kasai in 1983. Accumulating evidence presented at the symposium suggests that 8-hydroxyguanine is involved in the induction of various human cancers. The primacy of 8-hydroxyguanine as a key lesion in DNA is reflected by the presence of multiple enzymes involved in its excision. Sankar Mitra (University of Texas, Galveston, TX) showed that NEIL1, a homologue of OGG1, is a base excision repair enzyme that

removes 8-hydroxyguanine exclusively from the replicating genome. Yusaku Nakabeppu (Kyushu University, Fukuoka, Japan) reported that the excessive accumulation of 8-hydroxyguanine in DNA induces cell death or apoptosis. Teruhisa Tsuzuki (Kyushu University, Fukuoka, Japan) showed that knockout of the *Mth1* and *Msh2* genes in mice significantly enhances intestinal tumorigenesis, similar to that observed in human hereditary colon cancers. Thus, it is likely that both base-excision repair (BER) and mismatch repair (MMR) enzymes are crucial to guard the genome against 8-hydroxyguanine and related adducts.

DNA damage by environmental agents has been extensively implicated in causing human cancers. Hitoshi Nakagama (National Cancer Center Research Institute, Tokyo, Japan; Organizing Committee of the Symposium member) analyzed the mutations and tumors induced by PhIP, a heterocyclic amine present in cooked meat and fish. Minoru Takata (Kyoto University, Kyoto, Japan) reported on a high throughput method to identify carcinogens using chicken DT-40 cells that contain mutations in DNA repair enzymes. Miroslav Radman (University of Paris 5-7, Descartes Medical School, INSERM U-571, Paris, France, and Mediterranean Institute for Life Sciences, Split, Croatia) analyzed mechanisms used by organisms that are highly resistant to radiation. He proposed that “intrinsic aging” and age-associated diseases are the result of progressive oxidation of the proteome.

Mechanisms of DNA Repair

The repair of small nucleotide adducts in DNA is primarily mediated by BER and includes recognition by specific DNA glycosylases with overlapping specificities and re-synthesis by DNA polymerase beta (Pol β). Data presented at this meeting suggested that these overlapping specificities evolved to prevent mutations and cancer. Tomas Lindahl (Clare Hall Laboratories, London, U.K.) and Yusaku Nakabeppu (Kyushu University, Fukuoka, Japan) observed that mice lacking DNA glycosylase, OGG1, do not exhibit an increase in cancer frequency. However, if the expression of the backup enzyme, MUYH, is also diminished there is a 50% increase in malignancies. A similar situation exists with UNG and SMUG-1.

Samuel Wilson (National Institute of Environmental Health Sciences, Research Triangle Park, NC) in his Nakahara Memorial Lecture, presented the three-dimensional structure of Pol β with 8-hydroxyguanine in the catalytic site. From this detailed picture, we can now infer a molecular mechanism for misincorporation of adenine opposite 8-hydroxyguanine. The complexity of even the simplest mechanism of DNA repair, BER, was brought into focus by Eugenia Dogliotti (Istituto Superiore di Sanità, Rome, Italy) in studies showing the up-regulation of Pol β in cells that exhibit microsatellite instability, and by Sankar Mitra, who reported on the isolation of a mega-Dalton DNA repair complex by immune precipitation with antibody against the glycosylase NEIL-1. The purified complex contained, in stoichiometric proportions, both BER and DNA replication proteins as well as associated proteins including DNA polymerase- δ , FEN-1, RPA, PCNA, and DNA ligase-1.

Mismatch repair is a major mechanism for guaranteeing the fidelity of DNA replication. Misincorporated nucleotides are excised immediately after DNA synthesis. Mutations in MMR genes are the cause of hereditary nonpolyposis colon cancer and are associated with some sporadic colon cancers. Clearly, the mismatched nucleotides must be excised only from the newly replicated DNA strand; the signal for this selectivity is well established in prokaryotic cells but remains to be further defined in eukaryotes. Paul Modrich (Duke University, Durham, NC) established a system of purified mammalian proteins that carries out MMR *in vitro*. MutS α , RFC, PCNA, and EXO1 are sufficient to degrade the DNA stretch between a preexisting nick and the mismatch in a 5'→3' direction. MMR directed by a 3' nick requires, in addition, the latent endonucleolytic activity of MutL α , which introduces additional breaks into the nicked strand and thus generates new loading sites for EXO1. Another *in vitro* system, independent of EXO1, was also presented. It contains MutS α , RFC, and PCNA, also RPA and Pol δ , as well as FEN-1 that facilitates strand displacement.

The influence of structure on DNA repair was explored both at the level of noncanonical DNA structures in human DNA and the effects of chromatin remodeling. Karen Vasquez (University of Texas, Smithville, TX) found that multiple segments of H-DNA and Z-DNA in the human genome map to chromosomal breakpoints in human cancers. The scanning of the genome by RNA polymerase detects both blocking adducts and noncanonical DNA structures that serve to initiate transcription-coupled DNA repair. Philip Hanawalt (Stanford University, Stanford, CA), the discoverer of transcription-coupled DNA repair, postulated that noncanonical DNA segments cause multiple rounds of DNA repair and the entire process might account for mutations and rearrangements at these structures. The dependency of DNA repair on structure was further reinforced by a series of elegant studies by Akira Yasui (Tohoku University, Sendai, Japan). He quantified the time dependence of protein localization after DNA damage by laser micro-irradiation, and reported that, in addition to DNA repair proteins, there is an accumulation of chromatin remodeling factors implying that these factors may be future candidates for cancer therapy. This complexity of DNA repair was further considered by Leona Samson (Massachusetts Institute of Technology, Cambridge, MA). Inflammation results in DNA damage, alters multiple DNA repair pathways, and is associated with an increase in the incidence of 20 to 30% of human cancers. Her approach is to analyze the global response to DNA damage and interactions between multiple pathways in different eukaryotic organisms in response to DNA alkylating agents.

DNA Polymerases

In a series of studies with mutant replicative DNA polymerases, Thomas Kunkel (National Institute of Environmental Health Sciences, Research Triangle Park, NC) presented experiments to establish that Pol δ and Pol ϵ synthesize the lagging and leading DNA strands in eukaryotic cells, respectively. The alterations in the spectrum of mutations produced

by specific mutant DNA polymerases *in vitro* correlated with the spectrum of mutations observed during DNA replication of the lagging and leading strands *in vivo*.

So far, the only DNA polymerase found to be mutated frequently in human cancers is Pol β , which is believed to function predominantly in base excision repair. Mutations in Pol β are found in 30% of human stomach cancers. Joann Sweasy (Yale University, New Haven, CT) has shown that cells harboring a catalytically defective Pol β variant (present in 3% of the human population) are genetically unstable. The variant produces frameshift mutations; cells that overexpress the variant undergo chromosomal fusions and transformation *in vivo*. Future studies on the frequency of cancer in people that harbor this variant are clearly indicated.

In the past 10 years, the repertoire of DNA polymerases in eukaryotic cells has dramatically expanded. The Y-family of DNA polymerases, first identified by Fumio Hanaoka (Gakushuin University, Tokyo, Japan), are error-prone, and efficiently copy past bulky DNA adducts *in vitro*. The first of these polymerases, Pol η , is lacking in human cells from patients with hereditary XP, a disease characterized by UV-induced skin tumors. He showed that mice lacking both Pols η and ι also develop sarcomas upon UV-irradiation. The endogenous DNA alterations that have been the selective driving force for the members of the Y-family DNA polymerases have not been determined. Studies by Takehiko Nohmi (National Institutes of Health Sciences, Tokyo, Japan) indicate that Pol κ , and bacterial orthologs are efficient in copying past benzo[a]pyrene adducts in DNA. In contrast, Errol Friedberg (University of Texas, Dallas, TX) postulated that the natural "cognate" substrate is a cholesterol derivative that forms DNA adducts. Alan Lehmann (University of Sussex, Brighton, U.K.) projected a more complex picture, one in which Y-family DNA polymerases are organized in replication factories by binding to PCNA, and modulated by both ubiquitination and phosphorylation.

The fact that mutant replicative DNA polymerases could drive carcinogenesis in mice was established by Bradley Preston (University of Washington, Seattle, WA) using mice expressing mutations in Pol δ or ϵ that are defective in proof reading. These mutant enzymes confer a mutator phenotype and exhibit a high incidence of spontaneous cancers. Surprisingly, the cancer tissue specificity is different. Pol δ *exo*^{-/-} mice develop T-cell lymphomas, lung, and skin cancers, whereas Pol ϵ *exo*^{-/-} mice develop B-cell lymphomas, intestinal cancers, and sarcomas.

Kiyoji Tanaka (Osaka University, Osaka, Japan) reported his latest results on xeroderma pigmentosum (XP). Eight genetic complementation groups are known in XP. It is noteworthy that a variety of symptoms in XP and in related inherited diseases, Cockayne syndrome and trichothiodystrophy, cannot be explained by their nucleotide excision repair deficiency alone. He showed that XP complementation group G (XPG) forms a stable complex with transcription factor II H (TFIIH) and functions in maintaining the architecture of TFIIH, indicating that XPG also functions in transcription. Alex van der Eb (Crucell B.V., Leiden, The Netherlands) was one of the pioneers in studying XP and senescence in

diploid human fibroblasts. He exposed human fibroblasts that exhibit an extended life span but do not become immortal to high-LET (low energy transfer) radiation. These cells showed modest increase in p53 in the latest passage before senescence, and a sharp increase in p16 and p21 prior to senescence.

Targeting DNA repair

DNA repair processes are in part regulated by posttranscriptional modification of proteins. Minoru Takata (Kyoto University, Kyoto, Japan) presented studies indicating that both phosphorylation and ubiquitination regulate genes in the Fanconi anemia pathway; mutations in these genes result in malignant tumors and progressive bone marrow failure. Yukari Totsuka (National Cancer Center Research Institute, Tokyo, Japan) presented studies indicating that Pierisin-1, a 96-kDa protein derived from the cabbage butterfly, ribosylates the N² amino group of 2'-dG in DNA and is a potent inducer of apoptosis in mammalian cells. The importance of post-translational modification of DNA repair proteins by PolyCADP-ribose polymerase PARP-1 was emphasized by Mitsuko Masutani (National Cancer Center Research Institute, Tokyo, Japan; a member of the Organizing Committee of the Symposium). PARP-1 is involved in multiple DNA repair pathways; *parp*^{-/-} mice exhibit an increase in deletion mutations and develop multiple tumors after exposure to DNA alkylating agents. Drugs that inhibit PARP-1 now provide a paradigm for a new approach to cancer chemotherapy. Nicola Curtin (Newcastle University, Newcastle upon Tyne, U.K.) and Sydney Shall (King's College, London, U.K.) showed that PARP inhibitors are effective anticancer agents, particularly for breast cancer patients lacking BRCA1 and BRCA2. Considering the involvement of PARP-1 in activation of multiple DNA repair pathways, PARP-1 inhibitors may be effective in enhancing the effects of many agents that damage DNA. It should be noted that poly (ADP ribose) and its polymerase were discovered by Takashi Sugimura more than 40 years ago, and in a recent issue of *Science*, Bruce Alberts pointed out the strong therapeutic benefit of PARP inhibitors when used on tumors with defects in the DNA repair pathway. Agents that inhibit DNA repair pathways including the nonhomologous end joining (NHEJ) pathway are being investigated by Takashi Kohno (National Cancer Center Research Institute, Tokyo, Japan) to increase the effectiveness of irradiation to tumors and of anticancer drugs that induce double-strand breaks. Yasuhiro Furuichi (GeneCare Research Institute, Kamakura, Japan) designed a small RNA to inhibit RECQL1 helicase, which is involved in DNA repair-recombination pathways. The siRNA, together with liposomes, selectively prevented cancer cell proliferation in various mouse models of human cancer.

It has been argued that cancers arise from pluripotent stem cells and become "cancer stem cells." Peter Stambrook (University of Cincinnati College of Medicine, Cincinnati, OH) postulated and presented evidence that normal stem cells replicate their DNA with exceptional accuracy, greater than that exhibited by differentiated cells. The hypersensitivity

of normal stem cells to DNA damaging agents and their use of recombination-mediated repair provides mechanisms to maintain pristine populations. Stanton Gerson (Case Western Reserve University, Cleveland, OH) analyzed hematopoietic reconstitution by normal and tumor cells with deficits in DNA repair pathways. These studies reveal an age-dependent loss of DNA repair that precedes the development of hematologic malignancies. Mary-Claire King (University of Washington, Seattle, WA) pointed out the importance of DNA repair in human breast cancer; most of the 10 known genes mutated in hereditary human breast cancers are involved in DNA repair. Each of these genes is inactivated by a spectrum of mutations that are different in individual patients. This extraordinary heterogeneity presents a scientific challenge for both genetic counseling and for gene therapy.

General Discussion and New Directions

In general, discussions were centered on issues concerning the relationship of DNA repair to carcinogenesis. The thousands of DNA damaging events per cell per day require extensive and accurate DNA repair processes. The functional redundancy of DNA repair enzymes and even pathways has provided flexibility to repair diverse alterations in DNA and to meet the threats imposed by environmental changes. The crystal structure of DNA repair enzymes has influenced approaches to the design of new molecules that can target these proteins even if they are present in large complexes that patrol the genome. Despite this detailed three-dimensional knowledge, one of the authors of this meeting report argued that we still do not fully understand how DNA repair enzymes patrol DNA without excising normal nucleotides or how DNA polymerases distinguish a correct nucleotide from an incorrect one. With increasing understanding of the molecular mechanism of how chemotherapeutic agents act, we can more incisively synthesize analogous compounds with higher specificities. Poly-ADP-ribosylation by PARP inhibitors or termination of DNA replication by 5-fluorouracil has been studied extensively, and we still lack an adequate understanding of the mechanism of action of these important chemotherapeutic agents.

Multiple presentations indicated that the substrate specificity of DNA repair enzymes needs to be further delineated for designing inhibitors that target cells with accumulated DNA damage. This need was brought into focus by Dr. Errol Friedberg's presentation on the hunt for an endogenous substrate for DNA Pol κ . He might argue that the fact that it removes benz[a]pyrene might be irrelevant to the evolution of the enzyme. A more attractive endogenous substrate might be cholesterol adducts in DNA. Overlapping substrate specificities were further indicated by the multiplicity of enzymes that need to be disabled in order for oxidative DNA damage to result either in mutations, or in carcinogenesis. The discussion also centered upon the lack of reporting mutations in DNA repair enzymes and DNA polymerases in the Human Cancer Genome Atlas. Two arguments were brought forth. First, there are overlapping specificities; changes in the expression of a protein might not provide a selective advantage to the cells, even though it would generate

a large number of other mutations that would provide such an advantage. Second, it was argued that many of these mutations, while causing other mutations in cells, would have a clonal disadvantage, and thus would never be the most prevalent mutated genes in a population of cells and, therefore, not be detected by current methods of DNA sequencing. It is important to realize that current sequencing methodologies score for the most frequent substitutions at any position in a population of DNA molecules, and that mutations present in individual tumor cells or in a small percentage of malignant cells within a tumor may not be detected. Yet it is these mutations that could account for tumor heterogeneity and the rapid emergence of resistance to chemotherapeutic agents.

The final presentation was by Susumu Nishimura (University of Tsukuba, Tsukuba, Japan) on the impediments in discovering new anticancer drugs. The current molecular targeting strategy is focused on gene products that are specifically, or more highly, expressed in cancer cells and are required for tumor cell viability. Takashi Sugimura suggested that for many of these anticancer agents the so-called side effects are, in reality, their main effects; their anticancer effects are, in reality, their side effects. Moreover, Susumu Nishimura argued that many drugs with anticancer effects directed against specific molecular targets have limited therapeutic effectiveness in spite of their high cost. In most cases, molecular targeting by anticancer drugs is not highly specific to tumor tissues. The extension of survival by newly approved drugs may be limited to a few months or weeks, concomitant with a decreased quality of life. Many of the failings in cancer drug development arise from heavy reliance on animal models and also from over emphasis on the effects of drugs targeted against signal transduction pathways. By analogy, the Tokyo subway map reveals many interconnections similar to cellular signal transduction pathways. If one train is stopped, there is a temporary interruption before passengers find alternative routes. Thus, cancer cells have

evolved alternative pathways to thwart the effectiveness of the long-term administration of anticancer drugs against single molecular targets. In addition, the present preclinical reliance on experimental animal models, including tumor xenografts in nude mice or sophisticated genetically modified animal models, has severe limitations. For example, farnesyl-transferase inhibitors that were highly effective in MMTV-ras oncomice showed almost no efficacy in later clinical trials. The discrepancy may be due to the fact that growth of tumors in the mouse model is much faster than that of actual human cancers, thus exaggerating the efficacy of the inhibitors. Dr. Nishimura also emphasized the use of other tumor-specific phenotypes that may not be relevant to carcinogenesis. For example, the ABC transporter BCRP is reduced in many human cancers. If a drug is excreted by this transporter, it accumulates more in tumors, thus enhancing the efficacy of the drug.

In summary, this meeting emphasized the importance of understanding the mechanisms of DNA repair in normal and malignant human cells. Errors in DNA repair may be fundamental to mutation accumulation during tumor progression and provide a repository of mutant cells within a tumor that are resistant to chemotherapy. Inhibition of DNA repair provides an important mechanism for enhancing the therapeutic efficacy of many commonly used anticancer agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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