Chromatin and Cancer: The Eleventh International Symposium of the Hiroshima Cancer Seminar, November 2001

Wataru Yasui,1 Kazuhiko Igarashi,2 Masamoto Kanno3 and Eiichi Tahara4
1Department of Molecular Pathology, 2Department of Biomedical Chemistry, 3Department of Immunology, Hiroshima University Graduate School of Biomedical Sciences, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551 and 4Radiation Effects Research Foundation, 5-2 Hijiyama-Koen, Minami-ku, Hiroshima 732-0815

The Eleventh International Symposium of the Hiroshima Cancer Seminar (HCS) Foundation was held on 3 November 2001 at the International Conference Center, Hiroshima. The symposium consisted of seven special lectures; about 240 people were present and there were presentations and discussions on gene diagnosis. Prior to this symposium, an Open Lecture to the public was held on 27 October 2001. Kazuo Tajima (Aichi Cancer Center, Nagoya) and Masanori Fukushima (Kyoto University, Kyoto) spoke about cancer prevention and treatment to more than 180 people.

Wataru Yasui (Hiroshima University, Hiroshima), Chairman of the Organizing Committee of the Eleventh International Symposium, and Executive Director of the HCS Foundation, gave an opening address. Yasui briefly described the background and purpose of this series of symposia. Since the establishment of the HCS Foundation in 1992, annual international symposia have been organized to create an opportunity for basic scientists and clinical researchers to exchange ideas on cancer research, cancer prevention and cancer therapy. This year, the organizing committee planned to explore the very important issue of “Chromatin and Cancer.” Cancer researchers believe that epigenetic switches for cancer-related genes participate deeply in cancer development and progression. Among various epigenetic alterations, deregulation of gene expression through modifying chromatin structure by DNA methylation and histone acetylation has been highlighted, and other novel mechanisms must be involved. Knowledge of this topic will enhance our ability to control gene expression and its abnormalities in human cancers.

Special lectures on chromatin and cancer

Rudolf Grosschedl (University of Munich, Munich, Germany) described the mechanism by which enhancers and locus control regions (LCR) can augment the activity of linked promoters over large distances in nuclear chromatin. Regulation of higher-order chromatin structure by DNA methylation and nuclear matrix attachment regions (MARs) is involved. Interactions between enhancer- and promoter-binding proteins by DNA looping can result in reciprocal stabilization of protein complexes and recruitment of RNA polymerases. In nuclear chromatin, in which the binding of proteins to their cognate sites is impaired, chromatin-based mechanisms, including the spreading of alterations in chromatin structure to distal sites, may contribute to enhancer function. He took the immunoglobulin µ heavy chain locus as a model, and clearly demonstrated the importance of MARs, and also the correlation of the gene expression with DNA methylation and histone acetylation. The requirement of MARs in augmenting the enhancer-mediated activation of distal promoters can be observed in germ line transformation assays of the mouse, but not in stably transfected tissue culture cells. In early mouse development, DNA is extensively methylated, providing a repressed state of gene expression. A role of MARs in antagonizing the repressive effects of DNA methylation on long-range enhancer-promoter interactions was inferred from experiments in which µ genes were methylated in vitro prior to transfection into tissue culture cells. Premethylation of the µ gene was found to impart a requirement for MARs in activating the VH promoter in stably transfected B cells and to mimic the requirement for MARs in transgenic mice. He next described the role of protein inhibitor of activated STAT (PIAS) in the transcription machinery of lymphoid enhancer-binding factor-1 (LEF-1)/TCF, which is a crucial target of the Wnt signaling pathway. PIAS contains a ring domain and nuclear localization signal. PIAS associates with SUMO-modified LEF-1 via its ring domain. It represses β-catenin-mediated transcriptional activation independent of LEF-1 sumoylation sites.

Kazuhiko Igarashi (Hiroshima University, Hiroshima) presented data showing that architectural transcription factor Bach2 is a potential B cell-specific tumor suppressor. There are increasing examples of architectural factors that change higher-order structures of DNA and either enhance or repress gene expression. He first demonstrated that Bach1, a transcription factor with a BTB (broad complex, tramtrack, bric-a-brac/poxvirus and zinc finger) domain, mediates long-range interactions of cis-DNA elements by forming DNA loops through the BTB/POZ domain, indicating that it is an architectural transcription factor. He next suggested that B cell-specific factor Bach2, a relative of Bach1, might be a potential B-cell specific tumor sup-
pressor. Under normal culture conditions, Bach2 accumulates within the cytoplasmic region due to its Crm1-dependent nuclear export. Oxidative stress represses the export of Bach2, causing its nuclear accumulation. Overexpression studies indicated that Bach2 sensitizes cells toward oxidative stress-induced death. Full execution of the cell death program by Bach2 required its BTB/POZ domain, suggesting that the BTB domain-mediated reaction is important for oxidative stress-induced cell death. Under oxidative stress, PML protein and Bach2 are co-localized in the nucleus. Since Bach2 binds to DNA as heterodimers with the Maf oncoproteins to repress the 12-O-tetradecanoylphorbol-13-acetate (TPA)-responsive element, Bach2 may play a role as a tumor suppressor. This possibility is also suggested by the presence of Bach2 in the chromosomal region that undergoes frequent loss in B cell lymphomas, LOH of bach2 in B cell lymphomas, and induction of Bach2 expression in ST157I-treated CML cells. Further studies along this line may establish a connection between oxidative stress, cell death, and tumor progression.

Takashi Ito (Saitama Medical School, Saitama) examined nucleosome assembly and remodeling by nucleosome assembly protein-1 (NAP-1) and ATP-utilizing, chromatin assembly and remodeling factor (ACF) in a purified recombinant system. The assembly of genomic DNA and histones into chromatin is a fundamental process of eukaryotes that affects a broad range of biological phenomena including DNA replication, DNA repair and gene expression. Negatively supercoiled templates reveal distinct roles for NAP-1 and ACF in histone deposition and the formation of an ordered nucleosomal array. NAP-1 efficiently deposits histones onto supercoiled plasmids. Furthermore, NAP-1 exhibits a greater affinity for histones H2A and H2B than does naked DNA, but in the presence of H3 and H4, H2A and H2B are transferred from NAP-1 to the plasmid templates. ACF, composed of ISWI and Acf1, modulates nucleosomal spacing, and can do so well after histone deposition by NAP-1. Order-of-addition experiments indicate that prior activator-mediated, ATP-dependent chromatin remodeling by ACF is required for the acetylation of nucleosomal histones by p300. Chromatin remodeling, which requires a transcriptional activator, ACF and ATP, is an early step in the transcriptional process that regulates subsequent core histone acetylation. The acetylation of histones by p300 facilitates that transfer of H2A-H2B from nucleosomes to NAP-1. These results indicate a precise role for histone acetylation, namely to alter the structure of nucleosomes (e.g., to facilitate the loss of H2A-H2B dimers) that have been previously remodeled by the action of ATP-dependent chromatin remodeling complexes. He thus clearly demonstrated that transcription from the chromatin template is ordered and sequential, with precise timing and roles for ATP-dependent chromatin remodeling, subsequent histone acetylation and alteration in nucleosome structure.

Peter A. Jones (University of Southern California, Los Angeles, CA) described the importance of DNA methylation changes in human cancer. The methylation of CpG islands in promoter regions serves to ensure permanent gene silencing that is also linked with modifying chromosome structure through interaction between methyl-CpG binding protein (MeCP) and histone deacetylases. Scanning the genome by using a methylation-sensitive, arbitrarily primed PCR technique (MS-AP-PCR) demonstrated that a generalized defect in methylation patterns occurs early in malignant transformation, resulting in the aberrant methylation of CpG islands located in the transcribed regions of genes. He examined CpG island methylation in the promoter as well as exons of the p15 (INK4B) and p16 (INK4A) genes in colorectal tumors, leukemias and non-cancerous tissues or blood. While methylation of exonic CpG islands was detected even in normal colonic tissues and blood samples, two different patterns of promoter methylation distinguished leukemias from colorectal cancer. The exonic methylation does not serve to silence the genes, but rather indicates the presence of a methylation defect. Further alterations in methylation, which can occur within the promoter regions of genes, results in transcriptional silencing and this process has a significant role in cancer development. The de novo methylation of CpG islands requires active cell division and occurs in the S-phase, and continued cell division during the process of carcinogenesis is implicated in abnormal CpG island methylation, resulting ultimately in the silencing of gene expression important for cancer development. Therefore, methylation must be a good therapeutic target. The DNA demethylating agent Zebularine (Zeb) inhibits CpG island methylation of the p16 gene promoter, re-induces p16 expression and induces growth arrest of T24 bladder carcinoma cells. Zeb was confirmed to be an antitumor agent in a mouse system. For the practical usage of Zeb in cancer therapy, a prolonged demethylating effect must be guaranteed.

Tony Kouzarides (University of Cambridge, Cambridge, UK) presented the involvement of chromatin modifying enzymes in transcriptional control. A set of enzymes, acetylases and deacetylases, has important biological roles in controlling cell proliferation and differentiation through modifying chromatin structure, resulting in altered gene expression. In addition to histone acetylation, distinct modifications such as lysine methylation are also deeply involved in transcriptional regulation. Several lysines and arginines are known to be methylated on histone H3 and histone H4. SUV39H1, a mammalian homologue of Drosophila Su(var)3-9, is an enzyme that methylates lysine 9 of histone H3 and generates a binding site for heterochromatin protein 1 (HP1).
transcriptional silencing at heterochromatin sites. Methylation is defined as a process which sets up the heterochromatically repressed state and HP1 is the protein that executes this silencing, which is not unique to heterochromatin, but also takes place at euchromatic sites. Retinoblastoma protein (RB) recruits SUV39H1 and HP1 to the E2F-regulated cyclin E promoter, resulting in transcriptional repression via methylation of a specific nucleosome. On the other hand, SET1, a human set1 family member, methylates lysine 4 of the histone tail, and this methylation displaces the NuRD complex to positively regulate transcription. Arginines in the histone tail are also methylated to modify transcription. CARM1 is an arginine methylase which methylates arginine 17 of histone H3. The promoter of estrogen-dependent gene pS2 is activated by arginine 17 methylation by CARM1, which binds to histone acetylase, p300. These observations indicate that histone methylation on either lysines or arginines and HP1 association are common mechanisms for transcriptional regulation, together with alteration of histone acetylation.

Mitsuo Oshimura (Tottori University, Tottori) described epigenetic modification of genomic imprinting in relation to cancer. Loss of imprinting (LOI) is one of the important epigenetic abnormalities found in human cancers. For instance, LOIs of IGF2 and PEG1/MEST were frequently associated with lung adenocarcinoma. PEG3 was epigenetically down-regulated in a considerable number of glioma cells. An imprinted antisense transcript within the KvLQT1 locus has been recently identified on a human chromosome 11p15 region. The transcript, called LIT1 (long QT intronic transcript 1), is expressed preferentially from the paternal allele and is expressed in most human tissues, while an intronic CpG island is specifically methylated on the silent maternal allele. Wilms tumors exhibit normal imprinting of LIT1 and normal differential methylation, while most of the tumors show LOI of IGF2, suggesting that the imprinted gene domain at the KvLQT1 locus is discordantly regulated in cancer from the imprinted domain at the IGF2 locus. Considering that the expression patterns of the LIT1 and IGF2 are frequently disrupted in colorectal cancer, both must participate in cancer development. A targeted deletion of the LIT1 CpG island abolishes LIT1 expression on the paternal chromosome, accompanied by activation of the normally silent paternal alleles of multiple imprinted loci at the centromeric domain, including KvLQT1 and p57kip2. This deletion does not affect the imprinting of H19 and IGF2 located at the telomeric end of the cluster, indicating that the LIT1 CpG island can act as a negative regulator in cis for coordinate imprinting at the centromeric domain. Therefore, epigenetic abnormalities in modifying imprinted genes might be deeply involved in human carcinogenesis.

Masamoto Kanno (Hiroshima University, Hiroshima) described chromatin silencing and tumorigenesis by Polycomb group genes. Polycomb group (PcG) genes are known as negative regulators of the transcriptional memory mechanism through chromatin silencing in Drosophila. Mel-18 is a mammalian homologue of Drosophila PcG gene which encodes a transcriptional repressor via chromatin silencing, and participates in both the cell cycle and cell death. He recently found spontaneous breast cancer development in aged mel-18 +/- mice. The analysis of the remaining mel-18 gene locus revealed no point mutation in the entire mel-18 genomic region and mRNA, indicating that this model does not fit the “two-hit theory.” On the other hand, the destabilization of the huge nuclear Polycomb protein complex (Polycomb body, more than 6 MDa) leads to a deregulation of downstream target genes such as c-myc, cyclins, CDK inhibitors, and bcl-2 family. Kanno then proposed a “haplo-insufficiency model” in which destabilization of Polycomb silencing complex leads normal cells to become tumorigenic by changing the higher-order chromatin structure and silencing pattern of gene expression of downstream target genes. This could be another epigenetic modification (deviation of Polycomb protein concentration) for tumorigenesis.

Eiichi Tahara (Radiation Effects Research Foundation, Hiroshima) made closing remarks. The seven presentations on chromatin and cancer were summarized, and he noted that the active discussion had been fruitful in leading towards a detailed understanding of gene expression and its relation with carcinogenesis through modifying chromatin structure. Genes and molecules involved in these processes might be novel targets for new approaches to cancer diagnosis and therapy.

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