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Review

Epigenomics: Novel Aspect of Genomic Regulation

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An International Symposium on Epigenomics took place at Yonsei University, Korea in December, 2006. The meeting brought to light new aspects of genome regulation by DNA and protein modification.

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Introduction

Epigenetics can be defined as the study on alterations of gene function without changes of DNA sequence. Epigenetic regulation includes a number of processes that modify DNA and histone structures, such as DNA methylation, histone modification and remodeling, as well as gene silencing by small RNAs. Even though, they are not accompanied by changes in DNA sequence, epigenetic modifications are in many cases heritable, and cooperate to accomplish important physiological functions (Lund and van Lohuizen, 2004). For example, X chromosome inactivation, heritable imprinting and multi-potent cell differentiation are regulated by epigenetic changes (Lee *et al*., 2006a; Meshorer and Misteli, 2006; Heard and Disteche, 2006; Wood and Oakey, 2006). Aberrant epigenetic regulation often results in pathological disorders including cancer and chronic inflammatory diseases (Petronis and Petroniene, 2000; Feinberg and Tycko, 2004; Ballestar *et al*., 2006; Ting *et al*., 2006; Wilson *et al*., 2007). Indeed, since alterations of epigenetic regulation have emerged as major etiologic factors in cancer, the elucidation of the epigenetic regulatory mechanisms associated with the cancerous state is expected to provide important data for developing early diagnostic and prognostic markers. For this reason, there is a need to perform systematic searches for epigenetic markers of cancer on a genomic scale.

In order to meet the demand for epigenomic studies of cancer and related diseases, and to share novel insights regarding epigenetic regulation, the first International Symposium on Epigenomics took place at Yonsei University, Korea in December, 2006. The symposium dealt with many interesting questions, such as: how does the regulation of chromatin dynamics including modification and remodeling perform its biological roles? How can knowledge of these dynamics be applied to whole genome association studies? Can the identification of epigenetic status including mainly DNA methylation profiles facilitate the development of biomarkers? Finally, how can epigenetic approaches lead to clinical applications? Although, conclusive answers were not obtained, the symposium demonstrated that epigenomic studies have a promising future, and covered many exciting advances in the epigenomic field. We summarize some of the issues discussed below.

Chromatin modification and remodeling

Energy-dependent chromatin regulation. Histone modification plays an important role in the regulation of gene expression by controlling the packaging of chromatin. Phosphorylation, acetylation, and methylation are the most frequent modifications involved. It is intriguing that all these modifications rely on metabolic substrates, such as ATP, SAM (S-adenosylmethionine), and acetyl-CoA. This implies that if insufficient energy is available, cells may not be able to accomplish chromatin modification and raises the interesting question whether the status of chromatin modification is regulated by the metabolic rate of the cell?

The link between metabolic state and epigenetic gene regulation is still poorly understood. However, Hong-Duk Youn (Seoul National University) suggested that down-regulation of HAT (histone acetyltransferase) activity is a novel energy-dependent regulatory mechanism. CtBP (COOH-terminal binding protein) acts as a transcriptional co-repressor and is known to be a component of large protein complexes containing repressive epigenetic regulators such as HDAC (histone deacetylase), Lys4 demethylase, and Lys9/Lys29

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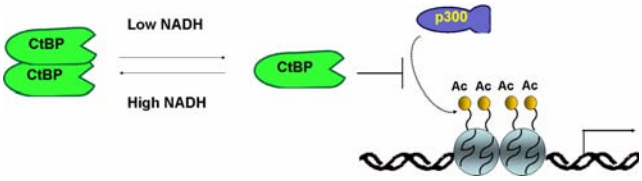

Fig. 1. CtBP, as a corepressor, blocks the accessibility of p300 to acetylated histone by binding to the p300 bromodomain in an NADH-dependent manner.

methylase. Dr. Youn showed that gene transactivation, which is mediated by p300 histone acetyltransferase, is inhibited by physical interaction between CtBP and the bromodomain of p300 (Kim *et al*., 2005) (Fig. 1). Moreover, this inhibition is dependent upon the redox state of NADH, a metabolite that reflects cellular energy status. Dr. Youn's results are consistent with a model in which cellular energy state-dependent signaling is transmitted to the CtBP repressive module, which then affects p300-regulated gene expression.

There is evidence that protein modification by p300 affects the activity of oncogenes including c-Myc, and the expression of cancer-related genes (Shao *et al*., 2005; Faiola *et al*., 2005). Dr Youn's findings suggest that the level of CtBP, and the NADH/NAD^+ ratio, are promising targets for cancer therapy. Also, this insight may aid in the treatment of diseases resulting from misregulation of NADH-associated enzymes. In other words, achieving some alteration of chromatin status by agents that influence epigenetic changes may be more efficient than targeting enzymes themselves.

Chromatin remodeling ATPases. Chromatin remodeling factors, as well as chromatin modifiers, play key roles in the global dynamics of chromatin structure by regulating access of transcription factors to gene regulatory elements. However, how chromatin remodeling processes that affect a broad range of chromatin can regulate the expression of specific genes is not well understood. Dr. Wagner's talk on the plant chromatin remodeling ATPase provided an interesting answer to this question.

Previous work indicated that SPLAYED is an important regulator of the pathway of Arabidopsis reproductive developmental. SPLAYED is a SWI/SNF chromatin remodeling ATPase that regulates transcription factor activity by altering the state of chromatin (Wagner and Meyerowitz, 2002). New data presented by Wagner (University of Pennsylvania) showed that SPLAYED and another SWI/SNF chromatin remodeling ATPase, BRAHMA, have unique, as well as overlapping, or redundant, roles in Arabidopsis and that these two ATPases target a very restricted number of genes. Furthermore, the genes that are involved are tightly regulated and are active in specific developmental pathways. These findings support a role for these two chromatin regulators in plant-specific developmental pathways (Bezhani *et al*., 2007).

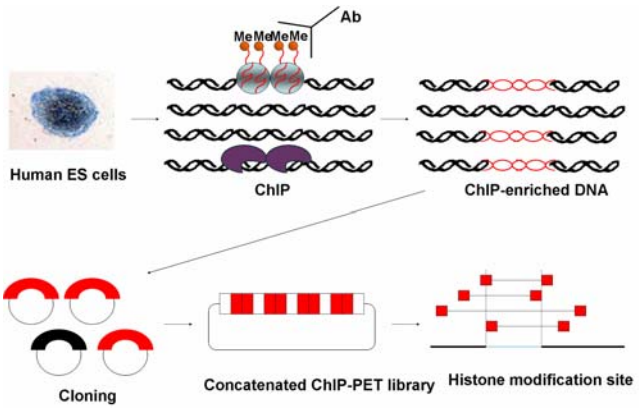

Fig. 2. Schematic diagram of genome-wide mapping of histone modification using ChIP-PET.

These two ATPases may tightly control specific groups of genes by associating with distinct cofactors. In the same context, there is compelling evidence that SWI/SNF complexes contribute to tumorigenesis by interacting directly with certain tumor suppressors and oncogenes (*i.e. RB*, *BRCA1*, c-*MYC*, *MLL*) (Roberts and Orkin, 2004). Although the chromatin remodelers are expected to act globally on the genome, Dr. Wagner's findings point to the striking possibility that distinct modules formed by the combination of specific SWI/SNFs and cofactors can regulate the expression of a few specific genes.

Whole genome mapping of histone modifications. Whole genome analysis is essential for the systems approach to understand complex biological processes. The study of chromatin dynamics in the field of epigenomics is no exception to this rule. This approach has in fact already been employed to investigate the genome-wide "epigenetic code" that operates in various biological processes, including tumorigenesis and differentiation.

Huck Hui Ng (Genome Institute of Singapore) used a novel ChIP-PET procedure (chromatin immunoprecipitation-paired end ditag) for mapping histone modifications over the entire genome of human embryonic stem cells. In this approach DNA immunoprecipitated by antibody to methylated histone is cloned into library vectors, and PETs created by concatenation are sequenced to characterize the immunoprecipitated DNA (Fig. 2). This approach can be combined with several genome-wide profiling methods to analyze the complex molecular networks more systemically (Loh *et al*., 2006). The mapping of histone modifications combined with gene expression profiling and DNA methylome and miRNA analyses should provide an exciting opportunity to elucidate the complex and dynamic processes underlying pluripotency and self-renewal in embryonic stem cells, as well, perhaps, as the uncontrolled multiplication of cancer cells.

DNA methylation and its therapeutic potential

Genome-wide genetic and epigenetic analysis in HNSCC (head and neck squamous cell carcinoma). Together with defective chromatin modification, altered DNA methylation status appears to be the major epigenetic change observed in many types of cancer. Paul M. Lizardi (Yale University) reported on an interesting feature of the DNA methylation profiles of the CpG islands in head and neck malignancies. The CpG islands examined encompass gene promoters and repetitive elements such as LINEs (long interspersed elements) and Alu repeats. The data obtained revealed hypermethylation of a variety of tumor suppressor genes, and hypomethylation of interspersed repeat loci. Dr. Lizardi and colleagues also attempted to detect changes in gene copy number in the head and neck tumors by using comparative genomic hybridization (CGH) arrays. This work underlines the importance of integrating both types of analysis. Despite the predominant role that epigenetic alterations play in tumorigenesis, the active multiplication of cancer cells is thought to depend on a combination of epigenetic and genetic plasticity (Feinberg *et al*., 2006). The data obtained from several types of HNSCC tumors were compared and analyzed to classify tumors, identify new tumor classes, and determine the prognosis of each class. These investigations will help to provide the basis for precise diagnosis and prognosis of patients suffering from head and neck cancer.

Genome-wide hypomethylation in human glioblastomas. As mentioned above, in addition to the hypermethylation of tumor suppressor gene promoters, global hypomethylation is also a hallmark of cancers. This hypomethylation frequently occurs in the repetitive elements that comprise about half of the human genome. Joseph F. Costello (University of California, San Francisco) reported altered methylation in certain cancers. In human glioblastoma multiforme (GBM), the most common and malignant type of human brain tumor, global 5-methylcytosine content was reduced. Furthermore, DNA hypomethylation was observed on pericentromeric, subtelomeric, and interspersed repetitive sequences. Significant hypomethylation in GBM was also associated with augmented proliferation of the cancerous cells and abnormal MTHFR (methylenetetrahydrofolate reductase) allele status. This abnormal status may disrupt normal methyl-group metabolism (Cadieux *et al*., 2006).

Although DNA demethylating agents have been exploited in recent cancer treatments, they may not be ideal because of the genomic instability induced by hypomethylation of the whole genome. Hence, the development of alternative epigenetic therapies using fine-tuned doses of drugs or combinatorial approaches taking into account genomic stability is essential.

Markers of carcinogen exposure and cancer risk: a novel link between bacteria and DNA methylation? As frequently reported, aberrant DNA methylation of the promoters of some tumor suppressor genes has been observed in cancer cells. These changes in DNA methylation are often induced in response to environmental stimuli. Can pathogens such as bacteria also alter DNA methylation patterns? And do DNA methylation patterns provide a "cellular memory" of past exposure to carcinogens? If so, how accurately can they reveal the likelihood and prognosis of cancer?

Work from Toshikazu Ushijima (National Cancer Center Research Institute) provided some clues. Their studies showed that infection with *Helicobacter pylori* (HP), a key gastric carcinogen, can induce DNA methylation in non-cancerous gastric mucosae. Thus, DNA methylation levels were much higher in cases positive for HP infection than in negative cases. They also showed that methylation levels could be decreased by successful HP eradication therapy (Ushijima, *et al*., 2006). Dr. Ushijima also presented evidence that methylation levels in gastric mucosae are strongly correlated with the risk levels of developing gastric cancers, and thus provide a powerful cancer risk marker (Maekita *et al*., 2006).

Pattern recognition receptors (PRRs) such as TLR and NOD recognize distinct PAMPs (pathogen-associated molecular patterns) in bacteria and viruses, as part of the mammalian innate immune response (Janeway and Medzhitov, 2002). Nod1 in particular is required for recognition of the peptidoglycan introduced by HP infection (Strober, 2006). Consequently, there is likely to be cross-talk between the Nod1-induced inflammatory response and the DNA methylation machinery. This notion introduces a novel therapeutic paradigm and provides reliable evidence for how excessive and chronic inflammation contributes to tumorigenesis at the epigenetic level.

Cancer epigenetics: DNA methylation and small RNAs. A major goal of epigenetic cancer research is to generate new diagnostic tools and to identify targets for therapeutic intervention. Accurate diagnostic tools are important since cancer recurrence is lowest when cancer is detected and treated early. Jingde Zhu (Shanghai Cancer Institute) described the diagnostic potential of DNA methylation analysis for several types of cancer using non-invasive sources of material such as urine and sputum. Dr. Zhu also demonstrated the possibility of combining global methylation profiling, using the CpG island array, and MBD (methyl binding domain protein)-based affinity chromatography. Thus, studies of DNA methylation promise to provide powerful approaches to the diagnosis and treatment of cancer.

In addition to the involvement of DNA methylation in tumorigenesis, the recently discovered regulatory small RNAs, and their powerful epigenetic effects on gene regulation, also appear to play a role in tumorigenesis (Esquela-Kerscher and Slack, 2006). In particular, Dr. Zhu has made a cDNA library of the endogenous miRNA and siRNA from an established hepatocellular carcinoma cell lines and identified several dozen novel miRNAs and similar amount of the siRNA candidates. Their roles in hepatocarcinogenesis are under a close examination at the present time. These research directions

will enable comprehensive investigation of the epigenetic mechanisms involved in cancer.

Epigenetics-targeted cancer therapy. Because cancer is initiated and progresses by a large variety of mechanisms associated with many different biological processes there are a plethora of targets to exploit in therapeutic approaches. There is no doubt that epigenetic silencing, such as that caused by hypermethylation of tumor suppressor gene promoters, and genomic instability caused by global hypomethylation, are major causes of commitment to transformation and tumor progression. Therefore, agents that control these epigenetic changes may yield promising anti-cancer drugs. Tae-You Kim (Seoul National University) described several potential therapeutic avenues related to epigenetic cancer mechanisms. First, Dr. Kim's work addressed a common and immense obstacle to tumor therapy, that of drug resistance. He identified a type of epigenetic therapy that has the potential to overcome resistance to fluorouracil (5-FU). This is significant since 5-FU is the most commonly used cytotoxic agent in the treatment of breast and gastrointestinal cancers. He found that TSA, a common histone deacetylase (HDAC) inhibitor, could reverse 5-FU resistance by down-regulating thymidylate synthase (TS) (Lee *et al.*, 2006b). TS is an oncogene and a well-known target of 5-FU. Because TS overexpression is a key factor in 5-FU resistance, the combination of of an HDAC inhibitor and a cytotoxic agent may provide a powerful new approach to cancer treatment.

Additional studies have demonstrated that some HDAC inhibitors can cause defects in mitosis in a tumor-selective manner. For example, Aurora-A kinase, which regulates chromosomal segregation during mitosis, leads to genomic instability when overexpressed. Importantly, Aurora-A is downregulated when cancer cells are treated with a HDAC inhibitor. Thus, HDAC inhibitors are prime candidates as therapeutic agents in certain cancers.

Since changes in DNA methylation patterns are associated with global cancers, Dr. Kim also has targeted mechanisms of DNA methylation in therapeutic approaches. He used direct DNMT (DNA methyltransferase) - targeted therapy rather than 5-aza-dC (5-azacytidine), which is known to cause DNA strand breakage as well as demethylation effect. He noted that DNMT1-targeted suppression induced promoter demethylation of genes silenced in gastric cancer cells, whereas DNMT3b did not. DNMT1 depletion also inhibited tumor proliferation and induced cell death without any DNA damage. Therefore, DNMT1-targeted therapy appears extremely promising as an approach to the epigenetic mechanism of tumorigenesis.

Concluding remarks

Recently, the journal *Science* suggested that "whole genome association studies" was the area to watch in 2007 (Science, 2006). In this context, epigenetic research at the genomic level is needed to decipher mechanisms of normal development and differentiation as well as the systemic epigenetic changes that occur in a variety of abnormal biological processes, such as cancer. In addition, detailed studies of spatially or temporally distinct systems will provide new information regarding genomic mechanisms and provide the basis for comparison with cancer cell genome studies. For instance, analyses of these systems, including elaboration of the "cancer stem cell hypothesis" as a model for cancer etiology, should be productive. Moreover, the compilation and construction of a series of databases would provide a powerful impetus in this area. Such epigenetic libraries could help devise therapy for individual patients based on the ability to predict the grade and class of each disorder. To realize the full benefits of these tools, the epigenetic databases should be freely accessible and the efforts of the Asian Epigenomic Alliance should be coordinated with those of the International Human Epigenome Consortium, led mainly by the US and Europe.

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