Epigenomics, Sequencing & SNiPs-2013 Meeting

Chromatin Methylation to Disease Biology & Theranostics

July 10 – 11, 2013, Harvard Medical School, Boston, Massachusetts, USA

Krishnarao Appasani

GeneExpression Systems, Inc.

PO Box 540170

Waltham, Massachusetts 02454 USA

Corresponding Author:

Krishnarao Appasani, PhD., MBA.

GeneExpression Systems, Inc.

PO Box 540170, Waltham, Massachusetts 02454 USA

Tel: 781-891-8181; Fax: 781-891-8234

E-mail: DrAppasani@expressgenes.com,

Introduction:

This meeting was the sixth international event of a themed conference that was designed for discussion and for scientific and business collaborations. The meeting was organized by GeneExpression Systems (USA) at the prestigious Harvard Medical School campus. The two-day intensive single-track meeting was arranged in seven scientific sessions, attracted about 100 scientists and brought together industry leaders and entrepreneurs, renowned international scientists from academia and industry. This report covers representative presentations from academia, and from the biotechnology and pharmaceutical industry.

Conrad Waddington, the last Renaissance evolutionary biologist coined the word *'Epigenetics'* in 1942. Epigentics is the phenomenon that describes, *"the heritable changes in gene expression that are not due to changes in DNA sequence".* Most of the non-heritable signals are resided and controlled by chromatin, which is the complex of DNA and protein that makes up chromosomes. Nucleosome is the fundamental unit of chromatin structure, which comprises a core of eight histones around which 146 base pairs of DNA wrapped in 1.75 spherical turns. Histones influence every aspect of DNA function. Chromatin involvement in development, differentiation, disease and genomic imprinting is still unclear and a mystery to biologists.

To understand the recent developments in chromatin biology and its emerging roles in various pathological sates, this theme conference was organized in a timely manner. This meeting differentiates from other events because of its careful integration of relevant emerging sequencing technologies, which is one of the strong technology platforms that has been continuously used today by various scientists to decipher the code of epigenomes in normal and cancer cells. Epigenetics is one of the fundamental mechanisms that involved in embryo development and differentiation of cell types. Applications of genomics approaches in the study of epigenetics to *'study the totality of epigenetic marks in a given cell type'* popularly now named as *'Epigenomics'*. This field has an impact in the modern biomedicine and commercial enterprise for the development of new theranostics (therapeutics and diagnostics) for several human diseases.

Chromatin Biology:

In general, covalent chemical modifications to the DNA and to histones, histone variants, nucleosome positions, small noncoding RNAs and the level of chromatin compaction all contribute to chromosomal structure and to the activity or silencing of genes. These chromatin-level alterations are defined as epigenetic when they are heritable from mother to daughter cell. The great diversity of epigenomes that can arise from a single genome permits a single, totipotent cell to generate the 250 cell types of an adult individual. The epigenetic changes are a prerequisite for reprogramming from one cell type to another during cell differentiation. In addition, maintenance of one particular epigenome during chromatin replication is crucial for clonal expansion of cell lineages and tissues. The conference was inaugurated by **Laurie Jackson-Grusby** (Children's Hospital Boston, USA), who presented an overview of epigenetic regulation in normal and cancer stem cells. Silencing of genes is associated with a small chemical modification to DNA termed DNA methylation, as well as other chemical modifications to proteins that bind to DNA and affect the packaging of DNA in the nucleus. Jackson-Grusby's group have developed mouse models in which the DNA methylation is erased to varying degrees. Her presentation also included interesting work on studying mouse brain tumour model, and developing treatment modalities for medulloblastoma using Hedgehog pathway inhibitor GDC-0449.

The compact conformation of chromatin presents a significant barrier to DNA dependent events such as DNA repair. Consequently, the repair of DNA double-strand breaks requires remodelling of the local chromatin structure at sites of DNA damage. **Brendan Price** (Dana-Farber Cancer Institute, Boston, USA) has showed the increase in H4 acetylation and H2A.Z exchange destabilizes the local chromatin structure, creating open, "relaxed" chromatin structures adjacent to the site of DNA damage. These relaxed chromatin domains then facilitate the further recruitment of DNA repair proteins and modification of the chromatin through histone phosphorylation and ubiquitination. This combination of histone modification and reorganization of the chromatin architecture leads to the assembly of a chromatin template, which is an efficient substrate for the DNA repair machinery. **Mariko Ariyoshi** (Kyoto University, Kyoto, Japan) showed that the SET and RING associated domain of UHRF1, which binds to hemi-

methylated DNA generated during replication, and mediates loading of the DNA methyltransferase 1 for faithful inheritance of DNA methylation pattern. The inter-module linker between tandem tudor domain and plant homeo domain finger maintains interaction with non-modified H3R2 and H3K9me3. Her structural and biochemical results suggest that the phosphorylation of a linker residue can modulate the relative position of the reader modules, which alters the histone H3 binding mode. This imply that the linker region plays a role as a functional switch of UHRF1 involved in multiple regulatory pathways such as maintenance of DNA methylation and transcriptional repression. To understand the biological implications of mononucleosome structure and dynamics **Thomas Bishop** (Louisiana Tech University, Ruston, USA) has developed tools those allowed to generate 3D models of chromatin and entire chromosomes from nucleosome positions. Additionally, he has demonstrated that nucleosome positioning and mis-positioning alters chromatin topology by exposing or hiding DNA defects.

Epigenetic Regulation:

Eukaryotic gene expression is tightly controlled to maintain proper cellular function. Eukaryotic genes are transcribed by RNA polymerase II and processed to generate mature messenger RNA molecules. Although the cellular machineries that carry out these reactions (RNA synthesis and RNA processing) have typically been studied as biochemically distinct reactions, they are, in fact, temporally and spatially organized to coordinately orchestrate the proper production of a fully-processed mRNA. One of the reactions that appears to be tightly coordinated with transcription is pre-mRNA splicing, the removal of non-coding introns from pre-mRNA, a reaction catalyzed by a remarkable macromolecular machine, the spliceosome. One of the critical challenges has been to identify specific factors that coordinate pre-mRNA splicing with transcription, particularly within the context of a chromatin template. **Tracy Johnson,** (University of California, Los Angeles, USA) showed an evidence that histone acetylation by the acetyltransferase Gcn5 influences ATP-dependent rearrangements at the heart of the spliceosome. Additionally her results support a model whereby co-transcriptional spliceosome assembly is intimately coordinated with histone acetylation and chromatin remodeling.

In heterogametic species, the process of dosage compensation is required to equalize transcript levels between the sex chromosomes in males and females. The *Drosophila* Male-Specific Lethal (MSL) complex increases transcript levels on the single male X-chromosome to equal the transcript levels in XX females. However, it is not known how the MSL complex is linked to its DNA recognition elements, the critical first step in dosage compensation. Therefore, **Erica Larschan's** group (Brown University, Providence, USA) has demonstrated that a previously uncharacterized zinc-finger protein, CLAMP Chromatin-Linked Adaptor for MSL Proteins functions as the key link between MSL complex and the X-chromosome. The discovery of CLAMP identifies a critical factor required for the chromosome-specific targeting of dosage compensation, providing new insights into how sub-nuclear domains of coordinate gene regulation are formed within metazoan genomes. On the other hand, **Garegin Papoian** (University of Maryland, College Park, USA) proposed a molecular mechanism for the way Lys-16 acetylation might lead to experimentally observed disruption of compact chromatin fibers. His results suggests that multiple acetylations may be highly non-additive, indicating that combinatorial post-translational modifications may regulate the tails in a very complex way.

Age related macular degeneration (AMD) is the leading cause of irreversible blindness in the elderly population worldwide. In order to study epigenetic regulation in AMD **Lai Wei** (National Eye Institute, Bethesda, USA) undertook DNA methylation microarray analysis on monozygotic and dizygotic twins who were discordant for AMD and identified methylated *IL17RC* promoters as being present only in non-AMD control individuals rather than in AMD patients. In addition, he also showed that the hypo-methylation of the *IL17RC* promoter in AMD patients led to elevated expression of its protein and mRNA in peripheral blood as well as in the retina and choroid, suggesting that the DNA methylation pattern and expression of *IL17RC* may potentially serve as a biomarker for the diagnosis of AMD and likely plays a role in disease pathogenesis.

Epigenomics in Cancer:

While breast cancer is a heterogeneous disease, it is invariably associated with genome wide changes to epigenetic modifications and transcriptional output. Of these changes, reprogramming of the epigenetic code at the promoters of tumour suppressor genes (TSGs), resulting in transcriptional silencing, is probably the most intensely studied epigenetic phenomenon in breast cancer cells. To date, it remains unclear precisely how these epigenetic events are initiated at TSGs, but several models have been proposed. It is hoped that understanding the mechanisms giving rise to transcriptional silencing in breast cancer cells will reveal new targets for anti-cancer therapy. Thus, **Michael Witcher** (The Jewish General Hospital & McGill University, Montreal, Canada) provided new insights into the mechanism whereby CTCF post-translational modification poly(ADP-ribosylation) (known as PARylation) is lost in cancer cells. His data also suggests that loss of CTCF PARylation impairs its function on multiple levels, which led him to predict potential new targets for anti-cancer therapy. **Xiaohong Zhao (**H. Lee Moffitt Cancer Center & University of South Florida, Tampa, USA) presented data on the gene silencing of *myc*-mediated miR-29 and histone modifications in lymphomas. She has investigated the transcriptional and epigenetic repression of miR-29 and other tumour suppressor miRNAs by MYC, HDAC3, and EZH2 in mantle cell lymphoma and other MYC-associated lymphomas. Her results for the first time defined a MYC-mediated miRNA repression mechanism that shed light on MYC lymphomagenesis mechanisms, and revealed promising therapeutic targets for aggressive B-cell malignancies.

Solid tumours exhibit chromosomal rearrangements resulting in gain or loss of multiple chromosomal loci (copy number variation, or CNV), and translocations that occasionally result in the creation of novel chimeric genes. In the case of breast cancer, although most individual tumours each have unique CNV landscape, the breakpoints, as measured over large datasets, appear to be non-randomly distributed in the genome. To shed light on the proximal cause of these breakpoint concentrations, **Nevenka Dimitrova's** group (Philips Research, Briarcliff Manor, USA) have performed a bio-statistical analysis on both high-resolution CNV and methylation data. Her group have identified a set of breakpoint enriched differentially methylated regions those were characterized by altered DNA methylation in cancer compared to normal cells. Head and neck squamous cell carcinoma (HNSCC) is

the sixth most common cancer in the world; currently there are no reliable biomarkers to detect this cancer at an early stage. Since, DNA promoter hyper-methylation of tumour suppressor genes occurs in cancer initiation and progression, **Chamindie Punyadeera** (The University of Queensland Diamantina Institute, Woolloongabba, Australia) adopted saliva-based biomarker approach. She analysed for the expression of CpG hypermethylation related tumour suppressor genes such as: *APC*, *p14*, *RASSF1a*, *DAPK1* and *p16* using sensitive methylation-specific PCR assay in the saliva samples collected from the cancer patients and healthy controls. She concluded that, the salivary DNA methylation biomarkers are clinically useful in detecting head and neck cancer in a non-invasive manner.

Epigenomics in Neurodevelopmental Disorders:

Pharmacotherapy for psychiatric disorders such as schizophrenia, bipolar disorder, and major depression has remained largely unchanged over the past 50 years, highlighting the need for novel target discovery and improved mechanism-based treatments. **Tracey Petryshen** (Massachusetts General Hospital, Boston, USA) examined in mice the impact of chronic, systemic treatment with Compound 60 (Cpd-60), a slow-binding, benzamide-based inhibitor of the class I histone deacetylase (HDAC) enzymes HDAC1 and HDAC2onpsychiatric-related behaviors. Her results provided evidence that selective inhibition of HDAC1 and HDAC2 in brain may provide an epigenetic-based target for the development of improved treatments for psychiatric disorders and other brain disorders with altered chromatin-mediated neuroplasticity. Epigenetics is a reversible system that can be affected by various environmental factors, such as drugs, nutrition, and mental stress, epigenetic disorders also include common diseases induced by environmental factors. **Takeo Kubota** (University of Yamanashi, Yamanashi, Japan) discussed the nature of epigenetic disorders, particularly neurodevelopmental disorders, on the basis of recent findings: (1) susceptible to environmental factors, (2) treatable by taking advantage of their reversible nature, and (3) trans-generational inheritance of epigenetic changes, (i.e., acquired adaptive epigenetic changes that are passed on to offspring).

Alterations in DNA methylation have been suggested to occur at a global, nuclear scale or in certain loci in the context of Alzheimer's disease. Therefore, **Philip De Jager** (Brigham and Women's Hospital, Boston, USA) utilized DNA methylation profiling at genome-wide scale to explore the role of brain's chromatin conformation in the pathophysiology of Alzheimer's disease. A sample of dorsolateral prefrontal cortex was obtained from each of 748 subjects, adopted the Illumina Humanmet 450K platform, and generated data for 486,428 CpG sites distributed throughout the genome for each sample. Using a novel chromatin state map of the frontal cortex, his group has identified 163 CpG sites those are distributed in a number of different chromatin states but appear to be enriched in polycomb-repressed and low-expression loci. His results suggest that there is a coordinated Alzheimer's disease-associated chromatin remodelling in aging brains that is present even in individuals with no cognitive impairment.

Epigenomics in Stem Cells and Metabolic Diseases:

Cytosine methylation in mammals is an epigenetic modification that is largely restricted to CpG dinucleotides and serves multiple critical functions including stable repression of target promoters, maintaining genomic integrity, establishing parent-specific imprinting patterns, and silencing endogenous retrotransposon activity. Generally, only a small fraction of CpGs switches their methylation levels as part of an orchestrated regulatory event. By contrast, DNA methylation is much more dynamic during mouse germ-cell and pre-implantation development. **Alex Meissner** (Harvard Stem Cell Institute & Harvard University, Cambridge, USA) presented data on methylation dynamics in stem cells and development specially by refining genome-scale mapping of DNA methylation in human pluripotent stem cells. Despite the explosive growth of genomic data, functional annotation of the regulatory sequences remains difficult. 'Comparative epigenomics' is an interspecies comparison of epigenomes. **Shu Xiao** (University of California, La Jolla, CA, USA) measured epigenomic profiles in human, mouse, and pig pluripotent stem cells. She suggested that the conserved co-localization of different epigenomic marks can be used to discover regulatory sequences. Additionally, the comparative epigenomics across species, allowed her to investigate regulatory functions.

Brown adipose tissue is attracting substantial attention for its anti-obesity function. However, its development and involvement in metabolic regulation remains uncomprehended. Using a special differentiation medium supplemented with hematopoietic cytokine cocktail, **Kumiko Saeki** (National Center for Global Health and Medicine, Tokyo, Japan) successfully induced a directed differentiation of human pluripotent stem cells, including embryonic stem and induced pluripotent stem cells, into highly functional classical brown adipocytes under a feeder-free and serum-free condition. Interestingly, the differentiated cells showed the characteristics of classical brown adipocytes from morphological and gene/protein expressional standpoints. Moreover, they improved lipid and glucose metabolisms in response to beta-adrenalin receptor agonist treatments. Our technique provides an excellent tool for epigenomics studies on human brown adipocytes development.

Obesity and Type 2 diabetes are common and lead to significant morbidity and mortality. While enormous efforts have been spent looking for the genetic and environmental underpinnings of these conditions, epigenetic causes have been largely overlooked. This is despite a wealth of evidence supporting epigenetic involvement in the etiology of obesity and diabetes. In a Keynote lecture, **Evan Rosen** (Beth Israel Deaconess Medical Center, Boston, MA, USA) summarized the utilization of chromatin state mapping of modified histones in order to predict novel transcriptional pathways in adipocyte development and physiology. He has also extended this approach to metabolic disease, specifically obesity and insulin resistance. Finally, Rosen indicated that a significant degree of epigenomic modification inherent in these conditions, and offer insights into possible therapeutic approaches.

Assays and Therapeutics:

Comprehensive understanding of mechanisms of epigenetic regulation requires identification of molecules bound to genomic regions of interest *in vivo*. However, non-biased methods to identify molecules bound to specific genomic loci *in vivo* are limited. To perform biochemical and molecular biological analysis of specific genomic regions, **Hodaka Fujii (**Osaka University, Osaka, Japan) developed insertional chromatin immunoprecipitation technology to purify the genomic

regions of interest. This method is not only useful for identifying interacting molecules (including genomic DNA, proteins, RNA, and others) with an emphasis on non-biased search using next-generation sequencing, microarrays, mass spectrometry but also useful for the analysis of genome-wide interactions with a specific genomic locus. Research into the role of epigenetics in disease could be significantly accelerated if chemical probes for such targets were available that were suitably selective and permeable for cell-based studies. **Ailan Guo's** group (Cell Signaling Technology, Inc. Danvers, USA) has developed specific motif antibodies against protein modifications that are highly relevant to epigenetic regulation including acetyl lysine antibodies, protein arginine methylation antibodies, and protein lysine methylation antibodies. Using their company's proprietary PTMScan technology, peptide immune-affinity enrichment and mass spectrometric analysis of protein post translational modification sites, they were able to identify protein acetylation and methylation in normal vs diseased tissues.

Pfizer is a member of a public-private partnership led by the Structural Genomics Consortium to help identify a suite of high-quality chemical probes for epigenetic targets. A number of epigenetic enzymes have now been identified that either introduce these epigenetic marks ('writers') or remove them ('erasers'). In addition, regulatory proteins have been discovered that directly recognize histone modification status ('readers') and drive the localization of complexes, which control gene expression. **Dafydd Owen's** (Pfizer Worldwide R&D, Cambridge, USA) presentation highlighted the academic and industry collaboration to discover novel chemical probes for epigenetic proteins those have an important role in human disease.

Genome Engineering & Sequencing Technologies:

Our ability to view and alter biology is progressing at an exponential pace faster even than electronics. Next generation sequencing (fluorescent and nanopore) can be used to assess a variety of DNA modifications. We can now systematically synthesize/edit millions of genomic variants, enabling us to move from correlation to causality studies — connecting genomics + environments via epigenomcs to traits. A growing set of CRISPR technologies enable similar numbers of epi-genetic variants to see where one can drive human pluripotent stem cells. In a keynote lecture, **George Church** (Harvard Medical

School, Boston, MA, USA) summarized the development and applications of CRISPR technologies and 3-D-RNA sequencing library *in situ*. Additionally, he elaborated on the use of Multi-plex Chip synthesis, for examining the role of long non-coding RNAs and epigenomic reprogramming in stem cells. The ability to introduce targeted modifications into genomes and engineer model organisms holds enormous promise for biomedical and biotechnological applications. **Feng Zhang** (Massachusetts Institute of Technology, Cambridge, USA) presented the development of an RNA-guided nuclease adapted from the bacterial CRISPR-Cas immune mechanism for efficient and multiplexable mammalian genome engineering. Through heterologous expression of three minimal components in CRISPR-Cas, he has shown that Cas9 can be programmed by custom RNAs to induce double strand break at endogenous mammalian loci with up to 59% cutting efficiency. In addition, using a single crRNA array to encode a pair of guide sequences, he showed that CRISPR can simultaneously and efficiently cleave multiple sites within the human genome. The tractability and multiplex capability of this system present unique possibilities for practical and therapeutic applications.

In recent years, our understanding of the Human Microbiome has advanced dramatically. What once was terra incognita is now known to be remarkably complex and diverse, with significant impact on human health, development and disease. However, we are still only scratching the surface of this key component of human biology. Advances in understanding the microbiome to date have been largely driven by improvements in technology. **Chad Nusbaum** (The Broad Institute of MIT and Harvard, Cambridge, MA, USA) discussed the role of sequencing technology in furthering insight into the microbiome research. **Barry Merriman** (Life Technologies, Inc. Carlsbad, CA, USA) discussed the latest advances in genome sequencing technology, especially around the history and status of the new Ion Torrent Proton Platform, and how this could be applied in a systematic way to solve genetic diseases for entire populations. Very interestingly, he gave a global perspective of genomics and developed a list of countries or regions those are good candidates to implement genomics based-personalized medicine. The high-throughput assessment of DNA-methylation across the genome is crucial to deepen our insights in the transcriptional regulation of essential genes across the genome. State-of-the-art methods to detect

DNA-methylation on a genome-scale can be roughly divided into two classes: bisulfite and methylation enrichment based methods. Upon bisulfite treatment, unmethylated cytosines are chemically deaminated into uracils, with further base-pairing behaviour as thymines, whereas methylated cytosines remain intact. To reduce costs only targeted regions of the genome are typically assessed using methods as reduced representation (RRBS) and targeted bisulfite sequencing. An alternative cost-efficient, putatively genome-wide, option is to assess only methylated fragments. Methylated DNA-fragments can be captured, after fragmentation, using either antibodies (methylated DNA immunoprecipitation, MeDIP) or Methyl-Binding Domains (MBD). This is typically followed by sequencing (MeDIP-seq or MBD-seq/MethylCap-seq) to obtain a genome-wide map of the sample's methylome. **Wim Van Criekinge** (MDxHealth, Irvine, CA, USA & Leige, Belgium) summarized the technical comparison of different technologies and highlighted his company's proprietary next-generation profiling methodology.

Outstanding Accomplishments and Final thoughts

On behalf of the scientific committee, Krishnarao Appasani (GeneExpression Systems, Inc, USA) honoured **Dr. George Church** (Harvard Medical School, Boston, USA) with '*Epigenomics Innovator Award'* for his contributions in (epi)genomics and sequencing fields. In addition, **Dr. Feng Zhang** (Massachusetts Institute of Technology, Cambridge, USA) was honoured with *"Epigenomics Young Innovator Award'* for his development of optogenetics and CRISPR technologies. The message from the meeting is there are lot of issues has to be addressed before we take HDAC-based therapeutics into the clinic to treat human diseases. Most of the attendees felt that this was a *"unique, coherent, well-organized, target meeting for learning cutting-edge technology and meeting authorities in the epigenomics and sequencing field."* I hope that the potential applications of epigenomics research in basic biology, agriculture, and biomedicine will come to a reality in the coming years; however, developing as drugs and therapeutics will take more time.

These opinions are exclusively of the authors and do not reflect those of GeneExpression Systems, Inc.