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ABSTRACTS

Epigenomics

045: Nuclear matrix, genome organization and regulation of genes

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Functional and spatial organization in the nucleus is supported by a non-chromatin nuclear structure called the nuclear matrix. Although precise composition of nuclear matrix is not known, proteins and RNA molecules are its major components. We have standardized procedure to prepare NuMat from Drosophila embryos by introducing several modifications in the published protocols and setting up several quality controls. We have established 2D profile of the NuMat proteome of Drosophila embryos and identified more than 150 proteins. While comparing the 2D profiles from different developmental stages, we noticed remarkable alterations in the composition of NuMat proteome during Drosophila development. While several studies have identified proteins from nuclear matrix, the nature of its RNA component is unknown. In this study we show that repetitive sequences are the major RNA component of the nuclear matrix of *Drosophila melanogaster*. We further demonstrate that these transcripts are essential for nuclear organization and viability. Identical DNA sequence of the genome is packaged in cell type specific manner resulting into corresponding epigenomes that in turn lead to cell type specific expression pattern. Our results identify key components of NuMat that help in packaging of the genomic DNA and enable chromatin mediated epigenetic mechanisms that regulate developmental gene regulation.

046: Epigenomic regulation of p53 pathway

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p53 regulates its target genes under various cellular stresses. To elucidate the p53 signaling pathway, we mapped p53 binding sites under various DNA damages in HCT116 human colon carcinoma cell, by chromatin immunoprecipitation (ChIP) followed by either microarray or high throughput sequencing analysis. In ChIP-chip assays, ChIP'd DNA, as well as randomly sheared DNA used as a reference control (input DNA), was linearly amplified using an in vitro transcription method, and hybridized onto the whole genome tiling arrays (Affymetrix WG10G), which cover non-repetitive whole human genome sequence on a set of 14 arrays. In the ChIP-seq analysis, adaptor-ligated DNA fragments of approximately 150–200 bps were isolated from agarose gels and sequenced using the Illumina 1G Genome Analyzer. A total of 1,642 ($p < 1e\text{-}7$) or 2,382 ($p < 1e\text{-}4$) putative p53-binding regions were identified with high confidence by ChIP-chip, while 2,885 ($p < 1e\text{-}25$) regions were identified from more than 10 million short reads by ChIP-Seq. Data from both platforms were well concordant, as 68% (1,143 common/1,642 ChIP-chip positive) were common on both platforms. In ChIP-seq uniquely positive regions, more than half lacked enough probes designed on the tiling arrays, partly due to presence of repetitive sequences. Conversely, we also found 499 ($p < 1e\text{-}7$) or 1,071($p < 1e\text{-}4$) ChIP-chip unique regions, where multiple genomic copies were often present, preventing from unique genomic assignment. Half of the p53 binding sites were located in the intergenic regions. To interpret roles of p53 binding throughout the genome, we examined histone modification patterns, e.g. H3 and H4 acetylation and H3K4me1 and K4me3 methylation. Nearly two thirds of p53 binding sites were accompanied by histone H4 acetylation, as demonstrated in our previous report on the ENCODE regions (Kaneshiro 2007), suggesting that p53 is likely to recruit histone acetyltransferase to its binding sites. Binding sites adjacent to the transcription start sites (TSSs) act as the promoter, where increased H4 acetylation and both H3K4me1 and K4me3 were observed. On the other hand, intronic binding sites and some of the intergenic sites were accompanied only with H4 acetylation and H3K4 monomethylation, implicating a role as the transcriptional enhancer. Finally, a subclass of retrotransposon sequences was enriched among the p53 binding sites as previously reported (Wang 2007), and might be involved in a newly acquired regulatory mechanism under p53.

047: DNMT3L: epigenetic correlation with cancer

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DNMT3L is an enzymatically inactive member of the DNA methyltransferase 3 family but acts as a regulator of the de novo DNA methyltransferases DNMT3A and DNMT3B and can therefore effect epigenetic reprogramming. We recently reported statistically significant loss of DNA methylation at the promoter of DNMT3L (DNA methyltransferase 3 like) in more than 90% of the cancer cervix samples we analyzed. In this study, we show that this loss of DNA methylation at DNMT3L promoter correlates with its increased expression. To examine whether increased expression of DNMT3L can play a role in cancer progression, it was over-expressed in cervical cancer cell lines, HeLa and SiHa. It was found that over-expression of DNMT3L in HeLa and SiHa cell lines was associated with increased cell proliferation and anchorage independent growth in soft agar assays. DNMT3L expressing stable HeLa clones also showed a proliferative advantage over its control counterpart. In addition, change in cell morphology was observed for HeLa cells over-expressing DNMT3L. Interestingly, these morphological changes were observed only after several passages and were concomitant with changes in gene expression at global level. The slow nature of morphological and global gene expression level changes observed in DNMT3L over-expressing HeLa cells correlate very well with what is known of genetic and epigenetic changes observed in cancer development.

048: High throughput sequencing technologies revealed correlation of histone methylation pattern on estrogen response element utilization of binding sites in MCF-7 human breast cancer cell line

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Integrating the genomic landscape of chromatin modifications with information on gene regulatory elements might provide important insights towards a better understanding of transcriptional regulation by specific factors. Epigenetic markers like histone methylations and acetylations provide additional information on local chromatin states and therefore have the potential to reveal functionality of transcription factor binding sites. Advances in high-throughput sequencing technologies combined with chromatin immunoprecipitation (ChIP) allow identification of transcription factor binding sites and histone modifications on a genome scale. Here, we aimed to define rules of ERE binding site usage in vivo by combining Chip-seq analysis of histone methylation marks with previously obtained ERα binding site (EREs) maps in MCF-7 cells. Using published data sets of ERα binding from two independent approaches (ChIP-PET and ChIP-on-chip data) as well as computationally derived binding sites, we constructed high and low confident sets of bound ERE sites and a set of non-bound EREs. We found different level of histone methylation marks for the sets of bound and non-bound EREs. Bound EREs were always found with higher level of H3K4me1 and me3 modifications compared to non-bound sites and there was better correlation between these histone modifications and transcription factor binding. On the other hand, average level of H3K27me3 and H3K9me3 modifications were not significantly different between bound and non-bound EREs indicating that these histone repression marks alone do not explain why the non-bound sites are not accessible for ERα binding. Therefore, it is plausible that chromatin signals other than H3K27me3 and H3K9me3 prevent these binding sites from being used by ERα and utilization of EREs is governed by additional mechanisms independent of theses histone marks. However, combinations of published ERα maps and these histone marks allowed us to describe distal regulators and enhancers of many ERα regulated genes.

049: The DNA methylation profile of genes from the inactive X chromosome from human fibroblasts

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X chromosome inactivation is a phenomenon occurring in female mammals for the purpose of equalising the dosage between the sexes, for genes on the X chromosome. X inactivation involves the transcriptional silencing of one of the copies of the X chromosome. There is a very clear correlation between the location of the gene on the X chromosome and the inactivation status of the gene. While it is reported earlier that the inactive X chromosome is highly enriched in five methylcytosine, much more so than what can be accounted for by all CG dinucleotides in the DNA sequence of the X chromosome, the precise role and localisation of 5 mC on the inactive X chromosome is still unknown. In this work we report the methylation profile of the X linked genes on the inactive X chromosome as well as the active X chromosome. We have employed a method involving a monoclonal antibody to 5 mC to detect the presence of methylation in the DNA hybridised to a human cDNA microarray. This method is not biased for methylation being restricted to any specific sequences like CpG islands or to restriction sites of methylation sensitive restriction enzymes, for it to be detected. Our method provides the information regarding the methylation content in the regions surrounding the genes in the hybridised DNA. The fluorescence intensity in the microarray readout would give a quantitative measure of the methylation content of the hybridised DNA. We have used DNA from fibroblasts having the karyotype 45 XO where there is no X chromosome inactivation. DNA from fibroblasts having the karyotype 47 XXX that has two copies of the inactivated X chromosome, was also used. The data from the 47 XXX and the 45 XO experiment together enabled us to derive a methylation profile of the X chromosomal genes when the chromosome is either inactivated or is active. We observed that the number of genes showing detectable is significantly higher on the inactive X as compared to the active X. We have investigated the correlation between the DNA sequence features in the neighbourhood of the genes and the methylation status. Our data taken together along with the correlation of the inactivation status to the X chromosomal location of the genes provides a valuable insight into the inactivation phenomenon. Our work thus provides a platform to understand the exact localisation and role of DNA methylation in the process of X inactivation.

050: Alteration of genome function by modulating epigenetic marks: implication in diseases

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The dynamic organization of chromatin plays significant role in the eukaryotic transcriptional regulation. Post translational modifications of chromatin proteins, ATP dependent remodeling factors and their interaction with proteins like histone chaperones confer a dynamic state to the chromatin and could thereby affect disease manifestation. We

have found that human histone chaperone NPM1, activates acetylation dependent chromatin transcription and also gets acetylated in vivo. Remarkably, hyperacetylation of NPM1 is a characteristic feature of a few types of cancer. Silencing of NPM1 and the subsequent gene expression analysis by microarray highlighted the modulation of expression of genes implicated in oral cancer. Dysfunction of chromatin modifying enzymes is often associated with several diseases like Cancer, Asthma, Diabetes and Neurodegenerative disorders. Small molecule modulators of HATs, HDACs, and HMTases thus are potential novel therapeutic molecules. We have identified a p300 HAT specific inhibitor, LTK14 that is nontoxic and specific for HAT inhibitory activity even in vivo. On expression analysis, most of the genes were downregulated as expected, however, few genes also showed upregulation indicating the importance of p300 in repressive mechanisms as well. Our analysis of oral cancer cell lines revealed histone hyperacetylation, which is the diagnostic marker of transcription activation. We found that histone acetylation could be inhibited potently in the oral cancer cell line by small molecule inhibitors. We have recently found a potent and specific inhibitor of histone arginine methyltransferase, CARM1. The role of this compound in methylation dependent activation of p53 responsive genes and its functional consequence is presently being investigated. The significance of these small molecular modulators as EPIGENETICS based drugs for development of new generation antineoplastic and anti HIV therapeutics and their role as biological switching molecules will be discussed.

050: Dynamics of genome organizations and epigenetic networks during pluripotent cell differentiation

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Understanding the unique genomic architectures crucial for maintaining the pluripotency nature of ES cells and their dynamic regulations are fundamental for the realization of the mammalian development and appreciation of their therapeutic potential. Key modulators, such as histone modifications, nucleus architectures and chromatin conformations are known to regulate genome organizations in stem cells critical for their functions in pluripotency nature and the cell fate decisions.

To characterize the chromatin structures and organizations pertinent to neuronal progenitor cell commitment, we profiled the whole genome enhancers (p300), promoters (H3K4me3), insulators (CTCF) and nuclear lamins binding sites in the context of spatial and temporal regulations within the nucleus of stem cells by ultra high throughput ChIP sequencing approach.

Our results revealed the specific genomic domains characterized by these landmarks. Unique transcriptional programs were established within such organizations that determine the characteristics of stem cells. Furthermore, the dynamics of such architectures between ES cells and neuronal stem cells demonstrate the plasticity of the stem cell genomes. Using such landscape, we further interrogated the high order architectures of chromatin interactions mediated by CTCF. Chromatin Interaction Analysis by Pair End diTag (ChIA-PET) was adapted to characterize the interacting chromatins tethered by CTCF in ES cells. In ChIA-PET analysis, tethered DNA by immunoprecipitated chromatins were joined in together by linker ligation. The ligated DNA was analyzed by pair end ditag based sequencing to reveal the long range interactions. Over 200 cis- and 500 trans-interacting loci were uncovered. We also associate these high order structures with global transcription expression changes, promoter activities, gene activities and major genomic features. Understanding the distribution, dynamics and impacts of such interactions mediated by chromatin modifiers should provide a framework that reveals the functional organizations and the molecular mechanism that establishes and maintains the nature of such specific genome organizations.