Epigenomics

325: 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 $\sim$150–200 bps were isolated from agarose gels and sequenced using the Illumina 1G Genome Analyzer. A total of 1,642 (p < 1e-7) or 2,382 (p < 1e-4) putative p53-binding regions were identified with high confidence by ChIP-chip, while 2,885 (p < 1e-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-7) or 1,071(p < 1e-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.

326: Methylation pattern of a few tumor suppressor genes in gall bladder cancer patients amongst North Central Indian population

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The recent reports and our prospective studies show high incidence of gall bladder cancer in various parts of India including North Central region (Madhya Pradesh, Uttar Pradesh and Rajasthan). Majority of the gall bladder cancer (46.42%) was found in the individuals of age group of 40–50 years, while patients with gall stone diseases were mainly in the age group of 30–40 years. About 53% of gall bladder cancer and 86% of gall stone patients were females. Highest numbers of gall bladder cancer (60.5%) were from rural areas; in contrast, the majority of gall stone (cholelithiasis) cases (55%) were from urban areas. About 84% of the females with gall bladder cancer were post-menopausal, while from those with gall stone diseases (61%) were pre-menopausal. About 96% of the gall bladder cancer cases were pathologically identified as adenocarcinoma. Epigenetic changes plays a major role in the molecular pathogenesis of cancer and varies within different ethnic population. The present study tries to elucidate the pattern of epigenetic alterations (like methylation of CpG islands) in the GBC and GSD cases. Methylation pattern of CDKN2B, CDKN2A, CDH1 and VHL genes were studied by highly specific novel methylation specific PCR in context to Indian version of the disease and establishes a comparative pattern of gene hypermethylations in carcinoma gall bladder and cholelithiasis amongst North Central Indian population. The detailed comparison of methylation pattern of these genes in the study population in comparison to other ethnic groups will be discussed during presentation.

327: A possible dual role of a chromatin remodeling protein INO80

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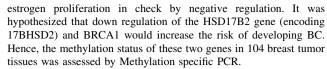
The role of global regulators like trithorax and polycomb complexes in epigenetic control of gene expression as components of chromatin remodelling complexes (CRM), and cellular memory modules in developmentally controlled genes in eukaryotes is well recognised. With the extensive similarity conserved in the developmental strategies across the phyla, the number of putative regulators predicted in the genome of Homo sapiens is limited in comparison to that in Drosophila. We mined the human genome sequence for novel trans-acting factors that could be part of the CRM complexes, identified and characterised hINO80 (SNF2-R), mapping to chromosome 15 of the human genome (OMIM 610169 INO80 HOMOLOG; INO80). The two hallmark features of INO80 subfamily are DNA dependent ATPase and the DNA binding activity which are conserved across the phyla. Towards the delineation of the function of INO80 in vivo we have taken dual approach:(1) exploiting the Drosophila system we analysed the function of INO80 in development by expression analysis and P-element mediated deletion of dINO80 and (2) using the cloned DNA binding domain (DBINO) of INO80 and SELEX approach we have identified the putative interaction sites of DBINO in the human genome. We conclude that dINO80 is essential for development in Drosophila based on, the ubiquitous expression of the of dINO80 during the Drosophila development and late embryonic lethality resulting from deletion of the gene. Its localization at a large number of sites on the Drosophila polytene chromosome is in contrast to other known CRM proteins and appears to be independent of one the recruiters of PRC complex, the PHO/YY1 protein with which INO80 is known to co-precipitate in ChIP assays. Concurrent with this we identify putative interaction sites for INO80 through, presumably its DBINO domain at multiple sites on the human genome which can also be mapped on the fly genome. These are distinct from the interaction sites of PHO/YY1. Our results predict dual function for INO80 protein both as a DNA dependent ATPase and a DNA binding protein, perhaps as a recruiter of CRM complexes in its own right.

328: Role of BRCA1 and 17BHSD2 methylation in the aetiology of sporadic breast cancer

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Carcinogenesis is a multistep process during which genetic and epigenetic alterations accumulate in a cell, resulting in the progressive transformation of normal cells into tumor cells. Breast cancer (BC) is the most common cancer among women. Increasing evidence indicates that intratumoral estrogens derived in situ may be responsible for mitogenic activity and carcinogenesis, irrespective of their serum concentrations in BC. 17Beta-hydroxysteroid dehydrogenases (17BHSDs) are a family of enzymes that regulate intratumoral estrogen production. 17BHSD2 catalyzes the conversion of active Estradiol (E2) to inactive Estrone (E1), while BRCA1 keeps the estrogen proliferation in check by negative regulation. It was hypothesized that down regulation of the HSD17B2 gene (encoding 17BHSD2) and BRCA1 would increase the risk of developing BC. Hence, the methylation status of these two genes in 104 breast tumor tissues was assessed by Methylation specific PCR.

In the present study 26% of breast tumor tissues exhibited a methylated BRCA1, while a similar percentage had a methylated HSD17B2, less than 2% of the tissues had both genes methylated. This suggests that epigenetic silencing of these two genes could explain the aetiology of more than 50% of BC. This is the first study to the best of our knowledge, assessing the methylation status of HSD17B2 gene and supporting the role of BRCA1 methylation in breast tumor tissue. Our results highlight the importance of epigenetics in intratumoral estrogen activity.

329: Epigenetic alteration of human DNA when chronically exposed to arsenic by drinking water

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Human exposure to arsenic is becoming common on a global scale mostly due to consumption of arsenic contaminated subsoil water. Several Districts of West Bengal in India are affected by such arsenic toxicity. We are investigating molecular changes associated with arsenic exposure among this population. Chronic arsenic exposure produces myriad clinical problems, including cancer of skin, bladder and lung. However, the molecular mechanism of arsenic induced carcinogenesis is not well understood. As arsenic is a poor mutagen, it does not induce significant point mutation. Therefore we are currently studying the role of epigenetic changes in arsenic induced carcinogenesis. We have shown earlier that, drinking arsenic contaminated water can be correlated with aberrant DNA methylation, which is related to arsenic level in water (1). We found significant level of methylation in the promoter region of TP53 gene and CDKN2A gene in this population. Moreover, here we show, that the Glutathione-S-Transferase (GST) polymorphism is apparently associated with arsenic induced methylation changes. When chronically exposed to arsenic, person with GSTM1 and GSTT1 null genotype shows DNA hypermethylation. This hypermethylation can be located to TP53 promoter region, but not restricted to it. We found clinical symptom score of arsenic toxicity to be high in GST M1T1 null genotype, when compared to wild type population. The level of total urinary arsenic was also found to be significantly higher in them. Comparison was made in GSTM1 or GSTT1 null polymorphic subjects with GSTM1T1 wild type subjects of same arsenic exposure group while drawing such conclusion. This may be one of the possible mechanisms underlying arsenic tolerance in endemic region.

330: CYP17A1 and CYP19A1 gene polymorphisms and breast cancer risk

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Background: Interindividual differences in susceptibility to breast cancer are partially mediated through the levels of endogenous and exogenous steroid hormones. The CYP17A1 and CYP19A1 genes encode P450C17alfa and aromatase proteins, which are involved in the metabolism of steroid hormones and thus contribute to the etiology of breast cancer.

Materials and Methods: In the present study, 250 cases of breast cancer patients as well as equal number of age matched healthy controls were analyzed for CYP17A1 (MspA1I) and CYP19A1 (Codon 39) polymorphisms by using PCR-RFLP and PCR-CTPP respectively.

Results: CYP17A1 polymorphism: There was a significant association between CYP17A1 polymorphism and breast cancer in the present study. The A1 genotype frequency was elevated in disease group as compared to controls whereas A2 genotype was not associated with breast cancer. The frequency of A2 genotype showed an increasing but insignificant trend in postmenopausal breast cancer women (4.2%) when compared to premenopausal cases (3.1%). The frequency of A2 genotype showed association with positive family history of patients (5.5%), obesity, estrogen receptor (6.2%) and progesterone receptor negative (5.0%) status as well as HER2/neu positive (7.7%) status, positive node status (5.0%) as well as advanced stage of the disease.

CYP19A1 Polymorphism: The TT (W39) genotype frequency was significantly elevated in disease group (90.8%) as compared to controls (68.5%). The frequency of TC was found to be increased in premenopausal cases (12.2%), patients with positive family history (10.8%), occupied in agriculture (14.8%), estrogen and progesterone receptor positive status and node positive status (9.8%). Higher frequency of both TT and TC genotype was observed in patients with high body mass index. The frequency of TT genotypes was also found to be increased in patients with advanced stage of the disease.

Conclusions: In the present study, A1A1 genotype of CYP17A1 showed a significant association with breast cancer, which was attributed to increased circular androgen levels associated with the genotype. The codon 39 polymorphism of CYP19A1 gene, W39 (T) with increased aromatase activity confers greater risk to develop breast cancer especially in postmenopausal women and might also contribute to the progression to advanced stage.

331: 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 5-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 5mC 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 5mC 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.

332: Dynamic of genome organization in mouse embryonic stem cells and neural-committed stem cells

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Chromosomes are organized into highly complex and dynamic structures. They undergo extensive alterations during development in response to lineage specific-gene expression program. Changes in chromatin structure have been detected during differentiation of embryonic stem cells. In vitro, mouse ES cells can differentiate efficiently into neural precursor cells upon withdrawal of serum or treatment of embryoid bodies with retinoic acid. Despite many studies in mammalian chromosome structure, only little is known about the overall chromosome organization in mouse ES and neural-committed stem cells, how it contributes to the characteristics of pluripotent and lineage-restricted stem cells.

In this study, chromatin immunoprecipitation coupled with ultra high throughput sequencing using Solexa Genome Analyzer Platform (ChIP-Seq) was applied to map the pattern of key histone modifications (H3K4m1, H3K4m3 and H3K36m3) and the binding loci of the enhancer activator protein (p300), repressor proteins (main components of the nuclear lamina, Lamin A/C and Lamin B) and insulator protein CTCF in mouse ES cells and neural stem cells.

In this approach, a total of 13–20 millions sequences containing 36 nucleotides were generated from each ChIP ∼75% of these sequences can be uniquely mapped to the mouse genome mm8. Using the optimized peak finding algorithm, over 18 K active promoters and 20 K open chromatin/enhancer regions were identified in ES and NS cells respectively. Among the 60 K insulator CTCF binding sites identified in ES and NS cells, differential binding regions were identified and their correlations with the gene expression regulation and nuclear lamin defined domains were analyzed.

These whole genome binding sites for key chromatin modifiers will provide insight into the dynamic organization of chromosome during differentiation of mouse ES cell into tissue-specific stem cells.

333: IGF2/H19 domain epigenetic alteration in sib pairs with schizophrenia

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Schizophrenia is almost certainly a multifactorial disorder with genetic and environmental elements contributing to overall risk of the disease. For instance, abnormalities of foetal growth have been found to be related to the risk of neuro-developmental disorder and schizophrenia and IGF2 genes are now known to be crucial for placental development and were the first imprinted genes to be identified as such. The methylation status of CTCF binding sites in the H19 imprinting control region contribute to the expression of IGF2. In this study, we investigated the status of DNA methylation of discordant sib pairs with schizophrenia around the sixth CTCF-binding sites in the promoter region of H19 using bisulphite sequencing and combined real time PCR. Among the 143 discordant sib pairs in our study, completely unmethylated maternal alleles were found in 22 patients (15.38%) and 13 normal controls (9.09%) p < 0.01. Among the 22 patients, there were nine patients with an affected mother, and one patient with an affected father. We hypothesised that the degree of aberrant methylation of IGF2/H19 might be related to the risk of schizophrenia. Further work on methylation profiles CTCF binding sites with respect to IGF2 expression is ill needed.

334: Analysis of promoter methylation of E-cadherin (CDH1) gene in esophageal squamous cell carcinoma

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Background: Esophageal squamous cell carcinoma (ESCC) is one of the most common cancers occurring globally and is a leading cause of cancer-related deaths in Jammu and Kashmir (J and K). Infection with high-risk oncogenic HPV types mainly HPV16 and HPV18 are found to be associated with this cancer. Epigenetic regulations such as promoter hypermethylation of tumor-related genes resulting in transcriptional silencing and gene inactivation play a pivotal role in tumorigenesis. In the present investigation, we have studied hypermethylation of CDH1 promoter in esophageal cancer. E-cadherin is one of the most important extracellular cell adhesion. Disruption in cadherins may cause uncontrolled cell migration and proliferation during tumor development and angiogenesis.

Methodology: We analyzed a total of 54 histopathologically confirmed esophageal cancer and 54 corresponding normal adjacent tissue biopsies collected from Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Kashmir. All biopsies were checked for HPV DNA sequences and aberrant DNA methylation in the CpG island of the E-cadherin promoter by chemical modification of genomic DNA using sodium bisulphite protocol and methylation-specific PCR.

Results: HPV16 DNA was detected in 17% (9/54) of the cases while no HPV DNA was detected in any of the corresponding normal adjacent tissue. Presence of promoter hypermethylation of CDH1 gene was observed in 59% (32/54) in tumor tissues and complete absence of hypermethylation was noted in normal adjacent tissue. Interestingly, all HPV16 positive cases showed hypermethylation of CDH1 gene. Analysis of hypermethylation of CDH1 with respect to different histopathological grades revealed hypermethylation in seven out of nine (78%) of poorly differentiated squamous cell carcinomas (PDSCC) while twenty two of thirty six (61%) cases were of moderately differentiated SCC (MDSCC). In contrast, only three out of nine (33%) cases showed promoter hypermethylation in well-differentiated SCC (WDSCC).

Conclusion: Preliminary study, therefore, suggests that hypermethylation of promoter region of CDH1 gene may play an important role in esophageal cancer progression and it appears to be associated with poorly differentiated state of the cancer as well as HPV16 infection. Sample size is being increased to determine the statistical significance of this lead indicating a possible role of CDH1 hypermethylation during esophageal carcinogenesis.

335: 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.

336: CpG island microarray based identification of differentially methylated genes during cervical cancer progression

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Carcinoma of the cervix is the leading gynaecological malignancy worldwide with ∼493,100 new cases and more than 273,000 deaths occur each year, out of which 130,000 new cases and 74,000 deaths are reported annually in India alone. Epigenetic alterations, especially DNA methylation, occur in both HPV (a risk factor) and host genome leading to silencing of tumor suppressor genes expression. On the other hand, global hypomethylation correlates to increased expression of oncogenes and genome instability. Since epigenetic changes occur very early, it, therefore, can be used as marker for early detection of cervical cancer. Identification of hypermethylated genes could offer novel means for screening the (pre)neoplastic cervical cancer lesions. Present study is targeted to examine the DNA methylation at the global as well as at the gene specific level and also to delineate the associated molecular pathways involved in the pathogenesis of cervical cancer using microarray techniques, and its further validation. Microarray based DNA methylation analysis showed that 245 hypermethylated and 123 hypomethylated genes in cervical squamous cell carcinomas. Gene ontology analysis showed genes related to physiological process, cellular process, development and regulation of biological process are highly altered. HPLC analysis showed a significant reduction in global methylation content in tumor samples. The average 5-methyl cytosine content was found to 3.5 and 2.8% in normal and tumor samples respectively. From MS-AP-PCR study, we have identified a number of cancer linked methylation changes within the unmethylated domains and novel CpG islands containing genes hypermethylated in cervical cancer. Out of fifteen differentially methylated fragments, ten fragments showed the characteristics of CpG island. MS-PCR results showed tumor suppressor genes, p16 (Cell-cycle) and CDH1 (Wnt-pathway), were differentially methylated in cervical cancer. Our results show that there are a large number of epigenetically modified genes which might play an important role in the development of cervical cancer. Hence, these altered sequences need to be validated in large number of cervical cancer samples before using it as marker and its possible involvement in signaling pathways related to cervical cancer. This study is funded by ICMR, Government of India, New Delhi.

337: 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.

338: 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.

339: 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.

340: Whole genome bisulphite sequencing by high-throughput oligonucleotide ligation sequencing

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Methylation of CpG motifs throughout the genome plays a critical role in many cellular processes within the cell, including differentiation, chromosome stability, regulation of gene expression and preventing transcription of repetitive or alien sequences. Accordingly, aberrant methylation patterns have been linked to many diseases, and play a major role in the initiation and progression in the majority of cancers. Bisulphite sequencing is the method of choice for characterizing the methylation status of each CpG in a given region, however until recently bisulphite sequencing has been limited to region specific analysis. The introduction of next generation sequencing technologies removes this limitation, allowing hypothesis-free analysis of the entire methylome. Here we present a technique that harnesses the ultra-high throughput of the SOLiD[TM] System, a sequencing platform based on massively parallel cycled fluorescent oligonucleotide ligation and cleavage, with the single base level resolution of bisulphite sequencing, enabling genome wide methylation analysis. A novel adaptation to the fragment library protocol enabled the DNA library to be treated with bisulphite after the addition of sequencing adapters. The resulting bisulphite converted library was then subjected to emulsion PCR and sequencing using standard sequencing protocols. Alignment of the resulting reads to the reference genome present some challenges due to the length of the read and the reduction in sequence specificity after bisulphite conversion. Methods have been developed to address these problems. We believe the combination of 'gold standard' bisulphite sequencing with the unprecedented throughput of next-generation sequencing removes long standing technical roadblocks to large scale methylation analysis at single base resolution, and will enable significant advances in the field.

341: TP53 gene polymorphisms and breast cancer risk

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Background: The TP53 tumor suppressor gene plays crucial role in maintaining genomic integrity and thus preventing cells from undergoing oncogenic transformation. A single nucleotide polymorphism at codon 72 of the TP53 gene results in either a proline or an arginine. This substitution occurs in the putative SH3 binding domain of p53, influencing binding capacity and thereby functional properties of p53. Apart from exonic variants, sequence variation in intronic region may also influence the gene regulation through aberrant splicing or through disruption of critical DNA protein interaction. The TP53 G13964C intron 6 variant polymorphism has been reported to influences the risk for breast cancer in patients with positive family history.
Materials and methods: A group of 250 breast cancer patients breast cancer cases and equal number of healthy and age matched women were selected to study the association of 72 codon polymorphism and G13964C intron 6 polymorphism using PCR-RFLP technique.
Results: TP53 codon 72 polymorphism: The frequency of Arginine genotype (33.2%) was increased significantly in breast cancer as compared to controls (19.6%). Arg genotype frequency was elevated in patients with Premenopausal breast cancer (41.1%) and advanced stages of the breast cancer. No significant association was observed with hormonal receptor status of patients and familial incidence of breast cancer. The heterozygote Arg/Pro frequency was found to be elevated in overweight and obese women with breast cancer.
TP53 G13964C intron polymorphism: No significant association was observed in relation to G13964C intron polymorphism and breast cancer. This may be due to the very low frequency of CC genotype. The breast cancer patient with CC genotype had premenopausal disease with estrogen receptor negative, progesterone receptor positive status. She was observed to be overweight and presented with advanced stage of breast cancer.
Conclusion: TP53 codon 72 polymorphism might predispose individual for the development of breast cancer as well as prognosis. No significant association was observed in relation to G13964C intron polymorphism and breast cancer.

342: Homocysteine mediated DNA methylation and its potential role in coronary artery disease

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A major role of Epigenomics in the manifestation of disease phenotype in complex diseases is increasingly being acknowledged. The identification of differentially methylated regions is a promising strategy to understand the epigenetic regulation of genetic disorders. Among the complex diseases, the incidence of coronary artery disease (CAD) is increasing worldwide at an alarming rate, especially in developing countries and is the largest cause of mortality and morbidity worldwide. The early onset of CAD along with its severity cannot be explained entirely by the classical risk factors and genetic variations. In coronary artery disease where hyperhomocysteinemia is recognized as an independent risk factor, epigenetic modifications like DNA methylation can be an indicator of the molecular consequences of the disease phenotype. However till date, there is no data on genome wide epigenetic changes in CAD patients. We evaluated the methylation status of genomic DNA from peripheral lymphocytes in a cohort of angiographically confirmed CAD patients and controls. DNA methylation in CAD patients was found to be significantly higher as compared to controls ($p < 0.05$). Since an elevated homocysteine level is considered to be an independent risk factor for CAD as well as a key modulator of macromolecular methylation, we investigated the probable correlation between plasma homocysteine levels and global DNA methylation and found a significant positive correlation between the two in CAD patients ($p = 0.001$). Further, within a higher range of serum homocysteine levels (>12–50 μM) global DNA methylation was significantly higher in CAD patients than controls. Thus the alteration in genomic DNA methylation associated with cardiovascular disease per se appears to be further accentuated by higher homocysteine levels. In an attempt to identify the genes/regions in the genome that are differentially methylated in CAD cases and controls, we performed a microarray based Epigenomic profiling using CpG island microarray after enrichment of the hypermethylated fraction of genomic DNA and obtained several differentially methylated regions that could be potentially associated with CAD. Among these differentially methylated genes a majority (55%) are involved in metabolism while 20% are involved in cell growth and maintenance, 20% in cell communication and 5% in cell death. Validations of these differentially methylated regions in CAD patients are currently underway.

343: MS-FLAG, a novel real-time signal generation method for methylation-specific PCR

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Cytosine-5 methylation of CpG dinucleotides is associated with the repression of gene expression and is widely recognized as an important event in carcinogenesis. DNA methylation represses transcription inducing a closer chromatin structure and/or altering the DNA binding site of some transcription factors. Aberrant promoter methylation is a major mechanism for silencing tumor suppressor genes in several human cancers. Detection of hypermethylation is used as a molecular marker for early cancer diagnosis, as a prognostic index, or to define therapeutic targets for reversion of aberrant methylation. We report on a novel signal generation technology for real-time PCR to detect gene promoter methylation. FLAG (FLuorescent Amplicon Generation) is a homogeneous signal generation technology based on the exceptionally thermostable endonuclease PspGI. PCR amplification is performed with special primers consisting of a target-specific 3′ region and a 5′ tail carrying a fluorophore/quencher pair separated by the recognition site of the

restriction enzyme. At each amplification cycle the complete double-stranded recognition site is created at both extremities of the amplified product and enzymatic cleavage results in a loss of quenching and production of fluorescence. We adapted methylation-specific PCR (MSP) applied on bisulfite-treated DNA to a real-time format (methylation-specific FLAG; MS-FLAG) for quantifying methylation in the promoter of CDKN2A, GATA5, and RASSF1 genes.

MS-FLAG was validated on plasmids and genomic DNA with known methylation status and then applied to detection of methylation in a limited number of clinical samples. Bisulfite sequencing was also conducted on these samples. Real-time PCR results obtained via MS-FLAG agreed with results obtained via conventional, gel-based MSP. The new technology showed high specificity, sensitivity (2–3 plasmid copies), and selectivity (0.01% of methylated DNA) on control samples. It enabled correct prediction of the methylation status of all three gene promoters in 21 lung adenocarcinoma samples, as confirmed by bisulfite sequencing. We also developed a multiplex MS-FLAG assay for GATA5 and RASSF1 promoters (Bonanno et al. Clin Chem. 2007 Dec 53(12)).

MS-FLAG provides a new, quantitative, high-throughput method for detecting gene promoter methylation and is a convenient alternative to agarose gel-based MSP for screening methylation. In addition to methylation, FLAG-based real-time signal generation may have broad applications in DNA diagnostics.

344: Epigenetic mapping of human Y-chromosome reveals the role of non-coding elements in genome organization

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Y-chromosome is unique as far as its evolution and sequence organization is concerned. 95% of the human Y-chromosome (MSY-Male specific region of Y-chromosome) does not undergo meiotic recombination while small part (pseudo autosomal region) on both ends of chromosome recombines with X-chromosome during meiosis. Because of this property, Y-chromosome is useful to study lineages and evolutionary consequences. In our lab we are working on different aspects of chromatin organization of Y-chromosome with respect to non-coding elements including repetitive sequences. We have analyzed the distinct structural features of Y-chromosome that contains gene expressed early during development and late during spermatogenesis. These features include chromatin domain boundaries that separate genes in domains or boundaries that separate euchromatin from heterochromatin. Only genome sequences are not enough to understand, how the genetic program is coded in the genome. Genome in an organism exists in heritable 'Epigenetic' state corresponding to diverse array of developmental stages, tissue types and disease states. Epigenetic messages are stored as chemical modifications to cytosine bases of DNA and to the histone proteins that make chromatin accessible or inaccessible to regulatory machinery. Histones are subjected to more than 100 different post-translational modifications including acetylation, methylation, phosphorylation and ubiquitination etc., at different amino acids. Here in this study we have targeted histone-3 lysine (H3K9 and H3K27) modifications and mapped it along the entire length of Y-chromosome using 'ChIP on chip' technique. Our data suggests the role of non-coding elements, repeats and histones in regulation of Y-chromosome genes and reveals several feature of epigenetic state regulating the packaging of the Y-chromosome.

345: Role of H2AX, a DNA damage response protein and a histone H2A variant, in sporadic breast cancer

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There is an increasing evidence for the role of chromatin in DNA damage responses, and several recent studies have implicated histone modifications and chromatin modulation to genome stability. One of the best-characterized chromatin modification events in DNA damage response is the phosphorylation of histone H2AX, (referred to as gamma-H2AX) by the ATR and ATM checkpoint kinases. In mouse, absence of H2afx gene results in increased genomic instability and radiosensitivity and also it was shown to behave as a dosage dependent tumor suppressor gene. Further, in human it maps to a cytogenetic position (11q23.3) that is deleted in most human cancers. With this background information from mouse studies and its functional implication during DNA damage signaling and repair, we aimed to systemically screen for gene mutations and copy number alterations in sporadic breast cancer patients. Although the whole H2AFX gene scans showed an absence of mutation in the studied samples, there was, however, alteration of H2AFX gene copy number in 37% of tumor samples. Analysis of clinicopathological association revealed a convincing correlation with positive ER/PR status. Further, a twofold reduction in gene copy number in MCF7 cell line strongly suggested an involvement of H2AFX alteration in breast carcinogenesis (Srivastava et al. Cancer Genet and Cytogenet 2007). To correlate the deletion in gene copy number with expression, the immunolocalization of H2AX and gamma-H2AX (phosphorylated form) in MCF7 cells is being analyzed. Preliminary examination does not reveal any reduction in protein level neither the mislocalization however; a possibility that response to DNA damaging drug may be compromised is being investigated. Further the possibility of promoter hypermethylation of H2AFX gene contributing to its haploinsufficiency in tumor condition is also being analyzed. Thus, the findings till date and analysis in progress would precisely dissect out the role of histone H2AX in human cancers and would also highlight the importance of chromatin alterations during tumor progression.

346: A reproducible high-throughput methylome analysis of a single cell

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Epigenetics is the study of epigenetic inheritance, a set of reversible heritable changes in gene function or other cell phenotype that occur without a change in DNA sequence. The amount of diseases suspected of being influenced by epigenetic factors is rising steadily and includes schizophrenia, bipolar disorder, Alzheimer's disease, diabetes, arthrosclerosis, cancer, Parkinson's disease, just to name a few. One of the best understood epigenetic modifications is DNA methylation, a chemical addition of a methyl group (–CH3) to the carbon-5 of the cytosine pyrimidine ring in CG dinucleotides. This modification is a very stable carrier of epigenetic information and plays a crucial role in the regulation of gene expression and the control of genome stability in higher eukaryotes. To study and ultimately understand complex diseases, we must be able to analyze DNA methylation patterns in a genome-wide method in a single cell (one-cell methylome). One rationale is that DNA methylation profiles are highly variable across individual cells, even in the same organ. To address these differences, single-cells have to be analyzed; there can be no doubt that the single-cell methylome promises to revolutionize biological and medical research, clinical diagnostics and drug development. Here we demonstrate a single-cell methylome technology based on the fact that each cell contains only one–two copies of each genomic locus. This provides the opportunity to get an 'all or nothing' information that can eventually be translated into a binary code and used for biomarker discovery. The main strategy of the method is based on the enrichment of the unmethylated fraction of the genome using the AmpliGrid platform, a chemically structured microscope slide where single cell PCR reactions can be performed. Since cellular methylation patterns are directly influenced by the interaction of cells with their microenvironment or handling procedure, we also applied our technology on AmpliCell, a cell culture system based on the AmpliGrid technology which allows to control the cellular environment prior to analysis without the need of sample preparation. Our approach offers many new roads to analyze complex disorders such as the identification of epigenetic drift markers, or the study of interactions between environment and the epigenome. Last but not least, the analysis of single cells can be highly cost-saving and high-throughput methods can be developed.

347: Epigenetic regulation of genes during development: a conserved theme from flies to mammals

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Eukaryotic genome is organized in form of chromatin within the nucleus. This organization is important for compaction of DNA as well as for the proper expression of the genes. During early embryonic development genomic packaging receives variety of signals to eventually setup cell type specific expression patterns of genes. This process of regulated chromatinization leads to 'cell type specific epigenomes'. The expression states attained during differentiation process need to be maintained subsequently throughout the life of the organism. Epigenetic modifications are responsible for chromatin dependent regulatory mechanism and play a key role in maintenance of the expression state—a process referred to as cellular memory. Another key feature in the packaging of the genome is formation of chromatin domains that are thought to be structural as well as functional units of the higher order chromatin organization. Boundary elements that function to define such domains set the limits of regulatory elements and that of epigenetic modifications. This connection of epigenetic modification, chromatin structure and genome organization has emerged from several studies. Hox genes are among the best studied in this context and have lead to the significant understanding of the epigenetic regulation during development. We will discuss the evolutionarily conserved features of epigenetic mechanisms that emerged from studies on homeotic gene clusters.

348: 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.