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EPIGENOMICS AND GENOMICS

IL.2 09.00 – 09.45

EPIGENOMICS AND EPIGENETIC THERAPY

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The abnormal methylation of CpG islands located near the transcriptional start sites of human genes plays a major role in carcinogenesis. The methylation of cytosine residues in these regions is associated with alterations in chromatin structure including the binding of methylated DNA binding proteins and changes in the state of modification of histone residues in nucleosomes. These alterations serve to reinforce each other and may lead to the heritable silencing of genes which can have profound implications for human cancer development. Unlike mutational changes, epigenetic alterations are acquired in a gradual process which is associated with cellular division. Thus, these progressive alterations are potentially susceptible to interventions to reverse silencing by epigenetic therapy.

FREE PAPER SESSION 1

O1 09.45 – 10.00

LONG RANGE EPIGENETIC SILENCING (LRES): A COMMON PHENOMENON IN PROSTATE CANCER

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Introduction: Our lab recently described that epigenetic changes in cancer are not always localised to individual genes, but can span large chromosomal regions resulting in silencing of neighbouring genes, due to a mechanism we termed "Long Range Epigenetic Silencing" or LRES (Frigola et al., Nat. Genet., 2006). Here we investigate how prevalent this phenomenon is in prostate cancer using a genome-wide approach.
Methods & Materials: Public expression microarray data from prostate cancer and normal samples were reanalyzed to identify megabase-size downregulated regions with LRES potential. The results were combined with new microarray expression data from prostate cancer cell lines and normal primary epithelial cell lines (Gene 1.0 ST arrays), and data for DNA methylation and chromatin modifications (using promoter tiling arrays), to validate if these regions were also epigenetically suppressed by LRES.
Results: Using our approach of combining public expression microarray data with new genome-wide epigenetic analyses of prostate cancer and normal cell lines, we have been able to identify numerous megabase-size regions in the genome that are associated with large domains of DNA hypermethylation and repressive histone. Within regions of LRES, DNA hypermethylation was observed for individual genes and clusters of genes, whereas depletion of active histone modifications and an increase of silencing chromatin marks were found to spread more uniformly across the regions. The gene expression levels, DNA methylation status and histone modification of several genes within these regions were confirmed by Real Time-PCR in clinical prostate cancer samples.
Conclusions: In prostate cancer, Long Range Epigenetic Silencing is a common phenomenon that is prevalent throughout the genome. We have generated a list of LRES regions and are now in the process of identifying common features to unravel the underlying mechanism, allowing a better understanding of disease development and progression and with true potential for applications in prostate cancer diagnosis and therapy.

O2 10.00 – 10.15

CLONAL HERITABILITY OF CTA EXPRESSION AND PROMOTER METHYLATION PATTERNS IN MELANOMA CELLS: BASES FOR EPIGENETIC THERAPY?

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Introduction: Cancer-testis antigens (CTA) expressed by tumor cells of different histotypes are suitable targets for cancer immunotherapy. However, their heterogeneous intratumoral expression may limit their clinical usefulness since the elicited anti-CTA immune reactions will eventually target only a portion of the tumor mass. Using a single cell clones model of intratumor heterogeneity of CTA expression we previously demonstrated that promoter methylation represents the molecular mechanism directly responsible for heterogeneous intratumoral expression of CTA in melanoma. However, it remained to be addressed whether, in the context of a constitutive intratumor heterogeneity, CTA expression and promoter methylation are stable throughout cellular division at single cell level.
Methods and results: Molecular analysis carried on sub-clones obtained by limiting dilution of Mel 313 melanoma clones demonstrated that presence and levels of CTA expression were highly maintained throughout cellular division and correlated with the single cell-level heritability of the promoter methylation status, as assessed by sequence analysis of genomic DNA treated with sodium bisulfite. Furthermore, the treatment of melanoma cell clones with the DNA hypomethylating agent 5-aza-2'-deoxycytidine (5-AZA-CdR) resulted in demethylation of CTA promoters that ended up in a stable co-expression of different CTA at single cell level. Finally, 5-AZA-CdR treatment consistently resulted in a de novo expression or up-regulation of all CTA analyzed, leading to a homogeneous expression of CTA among sub-clones analyzed.
Conclusions: CTA expression is stable throughout cellular divisions, therefore granting for a successful immunological targeting of melanoma cells in the course of the disease. Furthermore, the reported single cell-level heritability of CTA promoter methylation patterns and enduring sensitivity to the demethylating activity of 5-AZA-CdR suggest DNA hypomethylating drugs as effective modifiers of melanoma antigenic phenotype that may be utilized to design novel chemo-immunotherapeutic regimens that may improve the therapeutic efficacy of multivalent CTA-directed vaccines in metastatic melanoma patients.

IL.3 10.15 – 11.00

EPIGENETIC ACTIVATION OF HOXA9 DUE TO PI3K PATHWAY DYSFUNCTION HAS FUNCTIONAL AND CLINICAL RELEVANCE IN HUMAN TUMORS

Bruno M. Costa; Justin S. Smith; Ying Chen; Justin Chen; Heidi S. Philips; Ken D. Aldape; Giuseppe Zardo; Janice Nigro; C. D. James; Jane Ridlyand; Rui M. Reis; **Joseph F. Costello.**
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Glioblastoma (GBM) is the most common and aggressive primary brain tumor and existing therapy regimens are often ineffective. Improving therapeutic response rates for cancer patients depends in part on prognostic markers that allow tailoring of therapy to the specific alterations in the tumor. HOX genes encode transcriptional regulators with critical roles in embryonic development and tumorigenesis. We demonstrate that HOX gene clusters are aberrantly activated within confined chromosomal domains in some GBMs. In particular, activation of the HOXA cluster could be reversed by an inhibitor of phosphoinositide 3-kinase (PI3K) through reversible epigenetic mechanisms in GBM-derived cell lines and neurospheres. In contrast, inhibition of mTOR by rapamycin, a therapeutically relevant drug and derivatives of which are currently in clinical trials for GBM, failed to reverse aberrant HOXA gene activation. Expression of HOXA9, a potent oncogene in leukemias, was independently predictive of shorter overall and progression-free survival in three sets of GBM patients and proved to be a molecular prognostic tool that improves survival prediction by the currently most robust prognostic marker in GBMs, MGMT promoter methylation. In vitro functional studies showed that HOXA9 is able to decrease apoptosis,

increase cellular proliferation and promote chemoresistance.

11.00 – 11.30 TEA AND COFFEE

IL.4 11.30 – 12.15

EVOLUTIONARY COMPARISON OF EPIGENETIC PROFILES AT LARGE IMPRINTED DOMAINS REVEAL DIFFERING MECHANISMS OF ALLELIC SILENCING.

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Genomic imprinting is a developmentally important mechanism involving differential DNA methylation and allelic histone modifications. Through detailed comparative epigenetic characterization of two large imprinted domains, we have re-defined the extent of imprinting within the hChr11/mChr7 and hChr7/mChr6 regions, and show that they are subject to different imprinting mechanisms. The hChr11/mChr7 region harbors the Beckwith-Wiedemann syndrome related cluster and contains numerous maternally expressed genes. Imprinting within this cluster requires paternal expression of an ncRNA, *KCNQ1OT1*, and maternal germ line methylation of its promoter, the KvDMR1. It is postulated that this ncRNA recruits polycomb group proteins to the paternal allele, contributing to paternal repression of the remaining imprinted genes in *cis*. We show that the evolutionary discrepancy in imprinted gene expression between mouse and humans in this region is due to an absence of the allelic histone modifications H3K9me and H3K27me3. Next we characterized the hChr7/mChr6 cluster, a domain not known to contain a functional ncRNA. Similar to the *KCNQ1* domain, this region contains a single DMR. This CpG island is centrally located, flanked by six maternally expressed genes, and contains the promoters of the protein coding genes *PEG10* and *SGCE*, which are transcribed from the paternal allele in opposite directions. Similar to other ICRs, we find that the maternally methylated *PEG10* promoter allele is associated with H3K9me3 and H4K20me3, whereas the transcriptionally active paternal allele is enriched in H3K4me2 and H3K9Ac. Unexpectedly, there was a general lack of repressive histone modifications throughout the remainder of the domain, suggesting that this imprinted region may utilize a different silencing mechanism compared to other imprinted domains. Our work indicates that allelic histone profiles within imprinted regions not only allow for the potential identification of novel imprinted genes, but can also highlight-silencing mechanisms that account for imprinting discrepancies between species.

O3 12.15 – 12.30

INVESTIGATING THE CHROMATIN FIBRE STRUCTURE OF THE HUMAN X CHROMOSOME

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X chromosome inactivation is a highly complex and tightly controlled process in which one of the two X chromosomes in female mammals is silenced (Xi) to achieve dosage compensation with males. Maintenance of X inactivation has been shown to involve Polycomb group (PcG) proteins and the recruitment of factors and chromatin modifications, including association of histone MacroH2A1, histone hypoacetylation, DNA hypermethylation and H3K27 tri-methylation. This study aims to characterise whether the transcriptionally repressed Xi is associated with altered chromatin structure and if so, which marks on Xi are structurally important. The primary structure of chromatin, the nucleosome, is well understood. To understand further the secondary level of chromatin folding, the 30nm solenoid-like fibre, we have developed a sucrose-gradient based technique that enables us to fractionate compact and open chromatin fibres from bulk chromatin. DNA probes are derived from compact and open chromatin and their distribution across the genome is characterised by high-resolution microarrays. We have identified a clonal female lymphoblastoid cell line (SATO) in which the same X chromosome in each cell is inactivated. Open and compact chromatin probes, derived from SATO cells, are hybridised to Illumina Human HapMap 550K SNP arrays. Since we know the haplotype for Xi and Xa,

we have been able to map discrete regions enriched for compact or open chromatin on Xi. We are also interrogating further higher levels of chromatin structure by measuring the distance between pairs of probes to the X chromosome in interphase FISH. Additionally, to dissect the role of Xi associated epigenetic marks (e.g. MacroH2A1, H3K27 tri-methylation) on chromatin folding we are depleting them using RNAi and then examining the effect on secondary chromatin structure by sucrose sedimentation and subsequent SNP array structural analysis, and higher order chromatin structure by interphase FISH.

O4 12.30 – 12.45

SKEWED SEX RATIO AND X-INACTIVATION IN SPONTANEOUS ABORTIONS WITH TRISOMY 16 MOSAICISM

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Introduction: Current data concerning changes in number of active X-chromosomes in human triploids underlined the hypothesis about possible involvement of autosomes in the choosing of X-chromosome for inactivation (Migeon et al., 2008). However, the nature and localization of tentative trans-factor(s) remain to be determined. Skewed sex ratio (0.35-0.45) as well as skewed X-inactivation were reported previously in prenatally diagnosed fetuses with trisomy 16 mosaicism providing evidence for differential survival of females with this type of mosaic aneuploidy. Our study was aimed to analyze sex ratio among spontaneous abortions (SA) with pure and mosaic trisomy 16 and investigate the X-inactivation status depending on the level of aneuploid cells in the placental tissues.
Material and Methods: Extraembryonic mesoderm and cytotrophoblast of 28 first-trimester SA with trisomy 16 were studied. Frequency of aneuploid cells was determined by interphase FISH with D16Z1 probe in each tissue. X-inactivation was assessed by methyl-sensitive PCR of androgen receptor locus in 6 informative SA.
Results: Sex ratio among SA with mosaic trisomy 16 appears to be skewed with prevalence of male abortions (1.25) while females prevail among non-mosaic embryos (0.43). It seems that survival of aneuploid female conceptions strongly depends on the ratio of normal to trisomic cells. So the skewed X-inactivation would be expected in female SA with high level of trisomy 16. Indeed, asymmetrical X-inactivation was observed in embryos with more than 80.9% of cells with trisomy 16 in the placental tissues whereas no cases of skewed X-inactivation in SA with 52.4-80.8% of aneuploid cells were found.
Conclusions: Our results about skewed sex ratio among SA with pure and mosaic trisomy 16 are in direct complement to prenatal diagnosis data. Moreover, dose-dependent association of asymmetrical X-inactivation with the level of trisomic cells in the human placenta provides the first evidence for possible linkage of tentative X-inactivation trans-factor to chromosome 16.

O5 12.45 – 13.00

THE CHROMATIN BARRIER PROTEIN BGP1 MEDIATES PROTECTION FROM DE NOVO DNA METHYLATION

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Introduction: The HS4 insulator element from the 5' chromatin boundary of the chicken beta-globin locus serves as a CTCF-dependent enhancer blocker and as a barrier to the spread of chromosomal silencing, including CpG methylation. It has been shown that binding of USF proteins to a specific site within the HS4 contributes to barrier activity by recruiting active histone modifications, limiting the spread of condensed chromatin. USF-mediated histone modification is necessary, but not sufficient for barrier activity. Three further sites, all of which bind a zinc finger protein called BGP1, are also required for barrier activity. Neither of the BGP1 sites contribute to the recruitment of active histone modifications, so their function remained unclear.
Methods & Materials: We have analysed the DNA methylation status of the endogenous HS4 insulator element and of insulated transgenes using bisulphite sequencing.
Results: We have found that the CpG-dense HS4 element, and sequences up to ~500 bp on either side of it, are unmethylated despite being located within a heavily methylated region of the genome. We hypothesise that HS4-binding proteins including BGP1 may play a role in

preventing DNA methylation to restrict the encroachment of nearby condensed chromatin. We find that a reporter transgene is protected from de novo DNA methylation when flanked by HS4 insulators. Deletion of the CTCF site within HS4 is dispensable for barrier activity and has no effect on DNA methylation. Deletion of the USF site disrupts barrier activity and leads to transcriptional silencing. Despite this, the transgenic promoter remains largely unmethylated. Conversely, deletion of any one of the three BGP1 sites leads to complete methylation of the transgenic promoter. These findings demonstrate that the apparent protection from DNA methylation by BGP1 is independent of active histone modifications and transcriptional status.
Conclusions: We propose that the function of barrier protein BGP1 at the chicken globin HS4 is to prevent the spread of DNA methylation.

O6 13.00 – 13.15

HIGH-THROUGHPUT ANALYSIS OF METHYLATION PATTERNS TO TRACK CELL DIVISIONS

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Errors in epigenetic DNA methylation at non-functional or neutral CpG islands can be used as a "molecular clock" to track cell replication. Methylation errors occur on the newly replicated DNA strand during the reestablishment of the parental methylation patterns. Like nucleotide substitutions, methylation of neutral CpG sites is heritable and cumulative, but occurs at a much higher frequency; thus, the methylation state of 8-12 CpGs in a neutral CpG island can be used as a binary code of information to follow cell replication 'a posteriori' in somatic tissues. A series of studies have already recorded methylation patterns at 3 neutral CpG islands and modelled the replication history in different tissues of the human body. Methylation patterns in these studies were assessed by sequencing bacterial clones derived from bisulfite treated genomic DNA. In order to derive a more complete picture of the replication history based on many more cells and individuals, we developed a high-throughput assay using a new technology similar to the next generation sequencing platforms. Our assay amplifies individual molecules on magnetic beads in microscopic aqueous compartments of an oil-buffer emulsion, resulting in each bead being covered by many identical PCR copies. The methylation status of each original molecule can then be assessed by fluorescent probes under a microscope. With this technology we are capable of interrogating ~400,000 CpG strings in a single experiment. In addition, a tagging strategy allows analyzing simultaneously many individuals. We have tested the reliability of this technology on known mixtures of CpG strings. With this technology it should be possible to capture with great detail the diversity of methylation patterns observed in oncogenic tissues.

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13.15 – 14.30
LUNCH AND COMMERCIAL EXHIBITION

EPIGENETICS AND GENETICS

IL.5 14.30 – 15.15

POLYCOMB REPRESSORS CONTROLLING STEM CELL FATE: IMPLICATIONS FOR CANCER AND DEVELOPMENT

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Repressive Polycomb-group (Pc-G) protein complexes and the counteracting Trithorax-group (Trx-G) of nucleosome remodeling factors are involved in the dynamic maintenance of proper gene expression patterns during development, acting at the level of chromatin structure. As such, they are important controllers of cell fate.
When deregulated, these master switches of gene expression are strongly implicated in formation of a diverse set of cancers. An example is the Pc-G gene Bmi1 which is overexpressed in medulloblastoma, Non small cell lung cancer, hepatocellular carcinoma and breast cancer and Glioma and is causally implicated in leukemia. We and others have recently implicated Bmi1/Pc-G as a critical regulator of stem cell fate in hemapoietic stem cells, neural stem cells, mammary epithelial precursor cells and ES cells. In addition, we have shown that Bmi1 is regulated by the Shh pathway and that the Ink4a/ARF tumors suppressors are critical Bmi1 target genes in stem cells and in cancer formation.

However our recent work on brain cancer (Glioma) points to important ink4a/ARFindependent Bmi1 targets involved in adhesion and motility. Comprehensive profiling of Polycomb target genes in Drosophila revealed its crucial conserved role in repressing lineage differentiation pathways and morphogens, including Wg, Hh, Delta and Notch. Furthermore, we have characterized in detail an essential E3-ubiquitin ligase activity in the PRC1 Polycomb complex that consists of a functional Ring1B-Bmi1 heterodimer. This E3 ligase activity is required for maintenance of Polycomb repression in normal- and cancer stem cells and hence offers potential novel ways to target cancer stem cells or tumor re-initiating cells in which the activity of this E3 ligase is hyperactivated. This is further substantiated by a novel way by which the activity of the Ring1B.Bmi1 E3 ligase is controlled. The implications of these findings for stem cell biology, development and cancer will be discussed.

FREE PAPER SESSION 2

O7 15.15 – 15.30

POLYCOMB/TRITHORAX PROTEINS REGULATE NFIA EXPRESSION DURING NORMAL AND LEUKEMIC HEMATOPOIESIS

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Introduction: In view of their function and timing, Polycomb (PcGs) and Trithorax (TrxGs) proteins may favor the onset and progression of tumors by means of several mechanisms. Notably, the balance between PcGs and TrxGs activity regulate stem cell plasticity and the expression of lineage specific genes. Thus, anomalous PcGs and TrxGs activity might affect the expression of lineage specific genes impeding the maturation of stem cells and committed progenitors and allowing the onset and expansion of a "cancer stem cell". Among mammalian TrxGs, the dysfunction of the MLL gene product has been related with leukemogenesis. MLL rearrangements are associated with anomalous overexpression of HOX genes and aggressive leukemias. Recently, it has been reported the ability of PML-RARa to bind and to recruit the polycomb PRC2 complex at its transcriptional target RARß2. SUZ12 knock down reverses histone marks and DNA methylation status of the RARß2 promoter, resulting in its reactivation and granulocytic differentiation of APL blasts.
Results: Recently, our laboratory has shown that NFIA is involved in a regulatory circuitry controlling granulocytic differentiation with its translational regulator hsa-mir223 and the lineage specific transcriptional factor C/EBPa. Now we show that PcGs/TrxGs regulate NFIA expression through epigenetic modifications of NFIA promoter chromatin structure. A proper PcGs activity is required for NFIA downregulation and for the successful establishment of the granulocytic differentiation program during normal hematopoiesis and for the removal of the block of granulocytic differentiation in an acute myeloid leukemia cell line. Moreover, we show that aberrant expression of NFIA is measurable in human acute promyelocytic leukemia blasts, and that it needs to be downregulated during ATRA induced-granulocytic differentiation of APL blasts.
Conclusions: Thus, a proper regulation of NFIA expression by PcGs represents an epigenetic event linked to leukemogenesis and a new target for transcriptional/differentiation therapy of human leukemia.

O8 15.30 – 15.45

TOWARDS COMPARATIVE EPIGENOMICS: A SOFTWARE TOOLKIT FOR CROSS-SPECIES EPIGENOME DATA ANALYSIS

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Over the past years, research in epigenetics has gained prominence as epigenetics mechanisms emerged as fundamental factors contributing to human disease. With the methods now in hand for experimentally generating genome-wide datasets, multiple exciting research directions arise. On the one hand, ChIP-seq promises to make large-scale epigenome analysis available for a wide range of non-human species, thereby giving rise to the new field of comparative epigenome analysis. On the other hand, the shear amount of data arising from ChIP-on-chip and ChIP-seq analysis makes the development of appropriate bioinformatic tools for automated data analysis a major concern. In our research at the Max Planck Institute for Informatics, we focus on the genome-epigenome relations that are at the heart of both problems, the cross-species comparison as well as the

development of improved bioinformatic methods. In both cases, the sequenced genomes form the stable scaffold for analyzing and comparing epigenome data. Specifically, we have recently developed the EpiGRAPH epigenome analysis toolkit (Bock et al., in preparation), which can be used to reliably predict epigenetic states from DNA sequence (e.g. Bock et al. (2007) PLoS Comput Biol 3: e110). Furthermore, we have implemented a computational pipeline that integrates genome sequence, whole-genome alignments, and epigenomic data sets into a unified comparative epigenomics analysis tool. The resulting software can be used to analyze footprints of epigenetic features within the genomic sequence across multiple mammalian genomes. By applying this approach we found evidence for significant conservation of genomic footprints of CpG island methylation within the mammalian phylogeny.

15.45 – 16.15 TEA AND COFFEE POSTER VIEWING

IL.6 16.15 – 17.00

GENOME-WIDE MAPPING OF ESTROGEN RECEPTOR BINDING AND ACTIVITY

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Estrogen Receptor (ER) regulation of target gene transcription is a significant factor in tumor development and progression. Previous work on ER-mediated transcription focused primarily on promoter regions of target genes, although recent work from our lab combining ER Chromatin Immunoprecipitation (ChIP) with tiled microarrays covering chromosomes 21 and 22, showed that distal enhancers appear to be the primary ER binding sites, and that these sites require the Forkhead protein FoxA1. We have extended on these ChIP-chip studies to map all the estrogen-induced ER and RNA PolII binding sites in the human genome. Furthermore, we have combined these data with expression microarrays after estrogen-treatment, to provide a complete network of receptor binding sites and the subsequent target genes of these binding events. We find approximately 3,600 ER binding sites, almost all of which are distal from promoter proximal regions and more than 3,600 RNA PolII sites, which exist almost exclusively at promoter regions. The binding profiles, in combination with the expression data reveal important details about the mechanisms by which ER regulates gene transcription. Furthermore, analysis of the sequence information contained within the binding sites reveals a complex network of transcription factors that are involved in regulating ER-mediated transcription.

O9 17.00 – 17.15

HIGH-RESOLUTION ANALYSIS OF GENOMIC DISEASE ON CHROMOSOME 22 ON THE LEVELS OF DNA SEQUENCE VARIATION, DNA METHYLATION AND GENE EXPRESSION

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Urban, Grubert, Koga, Pelizzola, Chandra Erdman, Sherman Weissman, Michael Snyder Earlier we developed High-Resolution (HR-)CGH on isothermal high-density oligonucleotide arrays as a powerful method for the analysis of CNV and genomic disease, such as Velocardiofacial Syndrome (VCFS) on chromosome 22 [Urban, Korbel et al., PNAS 2006; Korbel, Urban et al., PNAS 2007]. HR-CGH revealed the existence of several additional levels of complexity that have to be taken into account when explaining the variability in penetrance and combination of symptoms: difference in endpoints of the main deletion and difference in CNV complement, along the chromosome and in particular within the VCFS critical region on the chromosome without the main deletion. Analysis of expression patterns on Affymetrix exon arrays showed changes in gene expression genome wide in the cases with genomic aberrations. Genes within the main VCFS deletion region mostly, but not always, changed their expression patterns as expected. However, on chromosome 22 the deletion-boundary on the DNA level did not completely delineate the changes in expression levels, with clusters of genes changing distant from the deletion but also nearby. To bridge between DNA sequence and gene expression we generated high-resolution DNA methylation maps for chromosome 22 for 3 cases of VCFS as well as for an unbalanced and a balanced translocation 22:11, respectively, and for controls. We used the same iso-Tm chromosome 22 arrays as for HR-CGH, applying genomic DNA that had been enriched for DNA-methylated regions using methyl-C specific antibody (MeDIP). Of particular

interest are again the breakpoint-regions and the non-deleted VCFS critical region, constituting yet another layer of complexity in the molecular endophenotype. The combined data allow building a model case for the integration of high-resolution information from the DNA sequence-, DNA methylation- and gene expression levels. Of particular interest is potential interplay of variation on the copy-number and DNA methylation levels followed by changes in the expression network.

O10 17.15 – 17.30

EPIGENOME PROFILING VIA DNA METHYLTRANSFERASE-DIRECTED LABELING

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Genomic DNA methylation is a key epigenetic regulatory mechanism in high eukaryotes. DNA methylation profiles are highly variable across different genetic loci, cells and organisms, and are dependent on tissue, age, sex, diet, and other factors. However, research into the epigenetic regulation is hampered by the lack of adequate analytical techniques. We aim to develop novel technological approaches to genome-wide profiling of DNA methylation for epigenome studies and improved diagnostics. Our approach involves i) selective labeling of unmethylated CpG sites in the genome using our innovative technique - methyltransferase-directed transfer of extended groups (mTAG) from synthetic cofactor analogs [1-3]; ii) enrichment and amplification of biotin-labeled fragments; iii) analyzing the enriched fractions on tiling microarrays [4]. To this end, we have chemically synthesized a series of novel cofactor analogs that permit sequence-specific covalent labeling of DNA with biotin. Protein engineering was used to achieve efficient labeling of unmethylated CG sites in model DNA substrates with bacterial DNA methyltransferases HhaI (target sequence GCGC), HpaII (CCGG) and SssI (CG). Labeled fragments can then be readily isolated thru binding to streptavidin beads, paving the way to profiling of genomic DNA methylation on tiling microarrays. This novel approach potentially offers numerous advantages over existing techniques, and may advance epigenomic research into a new technological level in terms of scope, scale, sensitivity, and cost efficiency. [1] Dalhoff et al. Nature Chem. Biol. 2006, 2: 31-32. [2] Lukinavicius et al. J. Am. Chem. Soc. 2007, 129: 2758-9. [3] Klimasauskas & Weinhold. Trends Biotechnol. 2007, 25: 99-104. [4] Schumacher et al. Nucleic Acids Res. 2006, 34: 528-542.

IL.7 17.30 – 18.15

DENSE CADM1 PROMOTER METHYLATION ASSOCIATED WITH REDUCED IMMUNO-EXPRESSION IN HIGH-GRADE CIN AND CERVICAL SCC

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We previously showed that silencing of TSLC1, recently renamed CADM1, is functionally involved in high-risk HPV-mediated cervical carcinogenesis (1). CADM1 silencing often results from promoter methylation. Examination of a 93bp promoter region revealed frequent promoter methylation in high-grade squamous premalignant lesions and squamous cell carcinomas (SCC; =CIN3). Here, we determined the extent of promoter methylation and its relation to gene silencing to select a CADM1-based methylation marker for identification of women at risk of cervical cancer. Methylation specific PCRs targeting three regions within CADM1 promoter were performed on PBMCs, normal cervical smears and (pre)malignant lesions. CADM1 immuno-expression in cervical tissues was analysed by immunohistochemistry. All statistical tests were two-sided. Methylation frequency and density increased with severity of cervical squamous lesions. Dense methylation (=2 methylated regions) increased from 5% in normal cervical samples to 30% in CIN3 lesions and 83% in SCCs. Dense methylation rather than occasional methylation was significantly associated with decreased CADM1 immuno-detection in both CIN3 and SCC ($p<0.00005$). Age-adjusted logistic regression analysis showed that frequency of dense methylation was significantly higher in =CIN3 compared with =CIN1 (Odd's ratio (OR)= 5.6, 95% confidence interval [CI]= 1.7-18.5, p=0.005). In adenocarcinomas, dense CADM1 methylation was significantly less common compared with SCC (23% versus 83%; OR= 17.2, 95% CI= 2.9-101.1, p=0.002). Currently, we are exploring a quantitative MSP approach to determine methylation

patterns of CADM1 in cervical smears representing the various stages of cervical disease. In conclusion, dense methylation of the CADM1 promoter associated with decreased immuno-expression of CADM1 provides a valuable diagnostic marker for the triage of high-risk HPV-positive women at risk of =CIN3. 1.Steenbergen RDM, Kramer D, Braakhuis BJM, Stern PL, Verheijen RHM, Meijer CJLM, Snijders PJF TSLC1 gene silencing in cervical cancer cell lines and in cervical neoplasia. J. Natl. Cancer Inst. 2004, 96, 294-305.

18.15 – 19.15
BOARD MEETING:
MEMBERS SCIENTIFIC COMMITTEE ONLY

19.30 – on
SOCIAL PROGRAM AND DINNER