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Tuesday May 6

NON-CODING RNAs

IL.8 09.00 – 09.45

Title: T.B.A.

George Calin
University of Texas, MD Anderson Cancer Centre, Houston, Texas, U.S.A.

No abstract available

FREE PAPER SESSION 3

O11 09.45 – 10.00

PHARMACOLOGICAL UNMASKING OF ABERRANTLY HYPERMETHYLATED miRNAs IN HUMAN CANCER METASTASIS

Amaia Lujambio; George A. Calin; Carlo M. Croce; Manel Esteller; Alberto Villanueva.
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MicroRNAs (miRNAs) are small noncoding RNAs that control a wide range of biological functions, such as cellular proliferation, differentiation and apoptosis. MiRNAs have been shown to act as tumor suppressors or oncogenes. It is widely known that the promoter CpG island hypermethylation of tumor suppressor genes is a common event in cancer. We have recently demonstrated, for the first time, that this aberrant hypermethylation occurs in microRNAs with tumor suppressor features, leading to their silencing. We have also functionally linked the epigenetic loss of the miR-124a family with the activation of cyclin D kinase 6, a bona fide oncogenic factor, and the phosphorylation of the retinoblastoma, a tumor suppressor gene. In conclusion, the study of the methylation status of microRNAs in cancer is a valuable tool in the search of new tumor suppressor microRNAs. Subsequently, we have analyzed the differential miRNA expression in a set of human metastatic cell lines, before and after treatment with 5-aza-2′-deoxycytidine, a demethylating agent that restores the expression of methylated genes. Among the upregulated miRNAs found in the cells treated with 5-aza-2′-deoxycytidine, we have focused our study on those microRNAs embedded in CpG islands. Bisulphite sequencing analysis has confirmed that five of these microRNAs are specifically hypermethylated in cancer. These miRNAs are also aberrantly methylated in human primary tumors and the methylation levels are significantly higher in those primary tumors that give rise to metastasis. From a functional standpoint, the reintroduction of the microRNAs decreases the invasion capability of the cells, both in vitro and in vivo. We have demonstrated that these microRNAs directly regulate target oncogenes, also closely related to metastasis. With this study we demonstrate that epigenetic studies can be used in the search for new metastasis suppressor microRNAs.

IL.9 10.00 – 10.45

SMALL RNAs IN GERMLINE DEVELOPMENT AND HUMAN CANCER

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The Piwi proteins of the Argonaute superfamily are required for normal germline development in Drosophila, zebrafish and mice and associate with 24-30 nucleotide RNAs termed piRNAs. Here we show that C. elegans Piwi is specifically expressed in the male and female germline and required for normal germline development. We identify a class of 21 nucleotide RNAs, previously named 21U RNAs, as the piRNAs of C. elegans. We find that piRNA expression is independent of many proteins in other small RNA pathways including DCR-1. Piwi is specifically required to silence Tc3, but not Tc1 or Tc4 DNA transposons in the germline. We do not find any evidence for a Ping-Pong model for piRNA amplification in C. elegans. Instead, we show that Piwi acts upstream of an endogenous siRNA pathway in C. elegans. These data provide new insights into piRNA biogenesis and function.

10.45 – 11.15 TEA AND COFFEE POSTER VIEWING

IL.10 11.15 – 12.00

Title: T.B.A.

Antonio Giráldez
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No abstract available

O12 12.00 – 12.15

DIFFERENTIAL EXPRESSION OF miRNAs IN HUMAN CERVICAL CANCER

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Introduction: MicroRNAs (miRNAs) are short (~ 22 nt) non-coding regulatory RNAs that control gene expression at the translational level (1). They play important and critical roles in various biological processes, including cell development, differentiation, apoptosis and cancer. To date, 5395 mature miRNAs have been discovered, through a combination of bioinformatics and cloning/sequencing approaches and their expression is being monitoring through Northern blot analysis, qRT-PCR and Microarrays. Nevertheless, their biological roles are still far from being understood. Despite this, important deregulation of miRNA expression has been discovered in a wide variety of tumours and it is now clear that they contribute to cancer development and progression (2). This prompted the development of miRNA-chips for cancer diagnosis or prognosis, opening a new door to understand carcinogenesis. Cervical cancer affects 17 out of 100,000 women in Portugal, which are the highest values in Europe (3). Therefore, there is a strong need for a non-invasive, fast and efficient method to diagnose the disease. We are investigating miRNA expression profiles in cervical cancer using a microarray platform developed in house containing probes specific for mature miRNAs. *Methods & Materials*: miRNA-chip was fabricated in house and includes human, rat and mouse miRNA mature sequences (Ambion). For this, 5' amine modified C6 oligonucleotides were printed onto Nexterion-E slides (SCHOTT) at 20 μM final concentration using a MicroGrid II compact spotter. Cervical cancer biopsies from 27 patients were obtained from the Tumour Bank of the Hospital of the University of Coimbra. Total RNA was extracted from biopsies, using mirVana miRNA isolation kit (Ambion), and was labelled using the miRacULS II miRNA labelling kit (Kreatech). miRNA-chip hybridization and washing was performed following manufacture's instructions. Slides were scanned and images were analyzed using specialist software for microarray data extraction (QuantArray). Slides were normalized using global median normalization and significant analysis of microarrays (SAM) was applied to normalized data.
Results: We used a miRNA-chip developed in house to evaluate miRNA expression profiles of a heterogeneous set of cervical tissues from 27 different patients. This set included 19 normal cervical tissues, 5 cervical adenocarcinomas, 6 high-grade squamous intraepithelial lesion (HSIL) and 11 low-grade squamous intraepithelial lesion (LSIL) samples. The unsupervised hierarchical clustering based on all the human miRNAs spotted on the chip, generated a tree with a clear distinction of samples in two main groups represented by normal and lesion samples. To identify miRNAs differentiating normal vs diseased tissue, we used SAM analysis. The results obtained from the 4 types of class prediction analysis were largely overlapping. The SAM comparison between normal tissues and each type of lesion identified some miRNAs differentially expressed. To confirm the results obtained by microarrays analysis we are carrying out qRT-PCR on some of the differentially expressed miRNAs.
Conclusions: A possible miRNA misexpression signature associated with cervical cancer was identified. We hope that the data will allow us to correlate miRNA expression with the metastatic, invasive or proliferative potential of the disease and will shed new light on the biological role of miRNAs in cervical cancer.
Acknowledgments: PMP is supported by the FCT grant SFRH/BPD/26611/2006. These studies are supported by FCT-FEDER grant POCI/SAU-MMO/55476/2004 and ACIMAGO.
References: 1. Bartel D.P. (2004) Cell, 116, 281-297. 2. Cho W.C. (2007) Mol Cancer, 6, 60. 3. Levi F., Lucchini F., Negri E., Franceschi S. and la Vecchia C. (2000) Eur J. Cancer, 36, 2266-2271.

O13 12.15 – 12.30

EXPRESSION OF THE miRNA CLUSTER MIR-17-92 IS ASSOCIATED WITH DNA COPY NUMBER GAIN OF 13Q DURING COLORECTAL ADENOMA TO CARCINOMA PROGRESSION

Begona Diosdado; Mark A. van de Wiel; Sandra Mongera; Cindy Postma; Beatriz Carvalho; Gerrit Meijer.
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A novel class of non-coding RNAs called microRNAs have shown to regulate central mechanisms of tumorigenesis and contribute to tumour development as tumour suppressors or oncogenes due to altered expression. We have documented that in colorectal chromosomal instable tumours 13q gain is a major factor associated with colorectal adenoma to carcinoma progression. Functional studies on the miR-17-92 cluster localized on 13q31 have demonstrated that its transcription is activated by the transcription factor c-myc and has antiapoptotic, proliferative and angiogenic activities. These results show an oncogenic function of this miRNA cluster. Therefore, we propose to investigate the contribution of the miR-17-92 cluster in the tumourbiology of colorectal cancer. RNA expression levels of the miR-17-92 cluster was determined in 55 colorectal tumours with array CGH data available and ten controls by quantitative RT-PCR. Identification of target genes was accomplished by integrating mRNA and miRNA expression data from the same tumours and miRNA target prediction programs. The miR-17-92 cluster shows increased expression in tumours showing 13q gain ($p<0.05$) compared to colorectal tumours without 13q gain. Five putative target genes, one of which has known tumour suppressor activity have been identified. The oncomir miR-17-92 cluster seems to play a role in colorectal adenoma to carcinoma progression.

IL.11 12.30 – 13.15

FUNCTIONAL GENETIC APPROACHES IDENTIFY CANCEROUS miRNAs

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MicroRNAs (miRNAs) are potent post-transcriptional regulators of protein coding genes. Patterns of mis-expression of miRNAs in cancer suggest key functions of miRNAs in tumorigenesis. However, current bioinformatics tools do not fully support the identification and characterization of the mode of action of such miRNAs. To perform genetic screens for novel functions of miRNAs we developed a library of vectors expressing the majority of cloned human miRNAs and created corresponding DNA barcode arrays. In a screen for miRNAs that cooperate with oncogenes in cellular transformation we identified miR-372 and miR-373, each permitting proliferation and tumorigenesis of primary human cells that harbor both oncogenic RAS and active wild type p53. We provide evidence that these miRNAs are potential novel oncogenes participating in the development of human testicular germ cell tumors by numbing the p53 pathway, thus allowing tumorigenic growth in the presence of wild type p53. Recently, we have used a novel functional genetic approach and identified miR-221 and miR-222 (miR-221&222) as potent regulators of p27Kip1, a cell cycle inhibitor and tumor suppressor. Interestingly, high miR-221&222 levels appear in signatures of poor prognosis cancers. Using miRNA-inhibitors we demonstrated that certain cancer cell lines require high activity of miR-221&222 for the maintenance of low p27Kip1 levels and continuous proliferation. Thus, high levels of miR-221&222 promote cancerous growth by inhibiting the expression of p27Kip1. Last, we performed experiments to uncover metastasis promoting miRNAs. We describe the role of miR-373 in cellular migration and metastasis of breast cancers. Thus, we find functional genetic experiments extremely usfule in the identification and characterization of cancerous miRNAs.

13.15 – 14.30
LUNCH AND COMMERCIAL EXHIBITION

IL.12 14.45 – 15.30

POSTTRANSCRIPTIONAL REGULATION OF DNMT3B mRNA BY THE RNA-BINDING PROTEIN HUR IN COLON CANCER CELLS

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The molecular basis of aberrant CpG island hypermethylation of tumor suppressor genes in human cancer is unknown. DNA methyltransferases (DNMTs) are believed to be responsible for the altered methylation levels of these genes since their expression levels increase during tumorigenesis. Three distinct families of DNA methyltransferase genes, DNMT1, DNMT2 and DNMT3, have been identified in mammalian cells. Here, we present evidence that the aberrant upregulation of DNMT3b in cancer is due in part to posttranscriptional mechanisms by an RNA-binding protein (RBP) and a microRNA. The RBP HuR binds to specific mRNA subsets and is increasingly recognized as a pivotal posttranscriptional regulator of gene expression. The presence of a putative HuR-recognition motif in the 3'UTR of the DNMT3b mRNA raised the possibility that this transcript could associate with HuR. The interaction between HuR and DNMT3b mRNA was demonstrated in vivo by immunoprecipitation of endogenous HuR ribonucleoprotein complexes followed by RT-PCR detection of the DNMT3b mRNA, and in vitro by pulldown of biotinylated DNMT3b RNAs followed by Western blotting detection of HuR. Importantly, binding of HuR to the DNMT3b mRNA stabilized the DNMT3b mRNA and increased DNMT3b protein levels. The microRNA miR29 was also shown to bind the 3'UTR of DNMT3b and to reduce DNMT3b expression. Because of the proximity of the HuR motif hit to the miR29 binding site on the DNMT3b 3'UTR, we have also studied whether HuR counteracts the function of miR29 family. In summary, our data identify DNMT3b mRNA as a novel HuR target and presents evidence of the posttranscriptional regulation of DNMT3b expression in colon cancer cells.

O14 15.30 – 15.45

EPIGENETIC DE-REGULATION OF MICRO RNA-223 IN MYELOID LEUKEMOGENESIS

Francesco Fazi; Serena Racanicchi; Giuseppe Zardo; Linda Starnes; Marco Mancini; Lorena Travaglini; Daniela Diverio; Emanuele Ammatuna; Guiseppe Cimino; Francesco Lo-Coco; Clara Nervi; Francesco Grignani.
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Hematopoiesis is a life-long, highly-regulated multistage process where a pluripotent self-renewing hematopoietic stem cell (HSC) gives rise to all blood cell lineages. In early hematopoietic progenitor cells, growth and maturation of erythroid, granulocytic, monocytic and megakaryocytic lineages are largely controlled by unique combinations of transcription factors that cooperatively regulate promoters and enhancers present on specific target genes. Several evidences have shown that microRNAs (miRNA) are differentially expressed in hematopoietic cells and play an important role in hematopoiesis. Thus, changes in specific transcription factors and miRNAs levels, and/or in local chromatin organization at their loci may affect proliferation, differentiation and genetic stability of HSCs resulting in myeloproliferative disorders and leukaemia. Deregulation of the DNA methylation and of the chromatin "histone code" at specific gene sites cooperate in the pathogenesis of leukemia. Leukemia fusion proteins derived from chromosomal translocation can mediate epigenetic silencing of gene expression, resulting in myeloid differentiation-block and acute myeloid leukemia (AML). In our studies the miRNA-223 induction by the myeloid-specific transcription factor C/EBPa is essential for granulocytic differentiation and its expression is epigenetically down-regulated by AML1/ETO, the commonest AML-associated fusion protein. In particular we show that miRNA-223 is a direct transcriptional target of AML1/ETO. By recruiting chromatin remodelling enzymes at an AML1-binding site on the pre-miRNA-223 gene, AML1/ETO induces heterochromatic silencing of miRNA-223. Ectopic miRNA-223 expression, RNAi against AML1/ETO, or demethylating treatment enhance miRNA-223 levels and restores cell differentiation. Together, these evidences highlight miRNAs, transcription factors and chromatin remodelling events as ultimate determinants for the

correct expression of cell type-specific genes and terminal differentiation, therefore providing new targets for the diagnosis and treatment of leukemias.

O15 15.45 – 16.00

IDENTIFICATION OF NOVEL BARRIER ELEMENTS ACROSS THE REGIONS OF THE HUMAN GENOME

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Introduction: Eukaryotic genes and gene clusters are proposed to be organised into distinct functional chromatin domains, which can be kept independent of their surroundings by means of insulator elements. The HS4 insulator element, which marks the 5' boundary of the chicken beta-globin domain, has two separable activities: it can act as an enhancer blocker to prevent inappropriate enhancer-promoter communication and as a barrier to the spread of repressive heterochromatin. We have recently determined that BGP1 interacts with HS4 sequences that are essential for its barrier activity. BGP1 is a very highly conserved zinc finger protein that is restricted to vertebrates. We hypothesised that we could use BGP1 as a bait to identify novel barrier elements across the human genome. Despite considerable progress in mapping CTCF-dependent enhancer blocking elements, no bona fide barrier elements have been identified in humans to date.
Methods: ChIP-on-chip experiments were performed to identify novel BGP1 targets. Briefly, BGP1 protein was in vivo cross-linked to its binding sites and precipitated using a specific antibody. Enriched DNA sequences were then purified and hybridised onto microarrays.
Results and Conclusions: We have mapped the in vivo binding sites of BGP1 across the ENCODE regions representing 1% of the human genome, in an early erythroid cell line K562. We have identified ~125 sites, which cluster into distinct groups consisting of known promoter elements (57%), known or predicted enhancers (15%) and other intergenic sites (20%). A significant proportion of sites in the latter group also bind the insulator-associated proteins USF and CTCF. We are testing whether these sites harbour insulator activities in a manner similar to the HS4 element. Furthermore, a considerable proportion of the BGP1 elements (55%) are unmethylated CpG islands, consistent with our findings that BGP1 can protect against de novo DNA methylation.

References: Yusufzai et al. Mol Cell. 2004 13:291-298. van Steensel B. Nat Genet. 2005 37: S18-S24. Kim TH et al. Cell. 2007 128:1231-1245.

16.00 – 16.30 TEA AND COFFEE POSTER VIEWING

O16 16.30 – 16.45

THE METHYL-CPG BINDING DOMAIN PROTEIN 2 (MBD2), A SPECIFIC INTERPRET OF METHYLATED LOCI IN CANCER CELLS

Amandine Masquelet; Emilie Auriol; Stephanie Bougel; Laury Perriaud; Joël Lachuer; Jean Benhattar; Robert Dante.
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In the past few years, several clinical trials have shown that targeting DNA methylation machinery might be of interest in cancer therapy to restore tumor suppressor genes expression and inhibit tumor growth. The methyl-CpG binding domain proteins (MBPs) are important constituent of the DNA methylation machinery since they are directly involved in the mediation of the epigenetic signal and therefore might represent an alternative target to DNA methyltranferases. Since MBPs seem to show some gene specificity, their inhibition should reactivate a limited number of genes and might be less toxic than global demethylation. Taken together these data suggest that MBPs represent potential new targets in cancer therapy and, therefore, new insights on MBPs specificities are, in this context, of importance. To this end, we have developed two different approaches: a candidate genes analysis and a genome-wide analysis by combining MBPs binding sites map and gene expression data, using HeLa cells. Chromatin immunoprecipitation (ChIP) assays showed that only MBD2 was associated with the methylated promoter region of NBR2, pS2 and hTERT. In MBD2 knockdown HeLa cells, transitionally or constitutively depleted in MBD2, neither MeCP2 nor MBD1 acted as a substitute for MBD2 at the NBR2, pS2 and hTERT methylated regions. Furthermore, depletion of MBD2 in these cells led to the suppression of NBR2, pS2 and hTERT repression that can be restored by expressing mouse Mbd2 protein. Our results indicate that MBD2 is specifically and directly involved in the transcriptional repression of the NBR2, pS2 and hTERT. Moreover, analysis of global gene expression patterns by microarrays indicates that MBD2 depletion does not lead to a global gene reactivation, since only a limited number

of genes (1-2%) are deregulated in its absence. In order to discriminate between indirect and direct effects, genomic targets of MBD2 protein are determined by ChIP on chip experiments. These data are currently under validation and cross-analysis between microarrray expression data should answer this question. Collectively, our data suggest that MBD2 has specific targets and its specific inhibition does not induce genome-wide reactivation.

O17 16.45 – 17.00

MOLECULAR DISSECTION OF THE ROLE OF HISTONE METHYLATION IN THE REGULATION OF TUMOUR SUPPRESOR GENES THAT BECOME HYPERMETHYLATED IN CANCER

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In cancer cells, the promoter CpG island of many tumour suppressor genes (TSG) undergoes a process of aberrant hypermethylation that leads to silencing through the modification of the histone modification patterns. We are investigating the connections between the pattern of activating lysine methylation marks in histones and the DNA methylation status of a set of promoter CpG islands of TSG. To this end, we have investigated the change in the pattern of all activating Histone methylations in TSG -specifically trimethyl K4 of histone H3 (H3K4me3), trimethyl K36 H3 (H3K36me3) and dimethyl K79 H3 (H3K79me2) - resulting from the abrogation of DNA methylation by pharmalogical inhibition or by genetic disruption of DNA methyltransferases (DNMT1; DNMT3B) in colon cancer cells. We have found that unmethylated promoters are characterized by the presence of H3K4me3 and H3K79me2 but not H3K36me3, which is present in the body of the genes. In contrast, hypermethylated promoters are devoid of active lysine methylation marks. Interestingly, depletion of DNA methylation at hypermethylated promoters is correlated with an incomplete re-establishment of the transcription levels which correlates with a partial recovery of the histone methylation signature, since only H3K4me3 but not H3K79me2 is re-established. We have demonstrated that the absence of H3K79me2 at those promoters is correlated with the absence of recruitment of DOT1L, the specific histone methyltransferase that modifies this K79 H3. To test the specific contribution of DOT1L, and H3K79me2, in maintaining the active status of promoters, we have performed a DOT1L knockdown. In DOT1L depleted cells, we have found a 50% reduction in the expression levels of unmethylated TSG, in a DNA methylation independent manner. Our data indicates that DOT1L and H3K79me2 are essential for the establishment of a proper euchromatic state and consequently in maintaining the transcriptionally active status of TSG.

O18 17.00 – 17.15

GENOME ACCESSIBILITY IN HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPC)

Gaetano Gargiulo; Gabriele Bucci; Sam Levy; Mauro Romanenghi; Fyodor Urnov; Lorenzo Fornasari; Marco Ballarini; Fabio Santoro; Pier G. Pelicci; Saverio Minucci.
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A major goal in genomics is to understand how genes are regulated in different tissues, stages of development and diseases(1). The key feature distinguishing epigenomic from genomic information is the dynamic pattern of epigenetic features, which impose cell-type specific expression, regulating cell function(2). Genome accessibility in the context of cellular chromatin is a well-established epigenetic feature of active regulatory DNA(3). We have used human HSPC as a model system to study which fraction of the genome undergoes changes in accessibility during differentiation. To this extent, we have developed a novel large-scale approach based on the use of restriction enzymes (RE) to probe and sequence DNA elements, which are accessible in chromatin(3). Among these sequences, we identified - in a tissue specific manner - known and novel cis-regulatory elements. Here we show that one single structural feature of the genome (accessibility) can provide a general picture of factor binding for all classes of transcription factors, including enhancers, silencers and insulators (validated by ChiP). Genome-wide analyses indicated that low nucleosome density is a common feature of gene promoters, even when poorly transcribed (as inferred by gene expression profile). In addition, a significant fraction (60%) of the DNA accessible in chromatin was located at intergenic sites and repeated DNA, suggesting that this feature may be far more complex then previously thought. We also mapped large genomic domains (1,2Mb wide) with differential accessibility in

early steps of hematopoiesis, which include, to some extent, clusters of genes involved in blood cell development and function. This study provides a framework for the application of comprehensive epigenomic profiling towards characterization of regulatory processes in primary cells.
References: 1. F. S. Collins et al., Nature 422, (Apr 24, 2003). 2. B. Li et al., Cell 128, (Feb 23, 2007). 3. H. Reinke et al., Biochim Biophys Acta 1677, (Mar 15, 2004).

019 17.15 – 17.30

GENOMIC REARRANGEMENT OF TMPRSS2 FUSIONS WITH ONCOGENIC ETS FACTORS: EVIDENCE OF EPIGENETIC PROGRAMMING OF POTENTIAL THERAPEUTIC SIGNIFICANCE IN PROSTATE CANCER

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Introduction: A unique gene fusions between the prostate-specific and androgen-responsive 5'TMPRSS2 gene and 3'ERG oncogenic ETS transcription factors represent a novel mechanism by which androgens promote carcinogenesis and the persistent "androgen dependence" of prostate cancers . Here, we explored the role of ERG-related oncogenesis using a combination array-CGH, gene expression arrays, in silico database searches, and testing of candidate compounds.
Methods and Results: First, using a rare set of 19 advanced prostate cancers, including hormone-refractory ones, we found 7 TMPRSS2-ERG fusions, 2 TMPRSS2-ETV4 fusions and none involving the ETV1 gene. Second, high.resolution array-CGH analyses showed that a small deletions and unbalanced intrachromosomal rearrangements underlied the fusion formation between the adjacent TMPRSS2 and the ERG genes. Third, to understand the downstream consequences and tumorigenic mechanisms of ERG fusions, we analyzed gene microarray data from a total of 284 prostate cancers. Significance analysis of microarrays (SAM), gene set enrichment analysis (GSEA) and various supervised clustering methods were applied to data analysis of three different cohorts of prostate cancers. Co-expression of ERG with HDAC1 was the most consistent feature in all three datasets.Using gene set enrichment analysis (GSEA), we found that the WNT and PITX2 pathways were significantly induced. In parallel, known HDAC1 target genes, and the CCR5, cell death, and TNF/FAS pathways were significantly repressed. A number of the genes that were most often silenced in prostate cancers by epigenetic processes such as GSTP1, negatively correlated with ERG overexpression. Finally, as the in vivo gene coexpression profiles suggested a role of epigenetic processes in the ERG-tumorigenesis, we tested well-known histone deacetylase inhibitors against ERG-positive VCaP and DuCap prostate cancer cell lines. Both cell lines types strong apoptotic responses with nanomolar concentrations of anti-HDAC drugs Trichostatin A and MS-275 . *Conclusions:* In summary, these studies imply that unbalanced chromosomal events underlie ERG fusion gene formation in prostate cancer, and that this leads to downstream events that involve several prostate cancer hallmarks, including silencing of apoptosis. The strong association with HDAC1 and its downstream genes, may indicate that patients with ERG-positive prostate cancer could benefit from epigenetic therapy with HDAC inhibitors alone or in combination with established therapies.

O20 17.30 – 17.45

GENOMICS OF MECP2 DUPLICATIONS

Marijke Bauters; Hilde Esch van; Michael J. Friez; Odile Boesplug-Tanguy; Martin Zenker; Angela M. Vianna-Morgante; Carla Rosenberg; Jaakko Ignatius; Martine Raynaud; Karen Hollanders; Karen Govaerts; Joke Nevelsteen; Kris Vandenreijt; Florence Niel; Pierre Blanc; Roger E. Stevenson; Jean-Pierre Fryns; Peter Marynen; Charles E. Schwartz.
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Recurrent submicroscopic genomic copy number changes are the result of non-allelic homologous recombination (NAHR). Non-recurrent aberrations however, can result from different non-exclusive recombination-repair mechanisms. We previously described small microduplications at Xq28 containing MECP2, in four patients with a severe neurological phenotype. Here, we report on the fine-mapping and breakpoint analysis of 16 unique microduplications. The size of the overlapping copy number changes varies between 0.3 and 2.3 Mb and FISH analysis on three families demonstrated a tandem orientation. Although 8 of the 32 breakpoint regions coincide with low copy repeats (LCRs), none of the duplications seems to be the result of NAHR. Bioinformatic analysis of the breakpoint

regions demonstrated a 2.5-fold higher frequency of Alu interspersed repeats (IRs) as compared to control regions, as well as a very high GC content (53%). Initial cloning efforts by long-range PCR allowed us to determine the junction in only one patient, which revealed non-homologous end joining (NHEJ) as the mechanism. Cloning of the breakpoints in two other patients, and detailed molecular analysis revealed a more complex rearrangement due to an alternative DNA repair mechanism during replication. This new recombination-repair model will be discussed. Our results demonstrate that the mechanism by which copy number changes occur in regions with a complex genomic architecture can trigger both the common repair mechanisms (NAHR, NHEJ) as well as a combination of common and alternative repair processes, yielding either a simple or complex rearrangement.

17.45
ANNOUNCEMENT SEPTEMBER WORKSHOP IN BRAGA, PORTUGAL

Rui Reis
University of Minho, Braga, Portugal

18.00
ANNOUNCEMENT NEXT CONFERENCE IN EDINBURGH, UK

Nick Gilbert
University of Edinburgh, Edinburgh, UK

18.00 – on
SOCIAL PROGRAM AND DINNER