

Thursday 3 May 2007

GENOME PROFILING TO IDENTIFY DISEASE GENES

IL2 08.45 – 09.30

MOLECULAR CHARACTERIZATION OF NOVEL AMPLIFICATION TARGET GENES IN PANCREATIC CANCER

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Pancreatic carcinoma is a highly aggressive tumor type with a survival rate of only about 3% and a median survival time less than 6 months. The disease is typically diagnosed in an advanced state when curative resection is no longer possible and it is also highly resistant to current chemotherapy regimens. Aneuploidy and increased genetic instability, manifesting as losses, gains, and amplifications, are common characteristics of pancreatic cancer. These genetic aberrations are likely to conceal genes involved in disease pathogenesis and uncovering such genes might thus provide targets for the development of new diagnostic and therapeutic approaches. In recent microarray based copy number surveys, we and others have identified a set of recurrent copy number changes in pancreatic cancer, including a novel amplified region at 19q13. Our subsequent studies have delineated the amplicon to a 1.1 Mb region containing a total of 39 transcripts. Comprehensive expression analysis using quantitative RT-PCT revealed a subset of genes with consistently elevated expression levels in the amplified versus non-amplified pancreatic cancer cell lines. Loss-of-function studies by RNA interference (RNAi) were then performed to identify functionally relevant genes within the amplicon. This screen highlighted a small set of biologically interesting genes that regulate key cellular functions, such as cell survival, cell cycle and apoptosis, in 19q13 amplified pancreatic tumors. In conclusion, this study demonstrates the power of combination of copy number and expression analysis together with targeted RNAi screen for rapid identification of putative amplification target genes in cancer.

FREE PAPER SESSION 1

O1 09.30 – 09.45

ANALYSIS OF BRCA1 AND BRCA2 MAMMARY TUMORS USING ARRAY CGH

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Individuals who carry a germline mutation in the BRCA1 or BRCA2 genes are predisposed to developing breast and ovarian cancer. Cells with mutations in these genes are unable to repair DNA damage, making them especially prone to acquire genomic aberrations. Certain aberrations may ultimately lead to activation of oncogenes or inactivation of tumor suppressor genes. To investigate specifically which genomic aberrations caused by nonfunctional Brca1 or Brca2 lead to malignancies in mammary epithelial cells, we generated conditional mouse models. We used the Cre-LoxP system to conditionally delete either the Brca1 or Brca2 gene with the p53 gene in the mammary gland epithelium thereby inducing the formation of mammary tumors. Because these tumors have arisen in genetically identical mice and carry the same conditional p53 deletion, it is feasible to identify specific genomic alterations that distinguish between the Brca1 and Brca2 tumor groups. We compared 35 Brca1;p53 tumors with 62 Brca2;p53 tumors, controlled by 33 p53 tumors. Analysis of these tumors by array CGH allowed us to detect specific DNA copy number changes. P53 loss renders tumors with a characteristic CGH profile and additional Brca1 or Brca2 loss intensifies this profile. Aberrations seen in the p53 tumor group are also found in the Brca1 and Brca2 groups, but more abundantly. For both the mouse and human tumor groups, we used our Kernel Convolution Method to identify genomic aberrations significantly different from random. When we compared these regions we found syntenic regions that were significantly deleted or amplified in both the mouse and the human tumor groups and identified the genes that lie on these regions. Expression microarray data from the mouse tumor panels enabled us to identify overexpressed or downregulated genes in the regions of these amplifications or deletions respectively. We have

identified a set of genes that lie on genomic regions that are commonly amplified or deleted in both murine and human Brca1 or Brca2 tumors. We are currently investigating which of these genes could be driving the selection of these different amplicons or deletions.

O2 09.45 – 10.00

COMPREHENSIVE CHARACTERIZATION OF GENOMIC ABERRATIONS IN GANGLIOGLIOMAS BY COMPARATIVE GENOMIC HYBRIDIZATION (CGH), ARRAY-BASED CGH AND INTERPHASE-FISH

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Introduction. Gangliogliomas (GG) are generally benign (WHO-grade I or II) neuroepithelial tumors frequently associated with epilepsy. They are composed of dysplastic neuronal and neoplastic glial elements. Because little is known about their molecular pathogenesis, we aimed to identify genomic aberrations involved in GG tumorigenesis.

Methods and Materials. Fifty-two GGs (47 WHO-grade I, 5 WHO-grade II) were screened for chromosomal imbalances by CGH. Twenty-one GGs with available high molecular weight DNA were analyzed by high-resolution array-CGH to an 8k-BAC-array. Interphase-FISH to tumor tissue-sections was performed.

Results. Genomic aberrations were detected by CGH in 60% of GGs with an average of 1.85 ± 0.34 (mean \pm SEM) alterations per tumor (range: 0–13). Recurrent gains were identified on chromosomes 7 (17% of tumors), 5 (13%), 8 (12%), Y (8% of tumors from male patients), 20 and X (8% each), 12 and 19 (6% each), 4, 9q and 17 (4% each). Recurrent losses were found on chromosomes 22q (17%), 9 (10%), Y (8% of tumors from male patients), 16 (8%), 17, 18, 20 and 21q (6% each), 10q, 13q and 19 (4% each). Combined gains of chromosomes 7 and 8 were detected in six cases. Array-CGH confirmed the aberration pattern and additionally detected four cases with smaller imbalances (dim10q21.1-q26, dim10q22.3-q25.3, dim15q11.2-q22.2, enh12q13.3-q14.1). By interphase-FISH, a subpopulation of glial cells was found to contain the chromosomal imbalances detected, whereas in the dysplastic neuronal cells no aberrations were found. Two GGs had recurred as malignant

glioblastomas (GBM, WHO-grade IV). These GBMs contained a loss of CDKN2A/CDKN2B in one case and an amplification of CDK4 in the other case. Interestingly, the loss of CDKN2A/CDKN2B and a gain of CDK4 were already detectable in the primary GGs of WHO-grade I and II. *Conclusions.* Our study provides the first comprehensive overview of genomic alterations in a large series of gangliogliomas.

O3 10.00 – 10.15

BAC TO INFINIUM: A COMPARISON OF NEAR-TILING PATH BAC ARRAYS AND ILLUMINA HAP300 SNP ARRAYS FOR MOLECULAR GENETIC PROFILING OF BREAST CANCER

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Introduction. Array-based comparative genomic hybridisation (aCGH) has proven to be a powerful tool to characterise the molecular genetic profiles of cell lines and human tumours, to fine map specific amplicons and to identify the likeliest 'amplicon drivers'. Whilst providing accurate copy number alterations within tumours, bacterial artificial chromosome (BAC) arrays do not provide information regarding copy neutral events (e.g., endoreduplication and mitotic recombination). On the other hand, profiles obtained with SNP platforms are reported to show a greater variation for regions with no copy number alterations. Our aims were to define whether Infinium SNP analysis accurately identifies single copy number gains and whether copy neutral events could be reliably identified.

Methods and Materials. We compared the molecular genetic profiles of a series of grade III breast carcinomas of distinct subtypes. Tumour samples were microdissected to ensure >90% of neoplastic cells and profiled with both platforms. Normalised and smoothed Log₂ ratios were converted into categorical variables (i.e., homozygous deletions, losses, no change, gains and amplifications) according to previously defined and FISH-validated thresholds. Results of BAC array analysis were directly compared to the copy number scores as generated by Illumina's proprietary software 'Bead studio v2' using the log R ratios and B allele frequencies. Amplifications were confirmed by means of in situ hybridisation.

Results. In general there was good agreement between whole arm gains and losses and high-level amplifications, however the Bead studio software failed to identify a number of low level gains and deletions detected by BAC arrays. Surprisingly, no significant increase in the resolution of the detected copy number changes was found with Infinium arrays. On the other hand, Illumina arrays identified regions with copy number silent LOH not detected by aCGH in 83% of samples. Identification of such events will enhance the understanding of the genetic evolution of the tumour and provide a greater insight into the mechanisms involved with inactivation of tumour suppressor genes. By comparing the log R ratios from the SNP chip analysis with B allele frequency, one can more readily discern copy number variation from the norm. However, in this study, it was observed that this approach is hindered by the inclusion of as little as 10% of normal cells within the tumour sample, making its application to micro-dissected tumour samples challenging.

Conclusions. Based on our analysis so far, it is apparent that the thresholds for determining copy number alterations are sub-optimal with the Beadstudio software v2. Our results also demonstrate that BAC arrays and SNP chips can be used in a complementary fashion. Deconvolution of the amplicons and identification of regions harbouring putative tumour suppressor genes in high-grade breast cancer samples using both high-resolution techniques is currently being undertaken.

O4 10.15 – 10.30
GENOME-WIDE SCREENING OF HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) USING SINGLE NUCLEOTIDE POLYMORPHIC MARKER (SNP)-ARRAYS

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Introduction. Genetic profiling of HNSCC can be performed using microarray comparative genomic hybridization (maCGH) that detects numerical chromosomal aberrations, but it underestimates allelic losses because of copy neutral events. Allelic loss is relatively frequent and occurs early in carcinogenesis. Moreover, established maCGH platforms do not always allow to use DNA from archival formalin-fixed paraffin embedded (FFPE) tissue. A recently introduced SNP

array platform allows for simultaneous detection of numerical and allelic aberrations. This is carried out on short (40 bp) segments of genomic DNA around the SNPs of interest, suggesting application for archival DNA. We aim to evaluate the performance of the Illumina SNP platform for FFPE HNSCC tissue specimen. We ultimately aim to elucidate whether the metastatic phenotype can be predicted based on a genetic profile of the primary HNSCC.

Methods and Materials. In total, 12 HNSCC and 12 corresponding normal DNA FFPE samples were analyzed. samples of primary HNSCC were from patients with more than three tumour-positive lymph nodes, who have a 50% risk to develop distant metastases. To evaluate the accuracy of the Illumina platform, samples were included from which the genetic changes were determined previously using established platforms. Three fresh-frozen samples were included for comparison.

Results. Analysis of SNPs showed a call frequency of 0.9931 for FFPE vs 0.9960 for frozen samples. Comparison of archival material to frozen material and to results obtained from frozen material of the same tumours with maCGH using BAC arrays or allelic loss determination by microsatellite PCR, shows that the genetic data obtained are practically identical.

Conclusions. These findings indicate that genome-wide SNP screening can be performed on FFPE-HNSCC material with high accuracy using the Illumina platform. Analysis of the results with respect to prediction of the metastatic phenotype on the basis of a genetic profile using archival HNSCC samples is currently in progress.

10.30 – 11.00 TEA AND COFFEE

O5 11.00 – 11.15
INTEGRATED GENOMIC AND TRANSCRIPTIONAL PROFILING YIELDS PUTATIVE MARKER GENES FOR CERVICAL CANCER

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A better understanding of the consequences of recurrent (epi)genetic alterations that occur during cervical carcinogenesis is essential in the search for novel biomarkers. In this study we determined genome-wide expression profiles of 10 squamous cell carcinomas (SCCs), 5 adenocarcinomas (AdCAs) and 6 normal epithelial samples. Expression patterns were subsequently combined with genome-wide chromosomal profiles in the same carcinomas.

Differential gene expression analysis identified 76 genes with altered expression in carcinomas compared to normal epithelium. Microarray results for a subset of these genes were validated by real-time RT-PCR. Among the differentially expressed genes a relative overrepresentation of genes located at chromosome 3q, one of the most frequently gained areas in SCCs, was observed (false discovery rate (FDR) <0.005). To further investigate the relationship between gene expression and chromosomal alterations 2 statistical approaches were used, i.e. differential gene locus mapping (DIGMAP) and the array CGH expression integration tool (ACE-it). Using these methods we found that increased gene expression was linked to increased gene copy numbers at 1q32.1, 3q13.32-22.3, 3q26.32-27.3, and 20q11.21-13.33, whereas a loss at 11q22.3-25 correlated with recurrent decreased gene expression. Seven genes with significantly higher expression in carcinomas compared to normal epithelium were located within these regions. In conclusion, integrated genome-wide chromosomal and transcriptional analysis of cervical carcinomas highlighted 7 genes of which further investigations are warranted.

O6 11.15 – 11.30
GAINS AND AMPLIFICATIONS OF THE ABL-NUP214 REGION IN BLAST STAGE OF CML

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Introduction. Chronic myeloid leukaemia (CML) is a pluripotent haematopoietic stem cell disorder defined by

expression of the BCR-ABL fusion gene, a constitutively activated tyrosine kinase. The fusion gene commonly results from formation of the Philadelphia chromosome (Ph) after a t(9;22)(q34;q11) or related variant rearrangement. The expression of the chimeric BCR/ABL gene is necessary but not sufficient to maintain the disease progression. The appearance of various chromosomal and molecular aberrations in the course of the disease is well documented but without any causal relationship.

Methods and Materials. We undertook array CGH analysis of samples from 48 CML patients (22 chronic phase and 26 blast crisis) and 12 CML cell lines. Two array platforms were used – 1 Mbp BAC chip (Spectral Genomics 2600) and 60-mer oligonucleotide (44B - Agilent). Experimental conditions followed manufacturers protocols. The same reference DNA (collections of 6 disease free individuals purchased from Invitrogen) was used throughout the study. Confirmation of the genome imbalances was sought using fluorescent in situ hybridisation and quantitative PCR.

Results. A number of recurrent genome imbalances associated with disease progression were identified. Among these were copy number variations of the 9q34 region harboured by the Philadelphia chromosome. Presented as gains and/or high-level amplifications, these cryptic aberrations involve the 9q34.12 sequences distal of the ABL breakpoint. These imbalances were found only in blast crisis patient's samples. Furthermore, in cases with follow-up samples, the 9q34.1 imbalances were only present in the advanced stage of disease, thus confirming their secondary nature. FISH demonstrated that these cryptic aberrations affect the Philadelphia marker without affecting the G banding appearance. A common amplicon covering the ABL-NUP214 region was identified with estimated size of 1.2 Mbp and found to contain three known genes, namely FIBCD1 (fibrinogen family), LAMC3 (laminins family) and the NUP-214. The latter is a nucleoporin gene, recently associated with T-cell acute lymphoblastic leukaemia. A dual colour FISH probe targeting the ABL-NUP214 region was applied to samples from (i) an additional twenty-three CML patients either in an accelerated/blast stage of CML or known to carry a deletion of der(9) chromosome or known to be resistant to Imatinib treatment and (ii) 12 CML cell lines. Duplications and amplifications of the ABL-NUP214 region were found in five BC patients and 4 cell lines.

Conclusions. The importance of these observations is two fold: firstly, the Philadelphia chromosome - a product of a balanced translocation is shown to be unstable and prone to secondary changes during disease

progression and secondly, the gains/ amplifications of the ABL-NUP214 regions are non-random event in CML, the role of which in remains to be elucidated.

O7 11.30 – 11.45
ESTROGEN RECEPTOR STATUS MODULATES THE GENOMIC PROFILE IN FAMILIAL AND SPORADIC BREAST CANCER: A STUDY BASED ON ARRAY CGH

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Introduction. Familial breast cancer represents 5-10% of all breast tumors. Mutations in the two known major breast cancer susceptibility genes, BRCA1 and BRCA2, account for a minority of familial breast cancer, while families without mutations in these genes (BRCAx group) account for 70% of familial breast cancer cases. Our aim is the profiling of the genomic changes present in the three familial breast tumor groups and in sporadic breast cancer.

Methods and Materials. We have analyzed 19 BRCA1, 24 BRCA2, and 31 BRCAx samples from familial breast cancer patients, and 19 sporadic breast tumors using a 1 Mb resolution BAC array-based comparative genomic hybridization.

Results. We found that BRCA1/2 tumors showed a higher genomic instability than BRCAx and sporadic cancers. There were common genomic alterations present in all breast cancer groups, such as gains of 1q and 16p or losses of 8p11-p12 and 16q. When we classified all tumors according to Estrogen Receptor (ER) expression status, we found that ER-negative tumors presented higher genomic instability and different altered regions than ER-positive ones, independently of the tumor type (familial or sporadic) and BRCA mutation status (BRCA1 or BRCA2).

Conclusions. We describe a set of common genomic aberrations that would characterize the breast tumor development. We suggest that the presence/absence of ER may play a crucial role in driving tumor development through distinct genomic pathways, rather than the BRCA mutation status. According to our results, the BRCA genes mutation status (mainly BRCA1) would

contribute to the genomic profile of abnormalities by increasing or modulating the genome instability.

O8 11.45 – 12.00
LOW-GRADE B-CELL LYMPHOMA SUBTYPES SHOW DISTINCT PATTERNS OF GENOMIC INSTABILITY

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Introduction. Low-grade lymphomas account for approximately 50% of all lymphomas. They are distinguished by a relatively low proliferative index, small cell size, large tumoural masses in hemopoietic organs, and a paradoxical combination of advanced clinical stages associated with low clinical aggressivity. Our aims are: 1) to characterize the genomic pattern (in terms of DNA copy number changes) of distinct groups of low-grade B cell lymphomas; and 2) to establish correlations between genomic aberrations and other histological, molecular or clinical features. We studied six different sub-types of low-grade B-cell lymphomas: follicular (FL), splenic (SMZL) and nodal (NMZL) marginal zone, lymphoplasmocytic (LPL), mantle cell (MCL) and B-cell chronic lymphocytic leukaemia (B-CLL).

Methods and Materials. We selected "gold-standard" samples from 10-15 different patients from each sub-type. Two independent pathological confirmed the presence of at least 80% of tumour cells and the complete absence of proliferation markers. A Whole Human Genome CGH Microarray that contains 44000 60-mer oligonucleotides (Agilent Technologies) was used to delineating the genomic pattern.

Results and Conclusions.

1. Varying degrees of genomic instability (assessed as gains and losses) affected all samples. The incidence of genomic aberrations ranged from 100% in MCL to 73% in SMZL.
2. Each subtype could be characterized by a distinct pattern of genomic aberrations. Among them, B-CLL and SMZL could be segregated in two classes that showed distinct numerical and structural genomic changes, probably reflecting different clinical behaviour.
3. On the other hand, we have identified some genomic aberrations that can be observed across most of the

subtypes. These changes (transversal aberrations) may represent progression markers.

4. We are elaborating a list of genes and/or genomic regions that may be involved in the understanding of low-grade lymphoma pathogenesis.

O9 12.00 – 12.15

COMPLEX GENOMIC ARCHITECTURE AT XQ28 RESULTS IN DUPLICATION OF MECP2 COMMONLY FOUND IN A SPECIFIC SUBSET OF MR PATIENTS

Marijke Bauters; Hilde Van Esch; Mike Friez; Odile Boespflug-Tanguy; Martin Zenker; Angela M. Vianna-Morgante; Jaakko Ignatius; Martine Raynaud; Karen Hollanders; Kris Vandenreijt; Pierre Blanc; Claude Moraine; Roger Stevenson; Peter Marynen; Jean-Pierre Fryns; Charles Schwartz; **Guy Froyen**.

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Recently, we identified a 2-fold increased dosage of MECP2 as the cause of severe mental retardation (MR) with progressive spasticity in 4 unrelated patients, thereby demonstrating a new disease mechanism in mental retardation. In order to assess the prevalence of submicroscopic duplications at Xq28, including the MECP2 gene, we screened well-defined groups of MR patients (12 positives) as well as a heterogeneous group of male MR patients (1 positive) with MLPA, array-CGH or quantitative PCR. This brings the total MECP2 duplication carriers to 17 unrelated cases/families, which enables a comprehensive genotype/phenotype analysis and the search for a common recombination mechanism. The duplication size varied from 0.3 Mb till 2.2 Mb with a common overlap of the smallest duplication. This 0.3 Mb interval contains 8 genes of which MECP2 is the only one that is highly expressed in brain. FISH in 3 patients showed a tandem orientation, and SNP and marker analysis suggests an intrachromosomal rearrangement inherited from the maternal line. Extensive in silico analysis of the breakpoint regions revealed the presence of numerous repeats, such as Low Copy Repeats (LCRs) and Alu repeats, thus rendering this region unstable and prone to rearrangements. More precise mapping of the breakpoints clearly showed clustering of at least one of the breakpoints in these repetitive regions in 14 out of 17 patients. Interestingly, we were able to clone the breakpoint in only one patient, strongly suggesting that complex rearrangements have

occurred. The single cloned breakpoint revealed a mechanism of non-homologous end joining (NHEJ). Therefore, MECP2 duplications most likely originate from combined homologous and non-homologous recombination, and are stimulated but not necessarily mediated by the complex genomic architecture surrounding the MECP2 gene.

IL3 12.15 – 13.00

TOWARDS CLINICAL GENOMICS: AN ARRAY OF POSSIBILITIES

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The development and evolution of array Comparative Genomic Hybridization (aCGH) has revolutionized the field of molecular cytogenetics. Chromosome abnormalities reflected as discrete copy number gains and losses can now be delineated on the sequence of the human genome at the molecular level. We have identified at high resolution, copy number changes in several thousand samples including 30 types of cancer, developmental disorders including autism, and various mental health diseases. DNA prepared from blood, solid tumors and hematologic malignancies, and FFPE tissues, including a subset with matched frozen tissue, were included. aCGH was performed on ~2000 cancer samples, ~500 developmental disorders and ~400 normal individuals utilizing a 19k BAC array developed at the Roswell Park Cancer Institute. Similarly, 244k oligonucleotide arrays (Agilent Technologies) were utilized for selected samples. The FFPE DNA was screened using the BioScore™ Screening and Amplification kit (Enzo Life Sciences) and those meeting the criterion for aCGH were subsequently assayed. All samples were fluorescently labeled and hybridized to BAC and Agilent oligonucleotide arrays. The copy number aberration profiles that have emerged from our large scale analysis will be discussed including: 1) tumor specific changes, 2) common changes within tumor types, 3) copy number changes in autism, 4) copy number variation in normal individuals, 5) FFPE derived DNA samples, and 6) BAC and Agilent oligonucleotide platform comparisons. In conclusion, array CGH has emerged as a tool not only for disease gene discovery but

as a robust technology that is amenable to automation for detailing genomic aberrations. As a result molecular karyotyping by aCGH will likely become a routine test in clinical labs for tumor and pre/postnatal diagnostics.

13.00 – 14.00

LUNCH AND COMMERCIAL EXHIBITION

STRUCTURAL VARIATION OF THE GENOME

IL4 14.00 – 14.45

LARGE-SCALE STRUCTURAL VARIATION AND IMPLICATIONS FOR THE PHENOTYPE

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Structural variation of the human genome was first noticed at the cytogenetic level. It is well known that certain chromosomal regions are very variable, e.g. the short arm of chromosome 15 and the pericentric region of chromosome 9, which may cause interpretation problems. The variation most often involves the heterochromatic regions, but euchromatin may also show variation that is visible in the microscope, so called heteromorphisms. More recent knowledge is the presence of sub-microscopic variation, its influence on the phenotype and importance for genome evolution. Variation at the single base-pair level was found during sequencing, and novel techniques have very recently revealed that there are many levels of variation ranging from SNPs to sub-microscopic level variations between 500 bp to 5 Mb. The human genome is rich in interspersed, duplicated sequences that are both intra and interchromosomal, containing blocks of DNA between 1-400 Mb in length with more than 90% sequence identity. They are called segmental duplications or low copy repeats (LCRs), constitute approximately 5% of our genome and have major implications for human disease and evolution. LCRs provide a substrate for structural rearrangements through non-allelic homologous recombination (NAHR), giving rise to deletions, duplications, translocations and inversions, depending on the orientation of the repeats. The proximal part of chromosome 22 is rich in LCRs, and as may be expected,

many recurrent rearrangements occur in this region. The most common and well described is perhaps the 22q11-deletion syndrome, but duplications of the same region are now being reported. In addition, one of the breakpoints of the most recurrent reciprocal translocation is located in 22q11, and rearrangement within this region also gives rise to the Cat-eye syndrome.

FREE PAPER SESSION 2

O10 14.45 – 15.00

MAPPING CHROMOSOMAL BREAKPOINTS AT SUB-KB RESOLUTION WITH HR-CGH

Alexander Eckehart Urban; Jan O Korbelt; Fabian Grubert; Christopher Hart; Sibel Kantarci; Rebecca Selzer; Todd Richmond; Roland Green; Barbara Pober; Beverly Emanuel; Kenneth Kidd; Mark Gerstein; Sherman M Weissman; Michael Snyder.

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We have developed high-density oligonucleotide tiling microarray based High-Resolution CGH (HR-CGH). This technology allows us to detect, with sub-kilobase accuracy, the presence and extent of chromosomal aberrations ranging in size from 600bp to several million bp, in confirmatory and ab initio studies. We first used arrays covering the β -Globin locus on chromosome 11 (9bp tiling density, isothermal tiling) and all of chromosome 22q (85bp tiling, isoTm), respectively. Analyzing full-complexity genomic DNA from patients with a variety of disease-causing aberrations (various heterozygous deletions, duplications, partial trisomies and partial tetrasomies) we could map breakpoints with high accuracy (typically up to 200 bp resolution as shown by DNA sequencing following vectorette-PCR) [Urban, Korbelt et al., PNAS 2006]. We have developed a Hidden-Markov-Model based set of algorithms, the BreakPtr-System, for the processing and analysis of HR-CGH data. With its help several new small and very small Copy-Number-Variants on chromosome 22q could be detected and validated either by qPCR or by vectorette-PCR and sequencing [Korbelt, Urban et al., submitted]. We have extended the use of these tools to other sets of samples and additional regions of the human genome. With the chromosome 22q HR-CGH array we could refine the mapping of breakpoints in a patient with Congenital Diaphragmatic Hernia and a predicted duplication in 22q. With an HR-CGH array covering

chromosome 21 (128bp tiling, isoTm) we are mapping the breakpoints in a panel of samples from patients with developmental abnormalities, especially such of the nervous system. Finally, with HR-CGH arrays covering chromosome X (overall 111bp and partially 35bp tiling, isoTm) and the ENCODE regions (38bp tiling, 50mers), respectively, we are mapping and cataloging Copy-Number-Variants and –Polymorphisms in healthy probands from various ethnicities (i.e. a subset of the HapMap panel as well as samples from S.W. Asia, Siberia, Pacific Islands and South America).

O11 15.00 – 15.15
RANBP17-TLX3: A HOT SPOT REGION FOR BOTH CONSTITUTIONAL AND ACQUIRED REARRANGEMENTS

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Background. It is well established that genomic structure can mediate recurrent chromosomal rearrangements leading to clinically recognisable syndromes (Lupski and Stankiewicz, 2005). Recent array CGH surveys have yielded less than expected so-called genomic disorders as most imbalances were found to be scattered in an apparently random fashion across the genome (Menten et al., 2006).

Furthermore, only few of the currently known chromosomal regions implicated in constitutional rearrangements also mediate tumor specific alterations. Here we report a new chromosomal region that appears to be prone to both constitutional and acquired rearrangements.

Objective. Molecular characterisation of a chromosomal region on 5q35.1 involved in recurrent rearrangements both in patients with mental retardation/congenital abnormalities and in patients with T-ALL.

Results. We report three deletions and one duplication affecting 5q35.1. Molecular delineation of the breakpoints was achieved by 244K Agilent genomic array analysis. Interestingly, for each of these rearrangements one of the breakpoints is located within a ~265 kb region encompassing the RANBP17 and the TLX3 genes. The same region is also involved in the cryptic t(5;14)(q35;q14) translocation, which occurs in ~20% of childhood T-cell acute lymphoblastic leukemias

(T-ALL) (Bernard et al., 2001). These data suggest that genomic architectural features of the RANBP17-TLX3 locus facilitate genomic rearrangements. In silico analysis revealed no segmental duplications in or near the breakpoint region. Further in-depth analyses are ongoing in order to determine to which extent the genomic structure at the breakpoint regions might predispose to these rearrangements.

15.15 – 15.45 TEA AND COFFEE

O12 15.45 – 16.00
PROGRESSION DYNAMICS OF EVOLUTIONARY-NEW CENTROMERES

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Introduction. Evolutionary-New Centromere (ENC) emergence, also called centromere repositioning, is the movement of a centromere along the chromosome without any change in the chromosomal marker order. The first clear examples of this phenomenon were reported in primates in orthologs of human chromosome 9 (Montefalcone et al. 1999). Since then, several other examples have been reported in primates and in non-primate mammals (Cardone et al. 2006, and references therein). Evolutionary history of human chromosome 6 in primates has been recently reported (Eder et al. 2003). Human chromosome 6 and the corresponding macaque chromosome 4 (*Macaca mulatta*, MMU) differ by a small inversion, which occurred in the Old World Monkey (OWM) ancestor. More importantly, both HSA6 and MMU4 centromeres are ENCs with respect to each other and to the ancestral chromosome 6, whose centromere was located in a region corresponding to the HSA6p22.1 (Eder et al. 2003). The MMU4 ENC domain corresponds to the human 6q24.3 euchromatic region. We investigated the organization of the MM4 centromeric-pericentromeric region with respect to the organization of the region in humans, in order to shed light to the progression dynamics of an evolutionary novel centromere.

Methods and Materials. FISH experiments were essentially performed as previously described (Ventura et

al. 2003). Briefly: DNA probes were directly labeled with Cy3-dUTP (Perkin-Elmer) or FluorX dCTP by nick-translation. Two hundred nanograms of labeled probe were used for the FISH experiments. Hybridization was performed at 37°C in 2 X SSC, 50% (v/v) formamide, 10% (w/v) dextran sulphate, 5mg COT1 DNA (Roche), and 3mg sonicated salmon sperm DNA, in a volume of 10 ul. Posthybridization washing was at 60°C in 0.1 X SSC (three times, high stringency). Washes of FISH experiments using macaque probes on human metaphases were performed at lower stringency: 37°C in 2 X SSC, 50% formamide (X3), followed by washes at 42°C in 2 X SSC (X3). Digital images were obtained using a Leica DMRXA epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments, NJ). Cy3 (red), FluorX (green), and DAPI (blue) fluorescence signals, detected with specific filters, were recorded separately as gray scale images. Pseudocoloring and merging of images were performed using Adobe Photoshop software. STSs were used to obtain PCR products to sequence. Analysis of STS amplification sequences was performed by MegAlign software in DNASTAR package (www.dnastar.com) using ClustalW method and default parameters for this method (15.00 gap penalty and 6.60 gap length penalty). Analysis of alpha satellite sequences was performed using BLASTN 2.2.13 version setting parameters to 1, -1, 2 and 2 for match, mismatch, open gap and gap extension respectively.

Results. BAC ends (BES) sequence analysis, STSs sequencing, and systematic FISH experiments using macaque BAC clones were used to build a ready-to-sequence BAC contigs, spanning the centromeric transition of the MMU4 ENC. The analysis revealed some key properties of this evolutionary new centromere: (i) a segment of about 250 Kb, constituting the seeding region, was found extensively duplicated, in the same orientation, on both sides of the MMU4 centromere; (ii) no other euchromatic sequences were involved in the shuffling; (iii) stretches of monomeric alpha satellite sequences were dispersed in the duplicated area; (iv) the seeding region is a gene-desert. Absence of genes around the ENC seeding point probably represents a key property playing a crucial role in ENCs progression towards a "mature" status.

Conclusions. Data from human genome have shown that pericentromeric regions are enriched for segmental duplications, and that, in these regions, the ratio of inter-versus intra-chromosomal duplications is about 6:1 (She et al. 2004). The MMU4 pericentromeric duplications detected by FISH were strictly intra-chromosomal, and originated only from the ENC seeding point. Elsewhere we report comparable FISH results on the ENC of

macaque chromosome 17 (human 13) (Cardone et al. 2006). However, only human probes were used in this study, and this bias precluded any conclusion on the absence of inter-chromosomal duplications. We have also shown that centromeres of human chromosomes 3, 6, 11, 14, and 15 are evolutionary novel centromeres (Cardone et al. 2007; Ederet et al. 2003; Ventura et al. 2003; Ventura et al. 2004). Chromosomes 3 and 6 match the pattern we found on MM4, while 11, 14, and 15 accommodate large blocks of inter-chromosomal duplications (She et al. 2004). A careful analysis of the evolutionary history of the latter chromosomes, however, showed that, very likely, large blocks of segmental duplications were already present, or simultaneously seeded, in the ENC seeding region (Cardone et al. 2007; Ventura et al. 2003). It could be therefore hypothesized that a novel centromere triggers only local duplication activity, while interchromosomal duplications are triggered by distinct forces, probably linked to intrinsic properties of specific sequences (Horvath et al. 2005) (Bailey and Eichler 2006).

O13 16.00 – 16.15

OVERALL IMBALANCE PROFILES IN CARCINOMAS OF DIFFERENT SITES: A DESCRIPTIVE META-ANALYSIS OF 6858 CASES ANALYZED BY CHROMOSOMAL CGH

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Introduction. Recurring chromosomal imbalances in human malignancies supposedly arise as the result of evolutionary pressure, acting on random or epigenetically induced genomic abnormalities during clonal tumor cell propagation. The Meta-analysis of genomic data derived from molecular-cytogenetic screening techniques may identify diagnostic markers and provide insights into pathogenic mechanisms.

Methods and Materials. As part the Progenetix project 14102 chromosomal CGH cases have been collected, representing approximately 50% of published data. For this study, the ISCN "rev ish" annotated data from 6858 epithelial neoplasias was converted into a chromosomal band specific aberration matrix (862 bands). Diagnoses and tumor loci were recoded according to ICD-O 3. For major disease entities as defined through the tumor sites, the frequency distribution of gains and losses was compared (667 breast, 600 prostate, 533 head & neck,

529 stomach, 508 colorectal, 489 ovary, 481 liver & intrahepatic, 351 cervix, 333 thyroid, 320 lung & bronchus, 257 kidney, 203 esophagus and 172 bladder).

Results. On average, cases had 5.8 imbalanced chromosomes (3.3 in prostate lesions to 8.4 in head and neck carcinomas). Gains involving 8q24 (overall 24.7%) were common in tumors from most sites, with the exception of thyroid gland and low frequency in cervix and kidney lesions. Most other frequent changes (+1q, +3q, +7, -13q, -17p, +20q etc.) varied considerably between the entities, while some abnormalities were described only for specific sites (e.g. -18q in colorectal and esophagus lesions). Detailed results will be presented.

Conclusions. Based on the large amount of accumulated CGH data, tumor type related chromosomal imbalance patterns can be drawn. This information should prove useful when assessing presumptive target genes, detected with high-resolution techniques but based on smaller series. In the future, advanced data analysis methods will be used for subset stratification. A general repository for supervised genomic array data should be implemented.

O14 16.15 – 16.30
CLASSIFICATION AND PROGNOSIS OF SARCOMAS USING DNA COPY NUMBER CHANGES IDENTIFIED BY ARRAY CGH

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Introduction. Human sarcomas, malignant tumours of mesenchymal origin, show recurrent copy number alterations of specific chromosomal segments. Leiomyosarcomas (LMSs) are tumours showing smooth muscle differentiation. Until recently, gastrointestinal stromal tumours (GISTs) were also classified as smooth muscle tumours, but now GISTs are recognized as a separate entity. Malignant peripheral nerve sheath tumours (MPNSTs) are of neuroectodermal origin, and arise sporadically or as part of the neurofibromatosis type 1 (NF1) or -2 (NF2) autosomal inherited disorders.

Methods and Materials. In order to identify novel alterations and target genes for the copy number changes, we have constructed a genomic microarray with bacterial- and P1 artificial chromosome (BAC and PAC)

clones covering the human genome at 1 Mb resolution, as well as the 1q12-q25 region at high resolution (tiling-path). We have used this genomic microarray for array comparative genomic hybridisation (array CGH) of a panel of 170 human sarcomas.

Results. We have analysed 12 LMSs and seven GISTs initially classified as LMSs. Hierarchical clustering of all samples separated GISTs and LMSs into two distinct clusters, and statistical analysis identified six chromosomal regions which were significantly different in copy number between GISTs and LMSs. We have in addition analysed seven MPNSTs, and correlated DNA copy number changes to patient outcome. A segment of 17q was gained in all patients who died of cancer. We are currently integrating mRNA and miRNA expression data in order to identify candidate targets.

Conclusions. Our work shows the potential use of array CGH to differentiate histologically similar tumours as well as patients with different clinical outcome, although further validation is required on larger tumour panels.

O15 16.30 – 16.45
DNA PROFILING OF SEROUS OVARIAN AND FALLOPIAN TUBE CARCINOMAS IDENTIFIES DISTINCT PATTERNS OF GENOMIC ALTERATIONS

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Introduction. Primary serous ovarian carcinoma (OVCA) and serous Fallopian tube carcinoma (FTC), both belonging to the BRCA-linked tumour spectrum, share many properties and are treated similarly. It has been suggested based on conventional chromosomal Comparative Genomic Hybridization (CGH) studies, that serous OVCA and serous FTC display a likeness in molecular pathogenesis. However, a detailed molecular comparison has been lacking.

Methods and Materials. We performed genome-wide, BAC-based array CGH on 14 serous OVCA and 14 serous FTCs, followed by targeted gene identification using a dedicated Multiplex Ligation-dependent Probe Amplification (MLPA) probe set.

Results. Array CGH analysis indicated that serous OVCA and FTCs displayed common but also distinctive

patterns of recurrent changes. Direct gene identification using MLPA identified EIF2C2 on 8q as a potential important driver gene. Other previously unappreciated gained/amplified genes included PSMB4 on 1q, MTSS1 on 8q, TEAD4 and TSPAN9 on 12p, and BCAS4 on 20q. SPINT2 and ACTN4 on 19q were predominantly found in FTCs. Gains/amplifications of CCNE1 and MYC, often in conjunction with changes of genes of the AKT pathway, EVI1 and PTK2, seemed to be players of earlier stages, whereas changes of ERBB2 were associated with advanced stages. The profile of the one BRCA1-mutated FTC paralleled that of the sporadic ones.

Conclusions. The data suggests that serous OVCAs and serous FTCs, although related, exhibit different genomic profiles. In addition to known pathways, new genes/pathways are likely to be involved, with changes of an miRNA-associated gene, EIF2C2, as one of important new features. Dedicated MLPA sets constitute potentially important tools for differential diagnosis and may provide footholds for tailored therapy.

O16 16.45 – 17.00
COMPLEX INHERITANCE PATTERN
INVOLVING A MICRODELETION IN
THROMBOCYTOPENIA-ABSENT RADIUS (TAR)
SYNDROME

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Introduction. Thrombocytopenia-absent radius (TAR) syndrome is characterized by hypomegakaryocytic thrombocytopenia and bilateral radial aplasia in the presence of both thumbs. Evidence for autosomal recessive inheritance comes from families with several affected individuals born to unaffected parents but several other observations argue for a more complex pattern of inheritance.

Methods and Materials, Results. In this study we describe a common interstitial microdeletion of 200kb on chromosome 1q21.1 in all of the investigated 32 TAR syndrome patients detected by microarray-based comparative genomic hybridization (array CGH) and quantitative PCR. Analysis of the parents revealed that this deletion occurred de novo in 25% of affected individuals. Intriguingly, inheritance of the deletion along the maternal as well as the paternal line was observed. The absence of this deletion in a cohort of control individuals argues for a specific role of the microdeletion in the pathogenesis of TAR syndrome.

Conclusions. Thus, TAR syndrome is most likely not a monogenic but a complex disorder. We hypothesize that TAR syndrome is associated with a deletion on chromosome 1q21.1; however, the phenotype develops only in the presence of an additional as yet unknown modifier (mTAR).



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