chromosomal regions of copy neutral loss of heterozygosity (LOH) (71%). This is in contrast to sporadic colon cancer, where physical loss is the main characteristic. The percentage of chromosomal amplifications (24%) is comparable to sporadic colorectal cancers with CIN. Furthermore, we verified our scoring of copy neutral LOH versus physical loss in MAP carcinomas by two methods: fluorescent in situ hybridization, and LOH analysis using polymorphic markers on carcinoma fractions purified by flow sorting. The results presented in this study suggest that copy neutral LOH is an important mechanism in the tumourigenesis of MAP.

439 Poster

Identification and characterisation of acquired structural rearrangements in cancer genomes using massively parallel paired-end sequencing

P. Stephens[1]
[1]The Wellcome Trust Sanger Institute, Cancer Genome Project, Cambridgeshire, United Kingdom

Acquired structural rearrangements including deletions, amplifications and translocations are commonly observed in cancer genomes. Although many are thought to be passenger events, a proportion clearly contribute to oncogenesis by mechanisms which include the amplification of dominantly-acting cancer genes, the inactivation of tumour suppressor genes and the generation of novel fusion proteins. Characterisation of the breakpoints of such rearrangements until recently has been possible only with low-throughput or low-resolution methods, and has primarily focused on leukemias and lymphomas. To investigate the feasibility of using the Illumina genome analyser as a high throughput method to identify and characterise cancer specific (acquired) structural rearrangements, we have undertaken massively parallel paired end sequencing of genomic DNA from multiple cancers. Paired sequences separated by a distance of ~500 bp are generated from cancer genomes, and aligned to the human reference genome sequence using MAQ. Alignments inconsistent with the expected insert size and orientation define potential rearrangements. Subsequent PCR amplification and capillary sequencing of breakpoint regions in tumor and normal DNA from the same individual defines both germline and somatic breakpoints to basepair resolution. Results that will be presented include the identification and characterisation of deletions, tandem and inverted duplications, internally rearranged gene transcripts, interchromosomal translocations, amplicons with complex structure, and potential fusion genes.

440 Poster

FAT tumour suppressor gene is mutated by both homozygous deletion and point mutation in a variety of different cancer types

H. Davies[1], G. Bignell[1], M.R. Stratton[1], P.A. Futreal[1]
[1]The Wellcome Trust Sanger Institute, Cancer Genome Project, Cambridgeshire, United Kingdom

As part of a study to identify changes in copy number in cancer, we screened 750 cancer cell lines for homozygous deletion using the Affymetrix Genome-Wide Human SNP Array 6.0. One of the deleted regions identified was situated on 4q35. Overlapping homozygous deletions were present in three cell lines, two head and neck cancers (SCC-25 and SAS) and a breast cancer (HCC1599). The minimal deleted region contains 2 genes, MTNR1A and FAT.

FAT is a member of the cadherin superfamily and is one of four human homologs of the Drosophila fat tumour suppressor. In Drosophila, fat is a member of the Hippo signalling pathway. FAT has recently been implicated as a tumour suppressor gene in oral cancer by complete or partial homozygous deletion of the gene (Nakaya et al Oncogene (2007) 26, 5300-5308).

To further investigate the role of FAT as a tumour suppressor gene, we screened the gene for truncating point mutations by resequencing all the exons of FAT in 785 cancer cell lines from a variety of tissues. We have identified 22 truncating mutations, 10 of which are homozygous or compound heterozygous mutations. We also identified 147 missense variants. However, since there was no matched normal tissue available for the majority of the lines it was not possible to determine if these are somatic mutations or rare SNPs. In addition, we are using multiplex PCR assays to screen for homozygous deletions of exons 1 and 4 which have been shown to be hot spots for deletion. To date we have identified one new homozygous deletion.

From our results the tumour type with the highest prevalence of truncating mutations and deletions of FAT was head and neck cancer, with mutations in 7 out of the 23 cell lines screened. However, truncating mutations were also found at a lower prevalence in a variety of other tissues, including thyroid, lung, cervix, vulva, prostate and skin. This is the first report to demonstrate that in addition to homozygous deletions of FAT, cancer cell lines can also contain truncating point mutations in this gene. These results suggest that FAT is a tumour suppressor gene in a range of different cancer types.

441 Poster

MGMT promoter methylation and TP53 gene mutations in glioblastoma

I. Zawlik[1], D. Jesionek-Kupnicka[2], E. Jesien-Lewandowicz[3], M. Szybka[2], D. Kulczycka-Wojdala[2], P. Rieske[1], P.P. Liberski[1], R. Kordek[2]
[1]Medical University of Lodz Poland, Department of Molecular Pathology and Neuropathology Chair of Oncology, Lodz, Poland; [2] Medical University of Lodz Poland, Department of Tumor Pathology Chair of Oncology, Lodz, Poland; [3] Copernicus Memorial Hospital Poland, Department of Radiation Oncology Chair of Oncology, Lodz, Poland

Objective: O6-methylguanine DNA methyltransferase (MGMT) enzyme reduces cytotoxicity of therapeutic or environmental alkylating agents by specifically removing methyl groups from the O6 position of guanine in DNA. MGMT promoter methylation has been associated with TP53 G:C to A:T transition mutations in various types of cancers, and with poor prognosis in patients who did not received chemotherapy. Glioblastoma patients with MGMT promoter methylation showed better response to chemotherapy based on alkylating agents and longer survival than patients without MGMT methylation. In this study, we examined if MGMT promoter methylation in primary glioblastoma is associated with TP53 mutations. We also determined whether MGMT promoter methylation and TP53 mutations correlate with survival of glioblastoma patients who do not receive chemotherapy.

Material and Methods: We examined 32 primary glioblastoma patients for TP53 mutation by using direct sequencing and MGMT promoter methylation by methylation specific PCR (MSP).

Results: MGMT promoter methylation and TP53 mutations were detected in 72% and 31% of primary glioblastoma, respectively. Although not statistically significant, the frequency of TP53 G:C to A:T mutations was higher in cases with (26%) than without (11%) MGMT promoter methylation. MGMT promoter methylation had no impact on patient survival.

Conclusions: Our results indicate that in primary glioblastoma MGMT promoter methylation is frequent, does not lead to G: C to A: T TP53 transition mutations, has no independent prognostic value and is not predictive marker unless glioblastoma patients are treated with chemotherapy.

442 Poster

Integration of genomic alterations and expression profiling in Glioblastoma Multiforme

M. de Tayrac[1], A. Etcheverry[2], M. Aubry[2], S. Saïkali[3], A. Hamlat[4], V. Quillien[5], J. Mosser[6]
[1]CNRS UMR6061 Institut de Genetique et Dévelopement, Transcriptional regulation and oncogenesis, Rennes, France; [2] OUEST-genopole®, Transcriptome Platform, Rennes, France; [3] CHU Pontchaillou, Department of Pathology, Rennes, France; [4] CHU Pontchaillou, Department of Neurosurgery, Rennes, France; [5] CRLCC, Centre Eugene Marquis, Rennes, France; [6] CHU Pontchaillou, Medical Genomics Unit, Rennes, France

Background: Glioblastoma Multiforme (GBM) is the most devastating and lethal form of glioma arising in the adult central nervous system. GBM is particularly known for its heterogeneity, which renders difficult its biological and molecular characterization and consequently its therapeutic management. Genomic surveys revealed the highly rearranged nature of GBM genome. However, the impacts of tumor DNA aberrations on gene expression remain unclear. Materials and methods: To investigate this relationship and to identify putative target genes in GBM, we performed a parallel copy number and expression survey in twenty GBMs using Whole Human Genome arrays and validated our findings with eighty-one GBMs from an independent microarray data set publicly available on Gene Expression Omnibus. Results: Loci targeted for high-priority minimal common regions (MCR) of recurrent copy number alterations were defined and combined with gene expression profiles performed on the same tumor samples. We first identified genes with concordant changes in DNA copy number and expression levels, i.e. over-expressed genes located in amplified regions and under-expressed genes located in deleted regions. Second, we defined genes within MCRs for which expression was directly linked to the corresponding genomic state (Pearson correlation). These 'cis-acting' DNA targeted genes are functional key elements of cancer cell biology and glioblastoma progression.Conclusions: This study shows the power of combining genomic alterations and gene expression to identify tumor biomarkers in cancer.