can be evaluated on clinical and histomorphologic grounds with contribution of immunoprofile and molecular profile (WHO 2001).

Design: Few studies used immunohistochemical expression detection for stratification of DLBCL, Barranas et al.(2002), Colomo et al. (2003), Linderoth et al. (2003), McClintock et al. (2003). Two major patterns of gene expression by gene array technology have been proposed, Alizadeh et al. (2000), Rosenwald et al. (2002), for dividing into prognostically significant subgroups in germinal centre (GC) a post-germinal centre (post-GC) DLBCL.

First publication discribing NFkappaB is mentioned in 1986 (Sen et al) and comprises family of transcription factors with important role in cell proliferation, antiapoptotic function and differentiation. NFkappaB signaling pathway is activated by numerous stimuli e.g. bacteria and viruses and is referred to as a central mediator of the immune response. NFkappaB signaling pathway regulates survival of normal and malignant B-cells by controlling the expression of cell death regulatory genes (Karin et al. 2002). Nuclear localization of NFkappaB leads to binding to the promoters of target genes, Li et al. (2002).

Summary: The sorting in GC and post-GC DLBCL group was used according the immunoprofile as decteted in GC and post-GC B-cells. CD10 is surface antigen that shows positive expression in non-neoplastic GC derived B-cells. Bcl6 is proposed protooncogeneic factor and shows nuclear localised positive expression in GC derived B-cells. MUM1 shows nuclear localised positive expression in post-GC B-cells. The GC group was signed if detected positive expression of CD10 and BCL6 and negative expression of MUM1. The post-GC group was signed if detected positive expression of MUM1 and negative expression of CD10 and BCL6.

Inactive NFkappaB heterodimers (c-REL/RELA, NFkappaB p50/p52, p65/RELA) reside in the cytoplasm, complexed with an inhibitor of IkappaB. The phosphorylation of IkappaB by IkappaK kinase results in the inhibitor`s dissociation from cytoplasmic NFkappaB heterodimer. Phosphorylated IkapaB is degraded via the proteasome. Free NFkappaB heterodimer translocates to the nucleus and induces the transcription of target genes. In our file of DLBCL we evaluated expression of NFkappaB (p50, p52, p65) and in some of cases in post-GC DLBCL group we detected it´s nuclear localisation.

Conclusion: Prognostic and predictive stratification of DLBCL in the group of post-GC DLBCL could be proposed to refine according to expression of NFkappaB family and also according to new available target of therapy, inhibitor of proteasome complex (bortezomib).

436 Poster

Mutations in the receptor tyrosine kinases in gastrointestinal stromal tumours from Russian patients

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BACKGROUND: Gastrointestinal stromal tumours (GISTs) are often show constitutive activation of either the KIT or PDGFRα receptor tyrosine kinases because of gain-of-function mutation. Aim of the study was to analyze KIT or PDGFRα mutations in GISTs from Russian patients and estimate their prognostic value.

METHODS: We have analyzed 90 DNA obtained from paraffin sections of GISTs in PCR with primers to KIT (exons 9, 11, 13, 17) and PDGRFA (exons 12, 14 and 18) followed with direct sequencing.

RESULTS: 96% of GISTs were CD117 positive. Seventy percents of GISTs harbor KIT mutations in exon 11, most of them were in-frame deletions or substitutions in the 5'-end of exon 11 in a region of 550-563aa. There was one gastric GIST with the deletion of 550-558aa that started in KIT intron 10 and involved the intron 10-exon 11 boundary. All GISTs with deletions in KIT exon 11 were highly malignant. Besides, 11% of GISTs had duplications of 1-12aa in the 3'-end of KIT exon 11 and were low malignant. These GISTs occurred predominantly in women over age 65. Mutations in KIT exon 9 (duplications of 502-503aa) were found in 27% of intestinal GISTs with aggressive behavior and metastases. Mutations in KIT exons 13 and 17 were found in one case each. PDGRFA mutations in exon 18 were found in 10% of GISTs. Typical substitution D842V was found only in two benign gastric GIST with epithelioid cell morphology, while other GISTs contain deletions, involving 842-846aa. There were wild-type KIT and PDGFRA in 13% of GISTs. We have found the additional mutations in KIT exon 17 (D820V and N822K) in GISTs treated with Gleevec that are associated with the secondary resistance to target therapy.

CONCLUSIONS: We have found some peculiarities in the variety of KIT and PDGFRA mutations in Russian patients, namely, high percent of GISTs with KIT exon 11 duplications, low percent of GISTs with D842V PDGFRA substitution, etc. The obtained results revealed some correlations between the type of KIT or PDGRFA mutation and clinico-pathologic parameters of GIST. They support the suggestion that mutational analysis of GIST is important for predicting GIST prognosis and the efficacy of target therapy.

POSTER SESSION

Oncogenomics

437 Poster

Breast cancer progression – genomic alterations in a continuum of stages

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Aim: To study the progression of genomic alterations in mammary epithelial cells from dense breast tissue to full blown cancers

Materials and methods: In total 127 breast tissue samples from three different series have been analyzed using 244K Agilent Human Genome CGH Microarrays (Santa Clara, CA). The samples comprise: 21 "normal" breast tissue (dense breast tissue, reduction mammoplasties, and normal tissue from mastectomies), 26 ductal carcinoma in situ (DCIS), and 75 breast carcinomas.

Results: Data analysis has been initiated using Nexus software from BioDiscovery (El Segundo, CA). Several of the "normal" samples show signs of alterations in areas known to be commonly altered in breast tumors. Hierarchical clustering revealed heterogeneity within each group of samples, suggesting further stratification. The "normal" samples clustered together with low aberrant tumor- and DCIS samples, while the highly aberrant tumor- and DCIS samples clustered together. Significance Testing for Aberrant Copy number (STAC) was applied to reveal common alterations within each group. Among the "normal" samples 118 genes were found to be in regions having significant frequency p-value ($p<0.05$) and being present in more than 35% of the samples, whereas the number was 105 for the DCIS and 245 for the tumors. Of these genes, 31 were overlapping between all groups. We identified group-specific events defined as at least 0.25-fold copy number change between two of the groups with a p-value < 0.05 using Fisher exact test. Genomic regions were found significantly altered in DCIS and breast cancer samples compared to the "normal" samples harboring 1341 and 2617 genes, respectively. Fewer genes (N=388) were identified in significantly altered regions when comparing breast cancer to DCIS. Enrichment analysis was performed to identify biological processes of significance.

Conclusion: Preliminary analyses reveal heterogeneity within each group and frequency of aberrations appears proportionally related to disease stage. Some genomic regions were found significantly changed in all groups. Most of these regions correspond to frequently observed copy number variations (CNVs) and might be candidate hotspots for early events of genomic rearrangements towards breast cancer development. More samples will be included and further stratification will be necessary to identify possibly important events that initiate and drive breast cancer carcinogenesis.

438 Poster

High frequency of copy neutral LOH in MUTYH-associated polyposis carcinomas

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Genetic instability is known to drive colorectal carcinogenesis. Generally, a distinction is made between two types of genetic instability: chromosomal instability (CIN) and microsatellite instability (MIN or MSI). Most CIN tumours are aneuploid, whereas MSI tumours are considered near-diploid. However, for MUTYH-associated polyposis (MAP) the genetic instability involved in the carcinogenesis remains unclear, as both aneuploid adenomas and near-diploid carcinomas have been reported. Remarkably, our analysis of 26 MAP carcinomas, using SNP arrays and flow sorting, showed that these tumours are often near-diploid (52%) and mainly contain

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

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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

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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

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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

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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.