804 Bioinformatic analysis of BRAFV600E vs RASG12V signatures in colon cancer cells reveals differential regulation of cellular pathways related to MSI or EMT

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Background: Sporadic colorectal cancer is a major cause of death worldwide. Cancer initiation and progression takes place in a sequential manner from benign adenomas leading to carcinomas. The Ki-RAS-BRAF-MAPK pathway is often mutated.
Materials and Methods: Intermediate adenoma colon cells have been stably transformed to express BRAF V600E, Ki-RAS V12 and Ha-RAS V12 oncogenes [1,2]. These cell lines have been studied in two sets of microarray experiments using Illumina microarrays.
Results: In the first set of experiments gene expression has been examined in Ha-RAS and Ki-RAS cell lines against a Caco-2 control. The Ha-RAS cell line has strong EMT phenotype. This phenotype is reflected in differentially expressed genes for this line. Network analysis has been carried out using these EMT linked genes to suggest functional connections [3].
In a second set of experiments using a 45,000 gene microarray, BRAFV600E cell lines (Caco-BR) have been compared to a Caco-2 control using multiple clones and replicates. Around 500 genes have been identified as consistently differentially expressed in our BRAFV600E cell lines. Notably, BRAFV600E has provided parental chromosomal instable (CIN) cells with High Microsatellite Instable phenotype (MSI-H), which is reflected in deregulation of expression of DNA repair pathways, as shown in this analysis (Joyce et al., in preparation). These results have been compared to single replicates for Ki-RAS, DLD-1 and HT29 cell lines using the same arrays.
Ingenuity analysis has also been carried out using data from both sets of microarrays to determine cross-talks with key functional pathways, such as the WNT signaling pathway.
Discussion: This study compares the effect of particular oncogenes in the same cellular background. Differentially pathways and novel markers by either RAS and BRAF oncogenes have been revealed which may be used in disease diagnosis and could provide new targets for disease treatment based on the mutation status of the individual tumour.

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805 Alterations in BRCA1, BRCA2, TP53 and ATM genes in sporadic breast tumours

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Background: Genome integrity is maintained by ensemble of genes including tumour suppressors *BRCA1*, *BRCA2*, *TP53* and *ATM*. These belong to hereditary breast cancer predisposing genes, however, their role in sporadic breast tumours remains elusive. The aim of this study was to analyze involvement of these genes in sporadic breast cancer tumourigenesis via their alterations – loss of heterozygosity (LOH), mutations and promoter methylation.
Material and Methods: 71 tumours and corresponding peripheral blood samples of unselected breast cancer patients were evaluated for mutations in *BRCA1*, *BRCA2*, *TP53* and *ATM* genes. Further, we studied promoter methylation and LOHs of microsatellites in the corresponding loci. The mutation analyses included entire coding regions of the studied genes and were performed using protein truncation test, MLPA and sequencing. Promoter methylation was determined by methylation specific MLPA (MS-MLPA) and bisulfite sequencing.
Results: Allelic losses of *BRCA1*, *BRCA2*, *TP53* and *ATM* were found in 14/65 (21.5%), 19/69 (27.5%), 23/62 (37.1%) and 15/70 (21.4%) informative tumour samples, respectively. In *BRCA1* gene two somatic (2/71; 2.8%) and one germline (1/71; 1.4%) mutations were found. In *TP53* gene nine somatic (9/71; 12.7%) alterations were revealed. The *TP53* frameshift mutation c.340–370del31 (p.L114AfsX46) was novel. We failed to detect any alterations in *ATM* and *BRCA2* coding sequences. Promoter methylation was found only in *BRCA1* (2/59; 3.4%) and *TP53* (2/59; 3.4%). One third of informative tumours (22/62; 35.5%) did not carry any alterations in respective genes. In addition, MS-MLPA revealed frequent promoter methylation of other genes, namely *CDKN2B*, *WT1*, *PAX5*, and *RASSF1*.
Conclusion: The high occurrence of allelic losses suggests the role of analyzed genes in sporadic breast tumourigenesis. However, acquired mutations were common only in *TP53* and promoter methylation was identified only two-times in both *BRCA1* and *TP53*. These results suggest that the role of analyzed genes is limited to the subset of sporadic breast tumours. MS-MLPA results indicate that other genes are involved and alternative ways of breast tumourigenesis should be considered.
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806 Identification of rare KRAS codons 12 and 13 mutations by shifted termination assay

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We have developed a sensitive and specific method for research studies, using Shifted Termination Assay (STA) to detect 12 possible KRAS mutations in codons 12 and 13. In this study, we analyzed 118 samples collected from formalin-fixed and paraffin-embedded (FFPE) metastatic colorectal cancer tumours.
The detection method includes carrying out a primer extension reaction in the presence of a wild-type DNA sequence. The primer extension reaction is stopped when encountered by a mutated base. If no mutation is detected the reaction terminates at the next nucleotide resulting in a large wild-type fragment. This creates a shift in the amount of labeled nucleotides incorporated on the primer extended product, and is then differentiated by fragment analysis using Applied Biosystems capillary electrophoresis systems.
Among the 118 samples tested, 32 were found to carry a mutation with 9 different variants. The most common mutations found in codon 12 were GGT>TGT (G12C, 9/32) and GGT>GTT (G12V, 8/32). Two rare variants in codon 12, GGT>GCT (G12A, 1/32) and GGT>CGT (G12A, 1/32), were also observed. For codon 13, the most common mutation found in the samples was GGC>GAC (G13D). In this study, we found 4 variants with 3 different types – 2 samples of GGC>AGC (G13S), 1 sample of GGC>GAC (G13D) and 1 sample of GGC>CGC (G13R). In summary, our data suggest that more KRAS mutations were observed in codon 13 relative to codon 12 in research samples, and the STA assay is an easy to use, robust method to detect possible KRAS mutations in codons 12 & 13. STA is currently for research use only, not for use in diagnostic procedures.

807 Comprehensive molecular analysis of oligodendroglial tumours – merging genomic, transcriptomic and metabolomic data

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Background: The challenge in post-genomic era is to integrate genomic, transcriptomic, proteomic and metabolomic data. It has been observed that Oligodendrogliomas (OT) are chemosensitive solid tumours and loss of chromosome (LOH) 1p was associated with chemotherapy response. The purpose of this study was to obtain a comprehensive genomic analysis of DNA copy number, gene expression, DNA methylation, and "*ex vivo*" and "*in vivo*" metabolic profiles in oligodendroglial tumours.
Material and Methods: Twenty-nine oligodendroglial tumours (19 pure and 10 mixed) were studied. SNP and expression arrays were used. *EGFR*, *CDKN2A* and 1p, 19q, 10q status were evaluated by Real Time PCR analysis. *TP53* and *IDH1* mutations were confirmed by sequencing. Genes more differentially expressed were selected and evaluated by PCA, Hierarchical Clustering and Functional Enrichment was determined. Methylation status was assessed by base specific cleavage and mass spectrometry. NMR metabolic profiles were performed according to eTUMOUR protocols.
Results: Three OT groups were detected. "*Neurogenic*" group, with samples showing 1p/19q LOH, over-expressed genes related to neurogenesis, showed *MGMT* hyipermethylation and *IDH1* mutation; this group had good overall survival. Tumours harboring 1p/19q ROH over-expressed genes linked to immune response and proliferation. This group could be further divided in two subtypes, "*Intermediate*" which did not show major genetic aberrations other than LOH and mutation at *TP53, IDH1* mutation and *GSTP1* hypermethylation in most samples. "*Proliferative*" group concentrated samples carrying several anomalies: LOH at 10q, *EGFR* amplifications, *MGMT* and *GSTP1* hypomethylation; worst prognosis and GBM features were displayed. *CDKN2A* was inactive in all samples; hypermetilation was more frequent in indolent tumour and deletion was higher in *proliferative* group.

OT with worst prognosis presented increased levels of Phosphocholine, Choline, Fatty acids and Alanine. In the same way OT harboring 1p/19q ROH present higher glutathione levels.
Conclusions: Each tumour is singular but alterations detected in this study depict the genetic landscape for oligodendroglial tumours and could reveal the divergent response showed by these molecular subgroups in survival and chemotherapy treatment.

808 Comparative proteomic study of multidrug resistance in chronic myeloid leukemia

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Background: Chronic myeloid leukaemia (CML)'s treatment has improved with the advance of Imatinib mesylate (Glivec®, IM, Norvatis). IM is a tyrosine's kinase inhibitor for CML biomarker, the BCR-ABL oncoprotein. Despite this improvement, BCR-ABL dependent and independent mechanisms of IM therapy's resistance are known to occur. The latter has been associated to multidrug resistance (MDR) phenotype emergence. MDR is known as the major cause of failure in cancer treatment, and it is most related with the expression of ABC transporters, such as P-glycoprotein (Pgp – *ABCB1*). Although the identification and the knowledge of ABC transporters, the resulting pathways in drug resistance in leukemic cells remain uncharacterized. In the present work, we investigated the possible relationship between MDR and resistance to IM therapy in CML.
Material and Methods: We screened drug transporters and *BCR-ABL* RNA transcripts levels, by real time Q-PCR, in the multidrug resistant cell line Lucena (K562/VCR) and verified its cellular viability, apoptosis and cell cycle after IM treatment. Then, we compared its proteomic profile to the parental cell line K562. Proteomics results were validated *in vivo* by real time Q-PCR and multivariate statistical analysis were applied.
Results: Our results demonstrate that MDR cell line Lucena has a resistant pattern to IM treatment. The proteomic approach resulted in identification of forty-six differentially expressed proteins. Among them, *LRPPRC, MCM7* and *RBM17*, jointly with *ABCB1* gene, were validated in fourteen CML patients and six donors. We found, through multivariate statistical analysis that, altogether, they were able to categorize patients' status as responsive or resistant to IM therapy.
Conclusions: By the data presented in this work, we showed that MDR can be closely attached to IM's failure, demonstrating its importance as a CML's prognostic factor. Moreover, the proteomic approach pointed out some new possibly markers associated in MDR phenotype, which could lead to additional information of this phenomenon and clinical improvement for MDR detection in patients.
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809 Prediction of lymph node metastases in small T1 breast cancers by expression profiling

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Background: The principal cause of mortality in breast cancer is distant metastases. Tumour size and lymph node positivity were classically used as prognostic factors for relapse risks. However, up to 30% of lymph node negative patients eventually develop metastases. We aimed to study breast cancers less than or equal to 2 cm where metastases to regional lymph nodes is generally uncommon, and hypothesised that these small tumours have acquired the ability to metastasise as an early event in oncogenesis.
Methods: Fresh frozen tissues from breast tumours that were $\leqslant$2 cm, positive (n = 23) and negative (n = 42) for lymph node metastases were expression profiled using the Illumina HumanWG-6 v3.0 Expression BeadChips.
Results: Distinct differences in the expression profiles between oestrogen receptor positive (ER+) tumours as compared to the oestrogen receptor negative (ER−) tumours were obtained by unsupervised clustering. As such, we subsequently performed supervised clustering on ER+ tumours (n = 47) and ER− tumours (n = 16) as separate subgroups using genes that were differentially expressed and with *P* values of <0.05. Our analysis showed segregation of breast cancers that were lymph node positive from those that were lymph node negative in ER− tumours. There were four HER2 positive tumours (defined as having immunohistochemical staining of 3+ or positivity with fluorescence *in situ* hybridisation) in this ER− subgroup. By selecting only genes that had at least 2-fold differences between the node negative and positive tumours, we identified 53 differentially expressed genes which were mostly involved in signal transduction, cell communication, metabolic processes and response to stimuli. Of these 53 genes, 13 were downregulated and 40 were upregulated in those with lymph node metastases.
Conclusion: Our results suggest that in ER− breast cancer, it may be possible to discriminate patients with or without lymph node metastases using gene expression profiling. The details of the differentially expressed genes will be presented at the meeting. We will perform further validation on an independent set of breast tumours.

810 Differential enrichment of pathways in association with TP53 mutation status of breast cancers

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Background: Various studies have so far tried to explain the biology of breast cancer associated with TP53 mutation status in terms of differential expression of genes. Keeping in view that altered functions of many genes belonging to specific biological pathways might result in a particular tumour phenotype, we attempt to infer the association of key pathways related gene sets that might play important role in development of breast cancers with wild type or mutant TP53 status.
Material and Methods: A single expression dataset based on Agilent whole genome microarrays platform, consisting of total 111 samples with 73 wild-type TP53 and 38 mutant TP53 status, was analysed by fitting a regression model as proposed by (Goeman *et al*., 2004, 2009) with gene expressions being the covariates and phenotypic data (TP53 mutation status of breast cancer) as the response variable. We also applied iterative signature algorithm (Bergmann *et al*., 2003, Csárdi 2009) in order to identify modules with enrichment of specific key pathways in our dataset.
Results: Using multiple test-corrected p-values based ranking, we identified top 20 important biological pathways (KEGG) and their associated genes. We also studied the extent of inter-sample similarity in pathway representation. Apart from p53 signaling pathway, we found differentially expression of purine metabolism; glycine, Serine and threonine metabolism; prostate cancer and vitamin B6 metabolism pathways. The biclustering analysis identified a module showing differential enrichment of key pathways − p53 signaling pathway, cell cycle and DNA replication and differential co-expression of corresponding genesets. Another module identified differential enrichment of immune response related pathways – such as cytokine-cytokine receptor interaction, T cell receptor signaling pathway, natural killer cell mediated cytotoxicity pathway.
Conclusions: Our findings from biclustering algorithm add valuable information to the findings from regression model-based pathways analysis and provide new insights about the potential pathway alterations that might be responsible for breast cancer development in association with TP53 mutation status. However, it remains to be established, which alterations are responsible for the cancer development and which alterations are consequences or mere associations to the TP53 mutation status. Therefore, we propose more detailed studies aiming at investigation of the association and possible role of certain pathways, such as purine metabolism and vitamin B6 metabolism pathway, immune response related pathways including natural killer cell mediated cytotoxicity pathway – in breast cancer development.

811 8q24 amplification in metastatic endometrial cancer

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Amplification of 8q24 is a hallmark of metastatic cancer. The target genes of 8q24 somatic amplification have not been precisely established. So far, investigations on different (but also on the same) cancer types have produced varying results. The *EIF3S3* and *ASAP1* have been proposed as targets in prostate cancer, the *PTK2* and *EIF3S3* – in hepatocellular carcinoma, the *BOP1* – in colorectal cancer, etc. The *MYC* and *PRL3* are two of genes in this region, known to be overexpressed in many cancer types, however this has not always been associated to gene amplification.
Endometrial tumours are particular for their paucity of genomic amplification, however overrepresentation of the 8q24 region has been described in metastatic endometrial cancer (EC). We compared the amplification profiles of 5 metastatic (MEC) and 20 metastasis-free EC samples (NMEC) by using Illumina 660K SNP-array. Tissue samples were obtained at the University Hospital of Obstetrics and Gynaecology "Maichin Dom", Bulgaria. All patients gave informed consent.