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.

Reference(s)

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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.