882 New insights into molecular pathways of colorectal cancer from genome-level expression data

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Background: Colorectal cancer (CRC) is the third leading cause of cancer-related death world-wide. So far, gene expression studies in CRC have mostly been focusing on individual genes in the carcinogenesis process and the identification of prognostic gene signatures. Differential expression has mostly been reported for genes with extreme changes, adjusting for multiple testing that is often conservative, and genes with small to moderate changes are subsequently not detected. However, small but coordinated changes within the same pathway may as well have an impact on the cell fate.
Material and Methods: We have used two gene expression data sets: a test set comprising 46 CRC and 4 normal colonic mucosa samples analysed by the Applied Biosystems AB1700 microarrays, and a validation set comprising 91 CRC and 6 normal colonic mucosa samples profiled on Affymetrix GeneChip Exon microarrays. Gene set enrichment analysis (GSEA) was employed for both gene expression data sets. This method focuses on coordinated changes within gene sets, which in this study were derived from KEGG pathways.
Results: The KEGG pathways scored as deregulated were highly reproducible between the two expression data sets. Our analysis supports previous findings for the deregulation of several pathways involved in cancer, such as cell cycle and TP53 signaling pathway. Interestingly, several metabolic pathways displayed highly coordinated deregulation of gene expression, while the effect on molecular signaling pathways was lower, but still highly reproducible. Among the metabolic pathways, aminoacyl-tRNA, N-Glycan biosynthesis and retinol metabolism were altered in CRC vs. normals. Among signaling pathways, MAPK signaling pathway has been scored as repressed, having more downregulated then upregulated genes in CRC vs. normals. Three defined sample clusters have been distinguished based on gene expression of MAPK signaling pathway. Two of the clusters have a comparable number of microsatellite unstable and stable tumours. Interestingly, CRC containing BRAF or KRAS mutations, PIK3CA and/or PTEN mutations were overrepresented in one of these clusters.
Conclusions: By exploring whole gene expression within the molecular pathways, we have identified the pathways involved in CRC carcinogenesis and extended previous research to gain insight at the pathway level. Gene clusters within the defined pathway, stratifies CRC according to mutation status of known genes in a novel manner. Currently, we are investigating and validating selected gene sets and associated individual genes which are deregulated in CRC.

883 Lack of interaction between functional polymorphisms in the MDM2 gene and exposure to 17-β estradiol in vitro

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Background: Lung cancer is the leading cause of cancer mortality in the world and although exposure to carcinogens is considered to be the main cause, genetic variation may contribute to risk. Murine double minute 2 (MDM2), is a key regulator of the TP53 signalling pathway. We have recently shown that a polymorphism in the promoter region of the *MDM2* gene (SNP309) was associated with increased lung cancer risk in women homozygous for SNP309 G/G having an odds ratio of 4.06 (1.29–12.8). However, the individuals with the T/T SNP were younger at the age of diagnosis of lung cancer. Estrogen signaling pathway has been implicated in regulation of the *MDM2* gene expression and some data show that MDM2 transcription may be induced by expression of the estrogen receptors (ER), especially ER-alfa (ER-α). It is also biologically plausible to suggest an interaction between estrogen and MDM2 functional polymorphisms in regulation of the MDM2 levels in vivo.
Materials and Methods: We have cloned SNP 309 as well as a second functional SNP (C1797G) in the promoter I of the *MDM2* gene in Luciferase expression vectors. Three ER-positive and three ER-negative human lung cell lines as well as one ER-positive and one ER-negative breast cancer cell line were transfected with MDM2 SNP309 or SNP C1797G luciferase expression vectors. The transfected cells were exposed to 0, 1 and 10 nM 17-β-estradiol for 3 hours and Luciferase levels were measured in cell extracts.
Results: For SNP 309 we found that the basal expression of Luciferase was higher from the T/T promoter variant compared to the G/G promoter variant. For the C1797G the C SNP was associated with higher expression of the Luciferase compared to the G SNP. Exposure of transfected cells to 17-β estradiol had no effect on the expression of neither SNP309 nor C1797G SNP. Furthermore, the ER status of the cell lines did not affect the expression levels.
Conclusion: Our results do not indicate interactions between estrogen exposure, ER status and functional polymorphisms of the *MDM2* gene in vitro.

884 Maintenance of the physiological integrin expression pattern in esophageal squamous cell carcinoma correlates with favourable disease outcome

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Background: The integrins are a family of cell adhesion molecules constituting heterodimeric transmembrane signaling receptors that mediate the adhesive properties of epithelial cells and affect cell growth and differentiation. Epithelial malignancies frequently exhibit altered integrin expression patterns, and often prognosis correlates with aberrant expression. However, until now little has been known about the integrin expression in oesophageal squamous cell carcinoma (ESCC).
Material and Methods: Establishing a quantifying immunofluorescence staining assay, we investigated the expression of the integrins α2β1, α3β1, α6β1, and α6β4 in the primary tumours of a cohort of thirty-six patients with ESCC who underwent esophagectomy. Intensity and distribution of the integrin expression were analyzed in the tumour samples and compared to the integrin expression in autologous esophageal squamous epithelium.
Results: Patients whose primary tumours maintained the physiological expression pattern shared a favourable prognosis in comparison to patients with aberrant integrin expression: Polarized expression of the integrin subunits α6, β1, and β4 was associated with significantly prolonged relapse-free survival. In contrast, patients with reduced focal α6 integrin expression at the tumour invasion front shared a reduced relapse-free survival compared to patients with strong α6 expression along the stromal surface of their primary tumours ($p = 0.001$). Finally, the maintenance of a strong α6 immunoreactivity at the invasion front of the tumours as observed in basal esophageal epithelium represented an independent prognostic factor for increased relapse-free and disease-specific survival in the multivariate analysis ($p = 0.003$).
Conclusions: Our findings suggest that alterations in both pattern and magnitude of integrin expression reflect ESCC disease progression and limited patient survival. The defined expression of the integrins α6β4 and α6β1 at the tumour invasion front as well as the maintenance of a polarized expression pattern in the tumour tissue appear to represent valuable new markers to assess the aggressiveness of ESCC.

885 High and persistent ERK phosphorylation induced by ursodeoxycholic acid inhibits proliferation of intestinal epithelial cells

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Introduction: Ursodeoxycholic acid (UDCA) prevents colitis-related colon cancer potentially attributed to enhanced proliferation and mutations during tissue regeneration. EGF-ERK pathway has been shown to regulate colonic epithelial cell proliferation. Therefore, we investigated the effects of UDCA on growth of normal rodent intestinal epithelial cells and on the EGF-ERK pathway.
Materials and Methods: Normal rat intestinal cell line IEC-6 was treated with UDCA (0 to 600 μM for 3 days) and total live cells were counted. Cell cycle and BrdU incorporation was measured by FACS. ERK-phosphorylation and localisation was determined by western blotting and immunocytochemistry. To check the association between cell cycle and ERK phosphorylation, cells were arrested through serum starvation and then released in medium with 10% FCS with and without UDCA. ERK-phosphorylation was monitored by western blotting and cell cycle by FACS. IEC-6 cells were treated with U0126 or PD0325901 for 3 days to inhibit ERK phosphorylation. IEC-6 cells were transfected with pSuper plasmids expressing sh-ERK1 or sh-ERK2 to suppress ERK protein, using Amaxa nucleofector.
Results: UDCA treatment for 3 days caused a 20% (400 μM) and 60% (600 μM) decrease of cell number. S-phase population and BrdU incorporation was reduced by 50%. The decrease in proliferation was associated with high and persistent ERK phosphorylation. There were more cells with strong nuclear expression of phosphorylated-ERK after UDCA treatment as compared to nontreated. Serum-starved cells released from G0/G1-arrest, entered the S-phase in 8 hours whereas in the presence of UDCA, they entered S-phase in 16 hours. The delay in cell cycle progression was associated with persistent and high ERK-phosphorylation. Inhibition of persistent ERK phosphorylation by U0126 or PD0325901 treatment or by suppression of