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

ERK1 but not of ERK2 protein partially abrogated the proliferation inhibition by UDCA.
Conclusion: High and persistent ERK phosphorylation is the likely mechanism of proliferation inhibition by UDCA.

886 Information function, carcinogenic action and radioprotective properties of chemical compounds

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Background: Actual problem of modern chemistry of biologically active substances is the problem of produce of preparations that effective in the radio protective relation. The basic requirements to these preparations are small doses, low toxicity and absence of collateral action. Purpose of this to pay attention to possible linkage of radioprotective action and carcinogenic activity of preparations. Quantitative characteristics of this linkage are resulted. An attempt made to construct a quantitative model of relationship of the carcinogenic and radioprotective properties of biologically active compounds with their electronic and information factors.
Materials and Methods: In this work the method is offered for revealing linkage between carcinogenic and radioprotective properties of drugs with their molecular structure. In this work the method is offered for revealing linkage between carcinogenic and radioprotective properties of preparations with their molecular structure. The approach uses the factorial characters: the mean quasivalency number Z of a molecule and information function H of Shannon–Wiener. We have analyzed more than 120 various chemical compounds.
Results: It is established that carcinogenic properties of chemical compounds and effective radioprotectors are overlapping with each other. Parameters Z and H statistically authentically separates the compounds having radioprotective effect from compounds do not having radio protective action. For overwhelming number of preparations having activity the parameters $Z < 3.0$ and $H < 1.79$ *bit*. Whereas for the chemical preparations which are not possessing protective activity $Z > 3.0$ and $H > 1.97$ *bit*. The suggested method of selection of preparations most effective for drugs which find out protective action at small doses ($\ll$1 mM/kg) and are inactive even at very large doses ($\gg$1 mM/kg). At the same time use of molecular characters Z and H for separating the carcinogenic compounds also leads to statistically authentic results. The information function for the chemical preparations possessing carcinogenic activity $H < 1.41$ *bit* and not possessing ones $H > 1.86$ *bit*.
Conclusions: Within the framework of an information approach and the statistical method of comparison of quality characters, a systemic factor is proposed that permits to reliable distinguish highly radioprotective agents and carcinogenic compounds among a series of chemical substances. It was shown that the correlation relationships are able apply to studies of the mechanism of action of the preparations and for the purposeful synthesis of new effective radioprotectors.

887 Alterations of copy number of methylation pattern in MMR genes by MS-MLPA methodsin cases of colon cancer

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Background: Like other tumour types, colon carcinoma is thought to arise following the activation of oncogenes and inactivation of tumour suppressor and DNA-repair genes. In addition to genetic alterations, epigenetic abnormalities, such as changes in genomic DNA cytosine methylation patterns, are associated with all cancer types. The syndrome is caused by germline mutations in DNA mismatch repair (MMR) genes, predominantly MLH1 and MSH2.1. More than 1,500 different variants in MMR genes have been reported, approximately half of which may be pathogenic.
Material and Methods: In our study with a diagnosis of colorectal cancer tissue samples embedded in paraffin were used. With total 70 samples were studied, adenocarcinoma is 49 (70.0%) of samples, carcinoma is 21(30.0%) of total consists of samples. A modification of the MLPA technique, MS-MLPA (methylation-specific multiplex ligation dependent probe amplification) allows the detection of both copy number changes and unusual methylation levels of 10−50 different sequences in one simple reaction. MLPA probes for methylation quantification are similar to normal MLPA probes, except that the sequence detected by the MS-MLPA probe contains the sequence recognized by the methylation-sensitive restriction enzyme HhaI. Gene methylation status was evaluated by (MS-MLPA), using the ME001 tumour-suppressor kit (MRC Holland). A total of 24 genes were studied, using 20–200 ng of sample DNA. The amplified products were analyzed by sequence-type capillary electrophoresis (ABI 310; Applied Biosystems, Foster City, California, USA). The peak sizes and areas were exported to an Excel file, and the normalized areas from the digested and undigested samples were compared to determine the methylation status of the genes in colon cancer patients.
Results: According to the results of this amplification mean MLH1 methylation rates (97.14%), MSH2 (24.28%), MSH6 (67.14%), MSH3 (78.57%), MLH3 (75.71%), PMS2 (65.71%), MGMT (82.85%) were found to be.
Conclusions: The Mismatch Repair (MMR) system is critical for the maintenance of genomic stability.

888 In vivo genotoxicity of deltamethrin, a synthetic pyrethroid

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Deltamethrin, an alpha-cyano class of pyrethroid insecticide is widely used in agriculture, public health and livestock due to its high activity against a broad spectrum of insect pests and low toxicity to humans. In the present study, by taking into consideration of the consumption risk of deltamethrin by mammals, the in vivo cytogenetic effect of deltamethrin was evaluated by assessing the ability of the insecticide to induce micronucleus formation in bone marrow and peripheral blood erythrocytes and splenocytes.
Deltamethrin was administrated to adult mice as i.p. doses of 50, 100, 200 mg/kg/bw in %10 tween 80. To the positive control group, Mitomycin C, which is a mutagenic agent, prepared in saline, was given in 2 mg/kg bw doses. Samples were taken 48 h after the treatment.
All doses of deltamethrin significantly ($p < 0.001$) increased the frequency of micronuclei in erythrocytes and splenocytes, compared with the control group. A linear relationship was evident between the doses of deltamethrin used and the frequencies of micronuclei. The micronucleus induction suggets a clastogenic potential of deltamethrin and indicates the in vivo susceptibility of mammals to the potential genetic toxicity of deltamethrin.

889 The involvement of miR-483 and its host gene IGF2 in development of adrenal cortical carcinoma

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Patients with tumours of the adrenal cortex may present incidentally detected or hormonally overproducing adenomas (ACA), or uncommonly the very aggressive carcinoma (ACC). Recent genome-wide studies of gene expression have revealed significant alterations in the different tumour entities of possible diagnostic and prognostic relevance. Of special interest is the frequent finding of *IGF2* over-expression, observed in the ACC entity only. The aim of this study was to identify molecular signatures of ACC based on microRNA (miRNA) expression profiling, and to determine the role of specific miRNA in the development of ACC and its relation to *IGF2*.
Global miRNA expression profiles were determined in a series of ACC, ACA and normal adrenal cortical samples by using two different platforms (miRNA oligoarray and miRNA qRT-PCR array respectively), and the results were confirmed by qRT-PCR. Distinct miRNA expression signatures were observed between the three sample groups. Over-expression of *miR-483−3p* was frequently observed in ACC samples, but not in their normal or benign counterparts. Interestingly, *miR-483−3p* is located within an intron of the *IGF2* gene. Given that in an extended series of 63 samples (ACCs, ACAs of incidentaloma, Cushing and aldosteronoma types; and normal adrenal) we showed striking co-expression of *miR-483−3p* and *IGF2*. Subsequent knocking down of *miR-483−3p* in the ACC cell line NCI-H295R resulted in significantly decreased cell proliferation as compared to the non-targeting sequence Anti-miR-transfected cells used as negative control. Current efforts are directed to analyzing the biological function of *miR-483−3p* in ACC development, which may lead to the identification of an important target for clinical intervention in ACC.

890 IDH1 and IDH2 mutations in Bulgarian patients with glial tumours

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Background: Gliomas, the most common type of primary brain tumours, include distinct disease entities that affect patients of different age and vary in prognosis. In the recent years many comprehensive studies were focused on genomic characteristics of gliomas. This led to the discovery of a variety of genes that were not associated with glial carcinogenesis before. Mutations in genes encoding isocitrate dehydrogenase isoforms 1 (*IDH1*) and 2 (*IDH2*) have been found in a large proportion of gliomas. *IDH1* and *IDH2* genetic alterations occurred early in tumour progression of brain neoplasias, but very rarely in other solid tumours and were associated with better outcome.