780 Rb1 in sporadic colon cancer

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Background: Mutations in numerous genes, oncogenes, tumour suppressor and mismatch repair genes are involved in colorectal carcinogenesis. It is considered that one of the gatekeeper genes involved in this process is *Rb1*. The aim of our study was to evaluate changes in *Rb1* at different levels in the cell in order to elucidate its role in colon cancer.
Material and Methods: Primer specific PCR was used to evaluate the loss of heterozygosity at the two polymorphic loci in 140 samples of normal tissue and corresponding tumour tissue from sporadic colon cancer patients. Rb1 mRNA expression was analyzed in 50 pairs of normal and corresponding tumour tissue samples using real-time PCR method. Rb1 protein expression was analyzed on 50 tumour paraffin sections using immunohistochemistry.
Results: Heterozygosity was detected in 63% and 79% of analyzed samples and LOH was observed in 17% and 12% of informative samples for polymorphic markers Rb1.2 and Rb1.20 respectively. Expression of Rb1 mRNA was higher in moderate and poorly differentiated tumours and tumours classified as Dukes' C. Rb1 protein was immunohistochemically positive in 83% of examined tumour samples. The expression of pRb was found higher in moderate and poorly differentiated tumours and it positively correlated with Dukes stage.
Conclusions: Our results support the thesis that malignant transformation in colon tissue is a consequence of more than one genetic alteration and suggest that *Rb1* plays a role in this multistep process.

781 Crosstalk between retinoid and steroid regulation pathways in the control of seminoma cell proliferation

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Background: In human males, an increase in the incidence of testicular cancer and infertility have been observed in many industrialized countries over the last 30 years. Such male reproductive disorders have been attributed to the increase in concentration of endocrine disruptor compounds (EDCs) in the environment and food. Epidemiological, clinical and experimental studies have suggested that excessive exposure to xeno-estrogens during fetal/neonatal life can lead to reproductive disorders in adulthood.
Material and Methods: Using the unique pure seminoma cell line TCam2 as a model for the main form of testicular germ cell tumour, we measured proliferation rate, activation of transduction pathway and target genes expression after cell exposure to natural steroid, retinoic acid or to several related classes of EDCs.
Results: We demonstrated that proliferation rate varies upon steroid or retinoid treatments through the activation of non-specific steroid receptor (GPER) and EGFR-dependent transduction pathways. Moreover, we point out crosstalk between retinoid and steroid-dependent regulation pathways. Taken together, these results allow classifying this type of testicular germ cell tumour as a hormone-dependent one.
Conclusions: The data also shed light upon possible mechanisms which could trigger carcinoma in situ cell proliferation and development of a testicular germ cell tumour in post-pubertal males. Therefore, we propose to use the TCam2 cell line as bio-indicator in order to characterize the effects of emerging pollutant mixes at low doses not only *in vitro* but also *in vivo* using xenografted Nude mice.

782 An automated analysis protocol for research of KRAS/BRAF mutation detection for data generated on capillary electrophoresis instruments

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Biomarker research continues to be an important focus in oncology studies, including the role of KRAS and BRAF mutations in CRC and other EGFR-associated cancers. This has lead to increased research of these genes as possible predictive markers and targets for continued study.
With this increased interest comes a need for automation of data analysis and report generation to decrease bottlenecks in the research laboratory by reducing manual review time. This poster will present an automated workflow for detection of KRAS and BRAF mutations and concise report generation in sample data generated on capillary electrophoresis instruments using fragment analysis software tools. We will demonstrate how key features in the software, such as sample quality values, allele binning and report analysis, enable this workflow to be a significant improvement over visual scoring methods.
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783 Epigenetic and genetic programs of osteogenic mesenchymal stem cell differentiation: a genome-wide integrative approach

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During malignant transformation the finely tuned processes of differentiation and proliferation gets out of balance. Understanding the normal balance is central in both oncology and normal biology. We here aim to identify genetic and epigenetic programs that control stemness and govern normal bone formation during mesenchymal development. These programs will be compared to those identified in bone cancer, specifically osteosarcomas, in order to reveal cancer specific events.
To study normal bone development, we have generated an immortalized human mesenchymal stem cell line that can be differentiated to the osteogenic lineage, evidenced by upregulation of osteogenic specific genes, induction of alkaline phosphatase activity and calcium deposition. Using high-throughput technology we have identified a subset of mRNAs and miRNAs that are up- and downregulated during osteogenic differentiation. The CpG methylation status of more than 14,000 gene promoters, including more than 900 cancer-related genes and 144 methylation hot-spots in cancer, have been mapped. Another epigenetic layer of information is contained in the covalent modification of histone N-terminal tails. Using chromatin immunoprecipitation combined with next-generation sequencing technology (ChIP-Seq) we are mapping the genome-wide profiles of histone H3 tri-methylation of lysines K4, K9, K27 and K36 and acetylation K9 at various stages of differentiation. Using bioinformatic approaches the different layers of information will be integrated to reveal regulatory networks governing stemness and osteogenic differentiation. In parallel, a corresponding set of data are being generated from a large panel of ostesarcoma cell lines, xenografts and primary tumours. Ultimately, data from normal and cancer cells will be compared to identify a set of genes specifically changed in osteosarcomas with the epigenetic regulatory mechanisms underlying cancer specific deregulation. Finally, we aim to develop a molecular staging tool for ostesarcomas, based on their differentiation status in the mesenchymal developmental hierarchy.

784 Clinical applications of molecular profiling of colorectal cancer

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Introduction: Despite developments in diagnosis and treatment, 20% of colorectal cancer (CRC) patients present with metastatic disease and 30% of cases recur after curative surgery. Furthermore, the molecular factors involved in prognosis and response to therapy in CRC is poorly understood. Objectives of the study were to quantitatively examine the expression of target genes in colorectal cancer and to correlate their expression levels with clinico-pathological variables.
Methods: A detailed analysis of published CRC microarray data was performed to identify the most prominent genes. The selected genes were validated in fifty-two pairs of fresh colorectal tumour and associated normal tissue specimens by RQ-PCR using TaqMan® assays. Statistical analysis and correlation with clinicopathological data was performed using SPSS software.
Results: Expression levels of *CXCL12* ($p = 0.000$), *CDH17* ($P = 0.026$), *MUC2* ($p = 0.000$), *L-FABP* ($p = 0.000$) and *PDCD4* ($p = 0.000$) were down regulated and IL8 ($p = 0.000$) was upregulated in tumours compared to normal colorectal tissues. No significant differences were noted in expression of *CEACAM5, CXCR4, CXCR7, TGFB1, TGFBR1* and *TGFBR2*. Furthermore, we found significant associations of gene expression levels and clinicopathological variables such as survival, tumour size, grade, invasion and lymph node status.
Conclusion: We identified a comprehensive list of genes with highly differential expression patterns in colorectal cancer that could serve as molecular markers to complement existing histopathological factors in diagnosis, follow up and therapeutic strategies for individualised care of patients.

785 HGUE-C-1 a novel colon carcinoma cell line

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HGUE-C-1 cells were obtained from ascytic efussion of a 76-year old colon carcinoma male patient. Citology of ascytic efussion obtained after paracenthesis confimed colon adenocarcinoma origin. Chemotherapy was then started with capecitabine and irinotecan, After a second cycle of chemoherapy, patient was admitted to the hospital. During admission, two parecenthesis of 5000 and 54000 ml of malignant hematic ascytis with 72 h interval were performed.