443 Poster

Transcriptional repression of cis genes via a new murine retrotransposon containing Snail- and bHLH-transcription factors binding sites

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Background: Transcriptional networks in cancer are deeply misregulated. Retrotransposons and other repetitive elements occupy more than 40% of the size of mammalian genomes, and their epigenetically silenced status have been recently reported to be lost in several cancer stages. Besides, their sequences are potential targets for transcription factors (TFs) regulation. In spite of this, little is known about how transcriptional networks are modulated by the presence of repetitive elements.

Materials and Methods: We used a mixed approach of genome-wide computational algorithms complemented with in vitro (DNA-binding affinity and luciferase reporter assays) and in vivo (real-time PCR and Chromatin Immunoprecipitation) experiments using the murine hepatoma (Hepa-1) cell line.

Results: We have identified the existence of a novel murine retrotransposon of the SINE B1 family characterized by the presence of functional binding sites for the Epithelial-Mesenchymal Transition regulator Slug/SNAI2 (Slug site) and the carcinogen-activated AhR (Xenobiotic-Responsive-Element, XRE) at 35 bp distance, forming the so-called B1-X35S (B1-XRE-35bp-Slug). This element is present in more than 1,300 mouse gene promoters.

Evolutionary studies revealed that B1-X35S is the only member of the B1 subfamily that has the Slug binding site, and that the X35S repetitive element within B1-X35S maintains a differential evolutionary pressure as compared to other family members.

In vitro, we detected the ability of both AhR and Slug to bind X35S and to down-regulate the expression of cis-reporter genes in a sequence-specific manner.

In vivo, we observed that AhR and Slug repressed the expression of three genes (Lpp, Tbc1d1, DAD1) containing the B1-X35S element at different distances from their transcription start site. Further, AhR and Slug were recruited to B1-X35S in these three genes during repression. Comparative genomic expression analyses predicted a potential genome-wide transcriptional modulation of B1-X35S-containing genes.

Conclusions: Some of the known effects of AhR and Slug TFs in cancer progression can involve or be mediated by the B1-X35S retrotransposon. Further studies are aimed to test the relationship or the causal role of this novel retrotransposon in the regulation of transcriptional pathways altered in pathological states like cancer.

444 Poster

Analysis of copy number independent regions of expression bias in breast cancer using partial correlation

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One of the major challenges in cancer biology is the identification and functional characterisation of the whole spectrum of pathologic changes in cells' genomes that can lead to malignancy. Recently using aCGH and DNA Microarray data a number of studies characterized genomic hotspots - regions of high correlation between amplification and over expression harbouring important oncogenes in breast cancer. These hotspots are well correlated with a number of histopathological features describing different stages of cancer progression and tumour subtypes. However, since the correlation between gene expression in these regions and cancer phenotypes is not ultimate, they can not account for all sources of pathologic abnormalities.

Here we apply technique of partial correlation to find copy number independent regions of gene expression bias (CNIREBS) in breast cancer using arrayCGH and gene expression data for 105 breast cancer tumours and 38 cell lines and correlate them with phenotypic outcome. The aim of our approach is to find highly correlated genomic regions that maintain their correlation after accounting for copy number changes, which suggests additional biological mechanism driving co regulation over contiguous genomic intervals. One plausible mechanism for such deregulation are regions of long range epigenetic silencing that have now being reported for a number of cancers. After estimating partial correlation coefficients and applying 0.05 p value cut-off (Fisher's z-transform of the partial correlation), 220 genes were found in the regions of expression bias that corresponded to 134 independent regions. Prioritization based on the number of other genomic features such as CpG islands density further narrowed down the number of plausible candidate CNIREBS to 50. Some of them like cluster of kallikrein-related peptidases on chromosome 19 were previously reported to be epigenetically silenced and 6 regions have strong correlation with estrogene receptor status. Further functional validation of the regions for histone modification and DNA methylation is under way.

445 Poster

Antiapoptotic genes are overexpressed in the group of patients with locally advanced rectal adenocarcinomas who do not respond to neoadjuvant chemoradiotherapy

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Purpose: Colorectal cancer is one of the most common malignities. The only curative treatment of locally advanced rectal adenocarcinomas (LARA) is radical surgery. To allow this, neoadjuvant chemoradiotherapy is performed to reduce tumor volume. This practice also increases a feasibility of sphincter-sparing surgery. Responsiveness varies from complete pathological response to resistance. Non-responders can be spared from toxicity, time, and expenses associated with the treatment. The aim of our study was to evaluate the capability of gene expression signatures to identify responders and non-responders pretherapeutically. Methods:164 patients (pts) with LARA treated with neoadjuvant chemoradiotherapy based on fluoropyrimidines were included. Response to the therapy was determined clinically (TNM) by trans-rectal ultrasonography and CT or MRI before and after therapy and histopathologically by TRG-scoring system (tumor regression grade 1-5) according to Mandard (Cancer 1994). Pts characterized by TRG 1-2 and improved T-stage (downstaging) were included to the responders group „R" and pts with TRG 4-5 and no signs of downstaging composed the group of non-responders „NR". Tumor biopsies were obtained before starting the therapy and stored in RNA later. RNA was extracted from each specimen and relative gene expression levels of 440 genes known to be involved in cancer biology were obtained by low-density oligonucleotide microarrays. Results: Downstaging was observed in 55% pts. Complete pathological remission (pCR, ypT0ypN0) after neoadjuvant chemoradiotherapy was seen in 23.3% pts and resistence to therapy in 10.3% pts. Gene expression data analysis of 20 pts based on SAM (Significance Analysis of Microarrays) and t-test methods identified 8 genes (lipocalin2, JUNB, RB1, MDM4, calnexin, MMP2, TCF7L2, PDGF-beta) with up-regulated expression in primary tumors of "NR". Conclusion: We identified 8 antiapoptotic genes that are significantly overexpressed in the group of pts with LARA who do not respond to neoadjuvant chemoradiotherapy. At the moment, validation of identified changes in gene expression is undergoing in our lab by more precise quantification method on the mRNA level (by Real-Time PCR) and also on protein level (by immunohistochemistry). We suggest that low-density oligonucleotide microarray technology could contribute to a better understanding of rectal cancer resistance at molecular level to neoadjuvant therapy. Supported by IGA MZ CR NR/9076–4

446 Poster

KIAA1199 is upregulated in colon adenocarcinomasand targets genes of the Wnt signaling pathway

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Early diagnosis and treatment of Colorectal cancer (CRC) requires the identification of new biomarkers as well as insights into the molecular mechanisms of human carcinogenesis. In previous microarray analyses on pooled samples we identified the KIAA1199 gene to be strongly upregulated in colon cancer stages I-IV. KIAA1199 seems to be a putative target of the Wnt signaling pathway, as inhibition of the Wnt-pathway by TCF4 proteins or β-catenin knockdown resulted in decreased KIAA1199 expression. Still, neither cellular function nor downstream target genes of KIAA1199 are known.

The aim of this study was to analyze KIAA1199 expression in adenocarcinomas and to identify KIAA1199 downstream targeted genes and associated pathways to elucidate the role of KIAA1199 in colon cancer.

Genome-wide transcript profiling studies of 379 adenocarcinomas using U133Plus2.0 arrays showed a strong upregulation of KIAA1199 compared to normal colon mucosas (n=10, median log2 3.8). The upregulation was more striking in microsatellite stable (MSS, n=78, median log2 8.8), than in microsatellite instable tumors (MSI, n=78, median log2 8.2, p=8.9E-04).