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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.

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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.

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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

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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).

Immunohistochemical analysis applying a peptide derived affinity purified antibody localized the KIAA1199 protein to the nucleus and the cytoplasma of tumor cells. Nuclear staining was strongest in stage I tumors and decreased in the higher stages. A multiple cancer TMA showed KIAA1199 to be upregulated in other cancers derived from kidney, lymphnode, stomach, skin and thyroid.

Cloning of the KIAA1199 gene identified an alternative splice variant in 2 out of 10 patient samples. The loss of one exon generates a stop codon, resulting in a truncated protein lacking the C-terminal GG-domain. Overexpression of wild-type KIAA1199 in SW480 (MSS) colon cancer cells showed a cytoplasmic localization. Moreover, the protein was found to be secreted into the culture media, and can thus be considered as a potential serum biomarker.

Expression profiling of SW480 cells overexpressing KIAA1199 showed a log2 6.3-fold upregulation of the gene compared to mock transfected cells. 2296 target genes were found to be differentially expressed and 338 genes showed significant expression changes between normal mucosas and MSS adenocarcinomas.

Potential target genes and results from microarray studies were classified by Ingenuity Pathway Analysis software and "Wnt/β-catenin signaling" was listed as a top canonical pathway. Among the KIAA1199 target genes we identified 17 known targets of the Wnt/β-catenin signaling, most were dysregulated in adenocarcinoma. A gene which was upregulated both by KIAA1199 overexpression and in our series of adenocarcinomas, was previously seen to correlate with the KIAA1199 expression in adenomas.

In conclusion, our data suggest that KIAA1199 is a modulator of the Wnt/β-catenin signaling pathway, and thus may play an important role in colon cancer.

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Mutational analysis of the tumor suppressor gene BRG1 in human lung primary tumors by next-gen sequencing technology

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The SWI/SNF chromatin-remodeling complex promotes gene expression in response to several stimuli by disrupting histone-DNA contacts in an ATP-dependent manner. Components of the complex such as INI1 are inactivated in human cancer and thus act as tumor suppressors. The gene SMARCA4 encodes for BRG1, which contributes the ATPase activity of the complex. Recently we performed a mutation analysis of BRG1 in 59 lung cancer cell lines and observed deleterious mutations in 24% of the cell lines. The alterations were significantly more frequent in the non-small cell lung cancer (NSCLC) type (35%) as compare to the small cell lung cancer (SCLC) type (5%). BRG1 was the fourth most frequent altered gene in NSCLC cell lines, strongly supporting that BRG1 is a bona fide tumour suppressor and a major factor in lung tumorigenesis. BRG1 mutations coexisted with mutations/deletions at KRAS, LKB1, NRAS, P16, and P53. However, alterations at BRG1 always occurred in the absence of MYC amplification, suggesting a common role in lung cancer development. The purpose of the present study is to drive our investigation a step further by confirming the mutational status of BRG1 in human lung primary tumors by exon-wise sequencing of genomic DNA using Next-Gen sequencing technology. The methodology used in this work includes the preparation of tissue microarrays (TMAs) from primary lung tumors and from associated healthy tissues and to test, by immunohistochemistry, the levels of BRG1 protein expression. In addition, genomic DNA from a panel of lung primary tumors was extracted for exon amplification/purification using specific intronic primer sets and a high fidelity/processivity polymerase. Finally, exons will be sequenced using the Next-Gen GS-FLX system from Roche and the output raw data will be analyzed using pertinent software. Immunostaining of BRG1 in primary tumors of the lung using TMAs has provided strength to our hypothesis that BRG1 is a bona fide tumor suppressor in lung carcinogenesis: among 122 lung tumors analyzed, 46 (38%) were negative for BRG1 immunostaining. By sequencing of BRG1 from primary tumors using the GS-FLX system we expect to provide the final evidence to the high relevance of BRG1 in lung carcinogenesis.

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Renal cell carcinoma primary cultures as in vitro model to study genomic profile of parental tumor tissues

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Clear cell renal carcinoma (RCC) accounts for 80% of all primary kidney malignancies. It is characterized by recurrent copy number (CN) alterations (amplifications and deletions) and loss of heterozygosity (LOH) events and many evidences suggest that this peculiar pattern of genomic instability may be useful in diagnostic and prognostic applications. However, molecular analyses of this pathology are complicated because bioptic tumor tissues are highly variegated and comprise a mixture of tumor and normal cells. In the context of an Italian oncological research project aimed to the identification of novel RCC molecular markers, we investigated the possibility to use short-term primary cultures as in vitro model of the parental tumors to study their genomic profiles and characterize their CN alterations. Using the Affymetrix 50K SNP Mapping microarray platform, we performed a high-throughput genomic profiling analysis of 10 pairs of RCC primary culture/original tumor tissue sample and assembled a genome-wide map of amplifications, deletions and LOH occurring in each sample by CNAGv3.0 software. Comparing each primary culture to the corresponding tissue, we found that 9 out of 10 cultures had a genomic profile concordant to the parental tumors: all CN alterations and LOH events occurring in matched tumor tissues were maintained and the typical RCC molecular signature was confirmed (e.g chromosome 3p loss and 5q gain); moreover, in 6 out of these 9 cultures CN alterations were better discriminated than in tumor parental tissues, and this phenomenon particularly affected the CN loss events. We observed that 4 cultures acquired additional CN alterations, such as amplifications or deletions on one or two chromosomes. Additionally, one RCC primary culture showed a diploid status as compared to parental tissue, suggesting the possibility that a normal clone population has been selected by culturing. We concluded that RCC primary cultures at early passages maintained the genomic profile of parental tumor tissues and showed an increasing cell homogeneity and enrichment in tumor cells. Thus, we suggest that the short-term RCC primary cultures are a reliable model to study this pathology and to identify novel genetic elements potentially involved in its etiology and useful in clinical applications.

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Patterns of copy number variation in cancer

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Allelic copy number variation in cancer was studied by running 781 cell lines across Affymetrix SNP 6 arrays for a range of tissue types. Results were investigated by first extracting copy number and allelic ratios, and then segmenting the data with hidden markov models. This allowed accurate identification of loss of heterozygocity, homozygous deletions, amplicons as well as major and minor allelic copy number. Examining the results across all the cell lines revealed a diverse pattern of copy number variation including polymorphisms, tumour supressor genes, amplified oncogenes and genomic fragility. Correlations of these effects with tissue type, mutation status and a range of genomic indices are discussed.

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Single nucleotide polymorphism in reuced folate carrier-1 gene and methyleneterahydrofolate reductase gene in patients with osteosarcoma

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INTRODUCTION: The introduction of systemic chemotherapy has significantly improved prognosis of osteosarcoma patients. Methotrexate (MTX) is an anti-folate chemotherapeutic agent and one of the key drugs to treat patients with osteosarcoma. The previous reports showed that the single nucleotide polymorphisms (SNP) of folate metabolic pathway genes, reduced folate carrier gene-1(RFC1) and methyleneterahydrofolate reductase (MTHFR), were correlated with therapy response and adverse effects of MTX for several diseases. The aim of study was to investigate retrospectively whether SNPs of RFC1 and MTHFR were correlated with distribution, therapy response, and adverse effect of osteosarcoma patients.

MATERIAL AND METHODS: Ninety-five Japanese patients with osteosarcoma were treated and acquired written informed consent at our hospital and 46 patients were received chemotherapy including MTX. For control, peripheral blood was also obtained from 188 Japanese healthy volunteers. Genomic DNA was isolated from frozen tissue obtained at operation by standard methods. PCR- restriction fragment length polymorphism (RFLP) analysis was used to detect polymorphisms in