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