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.

This work was supported by MIUR grants RBLA03ER38 and PRIN 2006.

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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

MTHFR C677T, MTHFR A1298C and RFC1 G80A. were evaluated. The differences in the distributions of the genotype between all patients and healthy controls were evaluated. For the patients treated with MTX, the association of the genotype with histological response, MTX plasma levels and adverse effects following as NCI Common Toxicity Criteria were examined by chi-square test.

RESULTS: The distributions of MTHFR C677T and A1298C genotypes were not different with those of controls, but the distribution of RFC1 genotype (GG in 22(23%), AG in 37(39%), and AA in 36(38%)) was significantly different with that of controls (GG in 54(29%), AG in 95(51%), and AA in 38(20%)). The ratio of homozygous for the RFC1 A80 allele was significantly higher in patients than in controls. Frequency of grade 3 or higher hepatic disorder after MTX administration was correlated with RFC1 AA allele in position 80 and without MTHFR genotypes in position 677 and 1298. Genotypes of MTHFR and RFC1 were no correlated with MTX serum levels, histological response and other adverse effects.

CONCLUSION: The distribution of RFC1 AA genotype in position 80 was significantly different between patients and controls in Japanese. The polymorphism of RFC1 gene influenced in hepatic disorder after MTX administration for osteosarcoma patients. This finding may be helpful to predict and to prevent severe hepatic disorder.

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Candidate molecular markers associated with endocrine resistance in breast carcinoma

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Gene-expression profiles could be a powerful predictor of the response of estrogen receptor (ER)-positive breast cancer patients to endocrine treatment. To this purpose, we used two different cell models of resistance to anti-estrogens: MVLN/CL6.7/CL6.8 cells and VP229/VP267 cells selected after exposure to tamoxifen (Tam), respectively in vitro and in vivo. We newly characterized the cross-resistance developed by the CL6.7 and the VP267 cells, but not by the CL6.8 cells, to fulvestrant (ICI 182,780), a pure ER antagonist used in second-line therapy for patients who have relapsed after Tam treatment. Using a candidate gene approach, we identified by RTQ-PCR 53 genes, the expression of which was deregulated in at least one of the three resistant cell lines (CL6.7, CL6.8 or VP267 cells). We selected 9 candidate genes as putative predictive/prognostic markers to further explore by RTQ-PCR their expression in ER-positive breast tumor samples from patients who relapsed or not under Tam treatment. Deregulation of expression of 7 genes was significantly associated with tamoxifen failure, relapse-free survival and overall survival. In conclusion, this study allowed the identification of new molecular markers associated with tamoxifen failure, and suggested their clinical potential in the management of ER+ breast cancer.

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The role of alterations in BRCA1, BRCA2, TP53 and ATM genes in sporadic breast tumors

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Background: The tumor suppressor genes BRCA1, BRCA2, TP53 and ATM are involved in maintenance of the genome integrity. They belong to predisposing genes in hereditary breast cancer, however, their role in sporadic breast tumors remains uncertain. The goal in this study was to analyze the role of these genes in tumorigenesis of sporadic breast cancer. Methods: We evaluated genetic material from 71 tumor and corresponding peripheral blood samples of unselected breast cancer patients for germline and somatic mutations in BRCA1, BRCA2, TP53 and ATM genes. Further, we studied promoter methylation and loss of heterozygosity (LOH) in the corresponding loci. The mutation analyses included entire coding regions of the studied genes and were performed using protein truncation test, MLPA and sequencing. Promoter methylation was determined by methylation specific MLPA and bisulfite sequencing. Results: Allelic losses of BRCA1, BRCA2, TP53 and ATM were found in 14/65 (21.5%), 19/69 (27.5%), 23/62 (37.1%) and 15/70 (21.4%) informative tumor samples, respectively. The sequencing revealed two somatic mutations in BRCA1 (2/71; 2.8%) and one inherited (1/71; 1.4%) and nine somatic (9/71; 12.7%) mutations in TP53. The TP53 frameshift mutation c.340-370del31 (p.L114AfsX46) was novel. We failed to detect any alterations in ATM and BRCA2 coding sequences. Promoter methylation has been found only in BRCA1 (2/59; 3.4%) and TP53 (2/59; 3.4%). No gene alterations were found in 22/62 (35.5%) informative tumor samples.Conclusion: The high occurrence of allelic losses suggests the role of analyzed genes in sporadic breast tumorigenesis. However, acquired mutations were common only in TP53 and promoter methylation was identified only two-times in both BRCA1 and TP53. These results suggest that the role of analyzed genes is limited to the subset of sporadic breast tumors and alternative ways of breast tumorigenesis should be considered.Supported by: IGA MZ 9051-3/2006, MSM 002160808

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Screening for inherited mutations in the Czech high risk breast cancer patients - analysis of 400 families

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Background: The role of genetic susceptibility in breast and ovarian cancer has been intensively investigated. In the Czech Republic, 3% to 5% of breast cancer cases are due to inherited mutations in BRCA1 and BRCA2 genes and the risk of breast cancer is also influenced by mutations in other genes. Inherited mutations in low penetrance genes, ATM and CHEK2, are associated with about 2-fold increase in breast cancer risk; germline mutations of TP53 are associated with high risk of early onset breast cancer. The aim of our study was to determine the frequency and types of cancer-predisposing mutations in high-risk breast and ovarian Czech families.

Materials and Methods: We evaluated DNA and RNA samples from 400 breast or ovarian cancer patients. A complete sequence analysis of the BRCA1, BRCA2, ATM and TP53 coding sequences was performed. Large deletions in BRCA1/2 and the most common mutation in CHEK2 (c.1100delC) were detected by multiplex ligation-dependent probe amplification (MLPA); other gene alterations were determined by protein truncation test (PTT) and DNA sequencing.

Results: Of the 400 analyzed families, 117 (29.3%) carried pathogenic mutations, including 86 (21.5%) in BRCA1, 23 (5.8%) in BRCA2, 5 (1.3%) in ATM, 2 (0.5%) in CHEK2 and 1 (0.3%) in TP53. One novel truncating mutation was found in ATM, three in BRCA1 and four in BRCA2 genes. The four most common recurrent mutations in BRCA1 (c.300T>G, c.1806C>T, c.3819_3823del5, and c.5385dupC) explained 70.9% (61/86) of BRCA1 related patients and the c.5385dupC was detected in 47.7% (41/86) of mutation positive women. Five different large deletions in the BRCA1 gene were identified in 9 families (2.3%); at BRCA2 no deletions were detected. Inherited deletions of BRCA1 varied in size (from 2 exons to 17 exons) and appeared among patients diagnosed before 40 years (7/9).

Conclusions: The MLPA ensures a sensitive test for screening of genomic rearrangements. In the Czech Republic, large deletions accounted for 10.5% (9/86) of patients with detected alterations in BRCA1.

Supported by grants: IGA MZ CR NR-9051-3/2006 and MSM0021620808

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Genomic data integration - application to understanding the biology of glioblastoma

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Technological advances has enabled scientists to collect an astonishing quantity of high-quality measurements from various biological process and events, such as gene expression, DNA copy number, miRNA expression, methlyation and so forth. A great challenge now facing the scientists is now how to analyze and integrate such data into a cohesive picture of the molecular state of the cell. We have developed novel methodologies and implemented these in a system called Nexus Copy Number to enable scientist to make such analyzes and integration. In this paper we will outline the process in analyzing large number of glioblastoma samples processed as part of The Cancer Genome Atlas (TCGA) project. This data set provides an ideal showcase for integration of data as it involves data coming from many different array platforms, including Agilent 244K, Affymetrix SNP 6, and Illumina 550K, as well as different data modality spanning copy number changes, mRNA and miRNA regulation and Methylation data. We will demonstrate how regions of genomic change can be identified and correlated with phenotype data. We will then use a novel "hotspot" detection algorithm to identify the regions where multiple genomic events coincide. The genes in these regions are then selected for enrichment analysis against various GO categories to yield possible biological processes that are involved in glioblastoma.