so altogether 18 CupPrint analyses had been carried out. The dropout of 12 cases is explained by paucity of tumor tissue and by insufficient RNA quality, possibly due to long storage time of tissue samples. Results were evaluated and discussed case-by-case on a consensus conference including two independent referees (Folprecht, Buettner). The information yielded by each method was regarded separately and reviewed by clinical experts based on the individual characteristics of the baseline data and clinical course of each patient.
Results. In 11 cases the results of IHC and CupPrint were concordant and matched also the clinical findings. In 1 of these cases, the results would have been beneficial to the patient, as he could have received a more specific chemotherapy. From the remaining 4 samples 1 can not be regarded as CUP anymore, as both IHC and CupPrint strongly favour the diagnosis of serous ovarian cancer, which would also be consistent with the clinical findings. In 2 cases the CupPrint was more concordant with the clinical findings than IHC. In 2 cases IHC was more concordant with the clinical findings than the CupPrint results.
Conclusions. IHC performed centrally led to more informative results than multicenter IHC. IHC and CupPrint microarray testing showed a high grade of concordance. Combination of the results of both methods led to a better definition of the possibly primary tumor, allowing a more specific therapy in some cases.

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Microarray gene expression analysis of human adrenocortical tumours

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Background: Adrenal tumours are common, occurring in 7% of patients over the age of 50 years. Adrenocortical carcinomas (ACCs), however, are rare, with an incidence of two per million population per year. The management of adrenocortical tumours (ACTs) is complex, compounded by the difficulty in discriminating benign from malignant tumours using conventional histology. The identification of a molecular marker which could reliably distinguish between the two groups would be valuable and would lead to improved clinical management of these patients. The aim of this study was to use microarray gene expression analysis to identify molecular markers which would discriminate between ACCs and adrenocortical adenomas (ACAs).
Materials and Methods: RNA was prepared from 6 normal adrenal cortices, 16 ACAs and 12 ACCs. Only samples with an RNA integrity number of 7.5 or greater were used. The samples were hybridised to Affymetrix HGU133plus2.0 genechips. Data analysis was performed with Partek and Affylmgui softwares. Seven genes were selected for validation studies with real time reverse transcription polymerase chain reaction (qPCR). Of these, three genes were also validated by immunohistochemistry (IHC).
Results: Using a cutoff of B > 2 and M > 2 or <−2, 217 genes were found to be significantly differentially expressed between ACCs and ACAs. Of these genes, 120 were unpregulated while 97 were downregulated. On qPCR, all seven candidate genes selected were significantly differentially expressed in ACCs compared to ACAs. All three candidate genes selected for IHC also differed significantly in their protein expression in ACCs when compared to ACAs and normal adrenal cortex.
Conclusion: We identified seven genes which were significantly differentially expressed between ACCs and ACAs using microarray gene expression profiling and confirmed the expression of these genes with qPCR and IHC. With further studies, these genes will provide greater insight into the pathogenesis of ACTs as well as having the potential to be reliable discriminators between ACCs and ACAs.

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Serum adipokine levels in colorectal cancer patients

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Purpose: Leptin, a product of the ob gene involved in the control of food intake and energy expenditure, may act as a potent mitogen and anti-apoptotic cytokine in colon cancer cell lines and promotes the invasiveness of familial adenomatous colonic cells. Adiponectin, in turn, may exert protective actions through its anti-proliferative and anti-angiogenetic effects. Despite a significant amount of in vitro data, direct and convincing evidence about their role in the development of colorectal cancer (CRC) is not available. Thus, the aim of this study was to evaluate the possible associations between leptin, adiponectin and clinicopathological variables of CRC.
Methods: Baseline serum leptin (DBC Inc.), adiponectin (BioVendor Inc.), TNF-alpha (R&D Systems) and carcinoembryonic antigen (CEA, Abbott Labs.) levels were analyzed in 90 patients with histologically diagnosed primary (Stages A: 7, B: 34, C: 19 and D: 13, with a single resectable liver metastasis) or metastatic (liver: 8, peritoneum: 5, lung: 1 and multiple: 3) CRC treated at “Tor Vergata” Clinical Center and followed for a median period of 3 years. The study was performed under the appropriate ethics approvals, and informed consent was obtained from each patient.
Results: Serum leptin and adiponectin levels in patients with CRC were 8.8 ng/ml [median, interquartile range (IQR): 3.7−17.6) and 8.06 μg/ml (IQR: 5.66−9.34). Of interest, median leptin (10.9 ng/ml), but not adiponectin levels of metastatic CRC were higher than those observed in primary CRC patients (7.7 ng/ml, $p = 0.034$). Leptin inversely correlated with adiponectin ($p = 0.002$) and directly correlated with TNF levels ($p < 0.05$) in all patients. In metastatic CRC only the correlation with TNF was retained. Of interest, 47% of non metastatic CRC had leptin levels above the median compared with 71% of metastatic patients ($p = 0.07$). Median follow-up of metastatic CRC patients was shorter (12.6 months) in patients with high leptin levels compared to those with normal levels (21.7 months, $p = 0.07$). Cox proportional hazard regression model including age, sex, leptin, adiponectin, TNF and CEA levels showed that leptin was an independent predictor for overall survival in metastatic CRC (Cox-Mantel test 2.03, $p = 0.042$).
Conclusions: These results suggest that serum leptin levels might have a role in the biology of CRC and may be regarded as a useful prognostic indicator in metastatic disease.

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Cetuximab-induced thymidylate synthase inhibition is associated with EGFR expression

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The monoclonal antibody cetuximab directed against the epidermal growth factor receptor (EGFR) is an attractive agent for targeted therapy in advanced colorectal cancer (CRC), especially when combined with 5-fluorouracil (5-FU)-based chemotherapy. However, the mechanisms of cetuximab activity as chemosensitizer remain poorly understood. Using proteome-fluorescence-based technology we found that cetuximab is able to suppress the expression of thymidylate synthase (TS) which is involved in the mechanism of 5-FU action. Caco-2, HRT-18, HT-29, WiDr and SW-480 CRC cells were found to express different levels of EGFR. SW-620 was used as EGFR-negative cell line. Only in EGFR-expressing cells cetuximab is able to inhibit TS expression. Combination treatment with cetuximab and 5-FU revealed an antitumor response that is closely correlated with the level of EGFR expression. Moreover, no correlation was seen between constitutive TS expression, cetuximab-induced TS downregulation and response either to 5-FU alone or in combination with cetuximab. We demonstrated that only high level of EGFR expression is important for the synergistic effects between cetuximab and 5-FU in the investigated cell lines and may represent a potential marker of response to cetuximab/5-FU-based chemotherapy in patients with advanced colorectal cancer.
This study was supported in part by the Austrian National Bank, project No. 11681, and the Austrian Cancer Society/Tyrol.

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Prognostic significance of Ki-67 expression in sporadic desmoid tumor

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Background: This study is conducted to evaluate the treatment outcome of sporadic desmoid tumor patients, and its association with Ki-67 expression.
Materials and Methods: From April 1999 to July 2005, 44 patients were pathologically diagnosed with primary sporadic desmoid tumor at Seoul National University Hospital. Among these, we analyzed the medical records of 38 patients and performed immunohistochemical staining for Ki-67 expression. Tumors were located in extra-abdominal areas (23 cases), abdominal walls (11 cases), and intra-abdominal areas (4 cases). Clinical or pathologic tumor sizes ranged from 1 to 13.5 cm in largest linear dimension

(median, 6 cm). Thirty-seven patients were treated with surgery. Surgical margins were negative in 19 patients, microscopically positive in 9 patients, and grossly positive in 9 patients. One patient received definitive radiation therapy. The Ki-67 expression was positive (>5%) in 9 among 38 cases (24%).
Results: The median follow-up period was 34 months (range, 7−75). Five patients developed local progressions and 9 experienced local recurrences. The 3-year disease-free survival rate and the 3-year progression-free survival rate were 54% and 55%, respectively. Positive Ki-67 expression ($p = 0.036$), tumor size more than 5 cm ($p = 0.021$), debulking surgery ($p = 0.021$), and extra-abdominal location of tumor ($p = 0.004$) were associated with poor disease-free survival with significance.
Conclusions: The current data suggests that patterns of Ki-67 expression are also a prognostic factor in addition to the gross anatomy in the sporadic desmoid tumors. Thus patterns of Ki-67 expression can be used as criteria for adjuvant therapy after surgery.

Table. Prognostic factors for disease-free survival

Variables		No. of patient	3Y DFS[a]	p-value
Location	Extra-abdominal	23	27%	0.004[b]
	Abdominal wall	11	100%	
	Intra-abdominal	4	50%	
Tumor size	<5 cm	10	90%	0.021[c]
	⩾5cm	28	37%	
Surgery	Wide excision	26	67%	0.021[c]
	Debulking	11	33%	
Surgical margin	Negative	19	69%	0.181[c]
	Positive	18	44%	
Ki-67 expression	Negative	29	66%	0.036[c]
	Positive	9	0%	

[a]disease free survival; [b]One-way ANOVA. Extra-abdominal vs abdominal wall; [c]Log-rank test

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Association of genetic polymorphisms with survival in Japanese pancreatic cancer patients treated with gemcitabine

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Background: Gemcitabine (Gem) is an anti-cancer drug effective against solid tumors. Nucleoside transporters including SLC29A1, cytidine deaminase (CDA), and deoxycytidine kinase (DCK) are involved in the transportation, detoxication or activation of Gem. We previously reported that a non-synonymous single nucleotide polymorphism (SNP), CDA 208G>A (A70T), affected the pharmacokinetics and toxicity of Gem [1]. Therefore, we investigated the effects of genetic polymorphisms and background factors on survival in pancreatic cancer patients treated with Gem.
Materials and Methods: The study involved 76 Japanese patients with stage IV pancreatic cancer, a subset of the patients reported previously [1], receiving Gem monotherapy and no previous radiation therapy. Plasma CDA activity was measured using Gem or cytidine as a substrate. Polymorphisms of the CDA, DCK and SLC29A1 genes were detected by dideoxy sequencing using genomic DNA obtained from peripheral blood leukocytes. The log-rank test or Cox proportional hazard models were applied for survival analyses. The ethics committees of the National Cancer Center and the National Institute of Health Sciences approved this study, and written informed consent was obtained from each patient.
Results: CDA 208G>A, which was previously reported to lead to reduced Gem clearance [1], was associated with prolonged survival (median for GG and GA; 165 and 606 days, $P = 0.042$), while an intron SNP of CDA, IVS1+37G>A, was associated with reduced survival (median for GG+GA and AA; 178 and 86 days, $P = 0.012$). A non-synonymous SNP of DCK, 364C>T (P122S), showed strong association with reduced survival (survival for CC and CT; 178 and 60 days, $P = 0.0028$). The allele frequency of DCK 364C>T was 0.061 in our study. No genetic polymorphisms of SLC29A1 showed any significant association with survival. Performance status, CA19−9, CRP, and plasma CDA activity also showed significant effects on survival ($P < 0.05$ for all). A multivariate Cox proportional hazard model suggested that CA19−9, CRP, the intron SNP of CDA, and DCK 364C>T are major factors determining the prognosis of pancreatic cancer patients receiving Gem monotherapy.
Conclusions: These observations suggest that genetic polymorphisms involved in the activation and detoxication of Gem, as well as some tumor markers, can be useful indicators of prognosis in patients with pancreatic cancer receiving Gem monotherapy.

References

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Epidermal growth factor (EGF) +61 A/G functional genetic polymorphism influences disease-free interval in androgen blockade treated prostate cancer patients

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Background: Most patients with Prostate Cancer (PCa) will evidence sometime along the course of their disease resistance to androgen-blockade therapy (ABT), emerging an androgen-independent state. EGF activates several intracellular pathways after binding to homo- (EGFR1) or hetero-dimmer receptors (EGFR1-HER2), ultimately leading to proliferation, differentiation and tumorigenesis of epithelial cells.
The EGF-EGFR/HER2 pathway seems to assume special relevance in androgen-independent Prostate Cancer state (AIPCa). EGF role in PCa oncobiology and the high frequency of AIPCa support the rationale for studying its potential as a molecular marker in prognosis and further evaluate pharmacogenomic application in ABT. Recently, a single nucleotide polymorphism (SNP) A/G in +61 locus of EGF gene was described, in which peripheral-blood mononuclear cells from A homozygous carriers expressed significantly less EGF mRNA compared to G allele carriers (AG/GG).
Materials and Methods: In the present study, EGF+61 A/G polymorphism detection was performed through Polymerase Chain Reaction – Restriction Fragment Length Polymorphism procedures in PCa patients submitted to ABT ($N = 124$), and in healthy controls without cancer evidence ($N = 152$).
Results: In the recessive model, genotype frequencies were similar between both groups (PCa group, AA = 27.4%, AG/GG = 72.6%; Control group, AA = 37.5%, AG/GG = 62.5%), without significant risk for being diagnosed with PCa in G-allele carriers (Odds Ratio, $OR = 1.59$, $P = 0.076$). Furthermore, there is an increased risk in AG/GG carriers for being diagnosed with a high grade PCa (Gleason ⩾7), compared to the control group ($OR = 2.49$, $P = 0.008$).
Disease free interval is significantly lower in AG/GG carriers compared with AA (mean±SEM, 32.1±7.1 and 71.3±13.2, respectively, $P = 0.008$). Kaplan Meier survival curves analysis and Log Rank test (Mantel-Cox) further support the influence of EGF+61 A/G polymorphism in disease free interval cumulative probability ($P = 0.018$).
Conclusions: In this sample, G-allele carriers have an increased risk for being diagnosed with high-grade disease and for developing precociously resistance to ABT. The results suggest that this EGF functional polymorphism may contribute to the establishment of a prognostic and predictive molecular profile in AIPCa patients submitted to ABT and support the involvement of EGF in an alternative pathway for tumor progression in androgen-independent prostatic tumors.

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An alternative splice variant of PIK3CD is common in neuroblastoma, colorectal and ovarian cancer

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Background: Alterations of the PI3K-Akt pathway in human cancers are very common. PIK3CD encodes p110δ, a catalytic subunit of type I phosphatidyl-inositol-3′ kinase (PI3K). The gene PIK3CD resides in chromosome region 1p36.2, a region commonly deleted in a variety of human cancers indicating that there is a gene in this region with a tumour suppressor function.
Material, methods & results: We have discovered an alternative splicesite in intron 5 of PIK3CD, resulting in an extra 163 bp insertion in the mature mRNA causing a frame shift and an early termination of the protein (302 aa compared to 1045 aa in p110δ). This splice variant encodes a protein that comprises a regulatory p85-binding domain but no catalytic domain. We can by cotransfection show that the protein resulting from the splice variant of PIK3CD colocalises with p85 in aggregates in the cytoplasm, whereas p110δ colocalises with p85 evenly distributed in the cytoplasm.