applied LOOCV, we were able to correctly predict the tumor behavior (T-level down-sizing) in 83% of patients ($p = 0.02$). We then observed that after a median follow-up of 34.5 months, all five patients with metastatic disease belonged to the group of non-responders. We again applied LOOCV and correctly predicted all five patients with recurrence. Furthermore, all 11 patients who were predicted to remain cancer free showed no evidence of recurrence.

Conclusion: Our results suggest that pretherapeutical gene expression profiling may assist in response prediction of rectal adenocarcinomas to preoperative CT/RT and in prediction of disease free survival if validated in larger independent studies.

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P68. GENE EXPRESSION SIGNATURE OF COLORECTAL CARCINOGENESIS

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Background: Colorectal carcinomas develop through the sequential stages of increasing morphological and molecular alterations. While the correlation of tumor phenotype with associated genomic alterations has been firmly established, the correlation with global gene expression profiles is less.

Methods: We analyzed tissue samples from 36 patients to identify sequential alterations of the genome and transcriptome that define the transformation of normal epithelium and the progression from adenomas to invasive disease.

Results: Comparative genomic hybridization (CGH) revealed patterns of stage specific, recurrent genomic imbalances. Gene expression analysis on 9K cDNA arrays identified 58 genes to be differentially expressed between normal mucosa and adenoma, 116 genes between adenoma and carcinoma, and 158 genes between primary carcinoma and liver metastasis ($p < 0.001$). Our analysis revealed a direct correlation of chromosomal copy number changes with chromosome-specific average gene expression levels.

Conclusion: Increasing genomic instability, a recurrent pattern of chromosomal aberrations and a specific gene expression pattern correlate with distinct stages of colorectal cancer progression. Chromosomal aneuploidies exert a direct effect on average expression levels of the genes residing on the aneuploid chromosomes, thereby contributing to a massive deregulation of the cellular transcriptome. The identification of novel genes and proteins might deliver relevant molecular targets for diagnostic and therapeutic interventions.

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P69. Ki-67 AUTO-ANTIBODIES IN COLORECTAL CANCER PATIENTS

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Background: Antibodies against the human nuclear antigen pKi-67 (Ki-67, MIB-1) are routinely used in oncology as immune-histological proliferation marker. PKi-67 is exclusively expressed in all active phases of the cell cycle (G1,S, G2, Mitosis). The Ki-67 index (relative number of positive stained nuclei) serves as an independent prognostic marker for certain tumor entities. We investigated whether colorectal cancer patients express auto-antibodies against pKi-67 and whether this has a prognostic relevance.

Methods: Auto-antibodies were detected by Western blot stainings from SW480 nuclear extracts with 36 pre- and 65 post-operative sera of colorectal cancer patients' sera. Sera of 20 voluntary healthy donors served as negative control. The same samples were simultaneously tested for p53 auto-antibodies.

Results: Thirteen percent of the sera proved to be positive for pKi-67 auto-antibodies while the control sera were completely negative. p53 auto-antibodies could be found in 53% of the patient sera. 75% anti-pKi-67 positive samples were also anti-p53 positive. For both antigens we found less positive antibodies in post-operative sera (pKi-67: 9%; p53: 42%) than in pre-operative sera (pKi-67: 19%, p53: 61%). There was, however, no significant correlation between pKi-67 positive sera and tumor stage (I: 13%, II: 4%; III: 23%; IV: 13%), grading or patient's prognosis. Remarkably, there is a significant ($p = 0{,}023$) correlation of pKi-67 positive sera of colon cancer patients (77%) in comparison to rectal cancer patients (60%).

Conclusion: PKi-67 auto-antibodies could be diagnostically valuable in the early detection of neoplasia and could be used as potential markers for recurrent or metastatic disease.

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P70. TRANSCRIPTIONAL AND MOLECULAR REGULATORS OF THE UROKINASE-RECEPTOR-(u-PAR)-GENE: FIRST ANALYSIS OF INDEPENDENT PROGNOSTIC RELEVANCE IN RESECTED COLORECTAL CANCER

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Purpose: Prognostic studies on transcription factors acting at specific promoter elements have never been performed so far. In previous studies we showed that the invasion-related gene *u-PAR* is regulated especially via an AP-2/Sp1(-152/-135)-, and an AP-1-promoter motif(-190/-171), mediating *u-PAR*-induction by

K-Ras and Src. The present study was performed to obtain first evidence for a prognostic relevance of transcription factors differentially bound to the *u-PAR*-promoter, and their molecular inducers, in colorectal cancer.

Patients and Methods: Tumors/normal tissues of prospectively followed 92 patients were analyzed for Src-activity, *K-ras*-mutations, and transcription factor binding to both *u*-PAR-promoter motifs (in vivo-gelshift, kinase-assay, PCR).

Results: Kaplan–Meier analysis (Mantel-Cox) showed a significant correlation between elevated binding of Sp1/Sp3 to region -152/-135 ($p = 0.002$, $p = 0.006$), the combinations of Sp1/AP-2 and Sp1/AP-1 binding to both motifs ($p = 0.010$, $p = 0.005$), and Sp1-binding/high Src-protein in tumors ($p < 0.001$), with poor survival. Survival risk increased with the number of bound transcription factors, the binding of three transcription factors to both *u-PAR*-promoter motifs defining a high risk group for survival ($p = 0.021$). In multivariate analysis, elevated binding of Sp1, or combinations of Sp1/AP-2-binding, Sp1/AP-1-binding, or Sp1-binding/high Src, were new and independent prognostic parameters besides surgical curability, and UICC. A first molecular staging model (CART) could define new high-risk groups from transcription factors bound to specific promoter motifs.

Conclusion: This is the first study to demonstrate an independent impact on clinical prognosis by transcription factors acting at specific promoter elements of an invasion-related gene, and mediating specific signaling, in colorectal cancer. Such analysis can select new high-risk subgroups for individualized targeting approaches.

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P71. TAMOXIFEN THERAPY STABILIZED CELL MEMBRANE PERMEABILITY FOR LDH IN PBL OF BREAST CANCER PATIENTS

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LDH is an intracellular enzyme that reflects important biological processes such as metabolic state, activation status or malignant process. The spontaneous LDH release from peripheral blood lymphocytes (PBL) of patients with breast cancer, lymphoma, head and neck, and malignant melanoma is associated with advanced clinical stage, bulky tumor mass, reflecting cell membrane alterations, possible by TNF. As breast cancer is a hormone-dependent tumor, in this study we investigate in vitro, effect of sex steroid hormone, 17-β estradiol, on membrane characteristics of PBL in 37 breast cancer patients relevant to clinical stage, menopause, Karnofski index before and undergoing tamoxifen therapy. We found that 17-β estradiol induced an increase in spontaneous LDH release activity of PBL at dose and time dependent manner in healthy controls with no changes in PBL cell cycle phase distribution (determined by Flow cytometry) and total intracellular LDH activity (determined from separated lymphocytes after lysis by ultrasound). Spontaneous LDH release activity from PBL of breast cancer patients with metastatic disease, undergoing tamoxifen therapy show significant decrease of spontaneous LDH release activity compared to untreated patients. In vitro treatment with 17-β estradiol of PBL had no effect on increase of spontaneous LDH release activity in breast cancer patients undergoing tamoxifen therapy, contrary to healthy control PBL. Based on these results we show for the first time that PBL of breast cancer patients undergoing anti-estrogen therapy show cell membrane stabilization. We conclude that determination of spontaneous LDH release activity in breast cancer patients is valuable in diagnosis and monitoring of the therapeutic effect of standard and anti-estrogen therapy.

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P72. PREDICTION OF RESPONSE TO PREOPERATIVE CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER

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Background: Preoperative chemotherapy is increasingly employed in operable breast cancer. Response or resistance cannot be predicted and treatment time may be lost if an ineffective regimen is selected. At our institute, prospective clinical trials were designed to identify gene expression patterns predicting response to preoperative chemotherapy in breast cancer patients.

Methods: Patients with operable or locally advanced breast cancer were either included in a randomized phase II study or received neoadjuvant chemotherapy off protocol. Treatment regimens were chosen dependent on the HER2 status of the tumor and response was evaluated by MRI. From all patients 14G core needle biopsies were taken before treatment and total RNA was isolated. Amplified mRNA was labeled and hybridized to 35k human oligo microarrays from our microarray facility.

Results: From 48 patients good quality RNA from biopsy tissue with more than 50% tumor cells was isolated. In a training set containing 11 pathological complete remissions (pCR) and 9 non-responders (NR) we were able to separate these groups by using 20 genes in a supervised classification with Euclidian distance and a 9-step cross validation. These results could be validated in an independent set of 11 samples (6 pCR, 5 NR). From 10 out of 11 samples, response status could be predicted correctly, independent from the treatment regimen administered. Although ER-positive tumors have a lower pCR rate than ER-negative ones, the steroid hormone receptors were not present in the classifier.

Conclusions: Neoadjuvant chemotherapy studies provide a good setting to identify gene expression profiles associated with response to specific regimens. Large series of patients (>100) are required to obtain reliable and reproducible gene expression signatures.

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