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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