genetic markers involved in the development and progression of breast cancer. The aim of our present study was to determine the association of combinations of TGFB1−509C>T (rs1800469) and IL10−592C>A (rs1800872) genotypes with the clinicopathological parameters and response to neoadjuvant chemotherapy.
Materials and Methods: Case patients were newly diagnosed breast cancer patients T1−4N0−2M0 (n = 186; age from 20 to 79 years) which were cared in Tomsk Cancer Research Institute. The healthy women (n = 190; age from 30 to 75 years) from Western Siberian region were used as the control group. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism method.
Results: Frequency of combination TGFB1(C/C) and IL10(C/A) genotypes was significantly higher in non-aggressive phenotype of breast cancer as defined by the absence of axillary lymph node metastasis ($p < 0.05$). A significant difference in distributions of genotypic combinations of TGFB1(C/T) and IL10(C/C) between women with multicentric and unicentric breast cancer was found (52.6% and 27.3% respectively; $p < 0.02$). We have revealed that high frequency of TGFB1(C/C) and IL10(C/A) variants was observed in patients with basal (hormone receptor-negative and HER2-negative) tumor against breast cancer patients with luminal subtype tumor (estrogen receptor-positive) ($p < 0.0004$). In contrast, 10.2% of luminal subtype patients were found to carry the combination of homozygous TGFB1(T/T) and IL10(C/C) genotypes compared to 2.3% of basal subtype patients ($p < 0.09$). In addition, this combination of TGFB1(T/T) and IL10(C/C) genotypes was related to a favorable response to neoadjuvant chemotherapy ($p < 0.05$).
Conclusion: Our data suggest that the genetic variants of TGFB1−509C>T and IL10−592C>A are associated with the progression of breast cancer. The combination of homozygous TGFB (T/T) and IL10(C/C) variants may be a potential prognostic marker for response to neoadjuvant chemotherapy.

PP61

Detection of breast cancer markers in serum by surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-TOF-MS)

D. Boehm[1], A. Lebrecht[1], K. Keller[1], R. Schwirz[1], M. Schmidt[1], F. Grus[2], H. Koelbl[1]. *[1]Department of Obstetrics and Gynaecology, University Medical Center Mainz, Germany; [2]Experimental Ophthalmology, Department of Ophthalmology, University Medical Center Mainz, Germany*

Background: Breast cancer is one of the most frequent and deadly cancers worldwide. Although the survival of patients has increased over the last decades, many patients die from metastatic relapse. Progresses in screening or early diagnosis will improve survival of breast cancer and reduces breast cancer-related morbidity. Although breast cancer biomarkers offer a promising means of detecting this disease at the earliest and most treatable stages there has never been any good serum tumor markers for early detection. The purpose of this study was to identify and evaluate a proteomics approach to search for new biomarkers in serum of breast cancer patients.
Materials and Methods: Blood samples of 50 women with breast cancer (CA) and 50 healthy women (CTRL), matched to the age, were drawn prior to surgery. We used SELDI-TOF-MS for protein profiling with three different active surfaces of the protein chips: cationic exchanger (CM-10), hydrophobic surface (H50) and a strong anion exchange surface (Q10) with different binding properties. Data were analyzed by multivariate statistical techniques and artificial neural networks.
Results: SELDI-TOF-MS could discriminate between serum of breast cancer patients and healthy women. We could generate a statistic significant ($p < 0.001$) panel with 15 biomarkers resulting of multiple peaks with different molecular weights. The diagnostic pattern could differentiate CA from CRTL with specificity of 77% and sensitivity of 85% in serum.
Conclusion: In this study a new biomarker panel in serum was successfully generated to allow breast cancer patients to be discriminated from healthy women. This promising approach provides a high sensitivity and specificity by a less invasive method similar to mammography that is used in screening programs. Therefore this study could also exemplify SELDI-TOF-MS as a potential screening method to detect breast cancer and for high-throughput biomarker discovery.

PP97

The human carcinoembryonic antigen (CEA) predicts therapeutic response towards VEGF-targeting therapies in colorectal cancer

K. Brämswig[1], G.W. Prager[1], A. Martel[1], M. Pöttler[1], G. Heinze[2], B.R. Binder[3], T. Brodowicz[1], G. Kornek[1], W. Scheithauer[1], C.C. Zielinski[1]. *[1]Department of Medicine I and Cancer Center, Medical University of Vienna, Austria; [2]Core Unit for Medical statistics and Informatics, Medical University of Vienna, Austria; [3]Department of Vascular Biology and Thrombosis Research, Centre for Bio-Molecular Medicine and Pharmacology, Medical University of Vienna, Austria*

Background: Angiogenesis represents a key element in the pathogenesis of malignancy. Studies from our laboratory have shown that carcinoembryonic antigen (CEA) functions as a major mediator of angiogenesis apart and independently from VEGF. As patients with metastatic colorectal cancer (mCRC) present with various plasma levels of CEA, we have analyzed whether the efficacy of anti VEGF treatment with bevacizumab was dependent upon plasma CEA levels.
Materials and Methods: To analyze a so far undescribed clinical relevance of CEA in angiogenesis, we retrospectively analyzed relevant data of 271 patients with mCRC, who were treated with bevacizumab plus chemotherapy consisting of fluorouracil and leukovorin or capecitabine in combination with oxaliplatin (FOLFOX, XELOX) or irinotecan (FOLFIRI, XELIRI) (n = 145) or − as control − chemotherapy (FOLFOX or FOLFIRI) plus cetuximab (n = 126). It was analyzed whether baseline CEA correlated with the overall response rate (ORR) consisting of complete remission (CR), partial remission (PR) or stable disease (SD) for at least three months according to RECIST (Response Evaluation Criteria In Solid Tumors). Patient cohorts according to CEA plasma levels were generated: <5 ng/ml (n = 36), 6−30 ng/ml (n = 47), 31–100 ng/ml (n = 26), >100 ng/ml (n = 36).
Results: Baseline CEA plasma levels inversely correlated with therapeutic response in patients receiving bevacizumab-based treatment (P for trend <0.001: OR = 0.52, 95% CI 0.36, 0.74). No such association was found in patients receiving cetuximab-based therapy. ORR in patients with mCRC receiving bevacizumab-based treatment in dependence upon plasma CEA levels can be seen as follows: with a CEA plasma level of <5 ng/ml the ORR was 92.7% (No. of Pat. 41), CEA plasma level 6−30 ng/ml the ORR was 80.4% (No. of Pat. 46), CEA plasma level 31–100 ng/ml the ORR was 60.9% (No. of Pat. 23) and CEA plasma levels >100 ng/ml the ORR was 59.0% (No. of Pat. 39).
Conclusion: Bevacizumab has been introduced as a potent anti-angiogenic therapeutic tool in the management of mCRC. CEA plasma levels might represent an important predictor of treatment response to bevacizumab-based treatment.

PP74

Detection of K-RAS oncogene mutations using PCR and pyrosequencing

L. Cai, L. Kam-Morgan, M. Eisenberg, S. Anderson. *Laboratory Corporation of America, USA*

Background: Mutations in the K-RAS oncogene are frequently found in human cancers, including colon cancers, lung cancers, pancreatic cancers, and other cancers of the GI tract. In these cancers, K-RAS mutations are often associated with patient prognosis or drug response. Several recent studies have shown that K-RAS mutation status is an important biomarker of response to monoclonal antibody based therapies, i.e., Erbitux™ (cetuximab) and Vectibix™ (panitumumab), which are targeted against the EGFR cell surface receptor. A variety of methods exist to detect K-RAS mutations in tumor samples including both PCR and sequencing based platforms. In this evaluation we have examined the analytical performance of both a PCR based method (DxS) and a pyrosequencing platform (Biotage).
Materials and Methods: DNA was isolated from formalin-fixed, paraffin-embedded (FFPE) specimens and analyzed for mutations in the K-RAS gene using either the ARMS (amplification refractory mutation system) in combination with a Scorpion primer or PCR and pyrosequencing technologies. The ARMS assay detects the 7 most common alterations in codons 12 and 13 of the K-RAS gene. The pyrosequencing method using the PyroMark Q24 software quantifies mutations in codons 12, 13 and 61.
Results: Intra-assay and inter-assay reproducibility for both methods was performed on pooled isolations from a series of tissues previously shown to have both wild-type and mutant versions of the K-RAS gene. These studies showed 100% reproducibility of both assay platforms. The accuracy of the ARMS assay was assessed using a series of previously characterized tissue samples and cell lines. The ARMS assay showed 100% concordance for the samples evaluated. For the pyrosequencing assay a series of sample previously characterized by the ARMS assay were evaluated

and shown to be 100% concordant. Dilution experiments demonstrated an analytical sensitivity of 1% mutant DNA in a background of normal gene sequence and the pyrosequencing method showed an analytical sensitivity of 2−5%.
Conclusion: The experiments performed in these studies showed that both the ARMS and pyrosequencing methods could detect K-RAS mutations in formalin-fixed paraffin-embedded tissues in a reproducible and robust fashion. The assays showed comparable analytical sensitivity for the common codons examined.

PP109

Comparability and reliability of different approaches to obtain gene expression measurements from formalin-fixed, paraffin-embedded breast cancer samples

M. Callari[1], V. Musella[1], P. Miodini[1], L. De Cecco[2], M.G. Daidone[1], V. Cappelletti[1]. [1]*Fondazione IRCCS Istituto Nazionale Tumori, Italy;* [2]*IFOM, Italy*

Background: The use of formalin-fixed, paraffin-embedded (FFPE) tumour samples, a valuable resource for gene expression studies on archival specimens or on limited amount of tissue as that obtained from a core biopsy, is no longer restricted by the poor quality of the extracted RNA. New technologies allow the performance of reliable gene expression quantification from FFPE samples. The choice of the technique to use for such studies on FFPE samples depends mainly on practical issues such as the available amount of sample and the biological end-point of the study. However it is very important to understand if gene expression information obtained by different methods is comparable.
Materials and Methods: We selected 16 breast cancer samples for which FFPE and snap-frozen tissues were available. Gene expression data for the FFPE tissues were obtained using the microarray-based cDNA-mediated annealing, selection, extension and ligation (DASL®) assay from Illumina, the QuantiGene® Plex 2.0 Reagent System from Panomics and qPCR. Gene expression profiles were also obtained from high quality RNA drawn from snap-frozen tissues (HumanHT-12® Expression BeadChip, Illumina). Frozen tissues were also analysed using the QuantiGene® Plex 2.0 Reagent System and qPCR.
Results: After adequate pre-processing of data, we used a Pearson correlation analysis to assess DASL® and QuantiGene® reproducibility and intra-assay discrepancy when using frozen or FFPE derived RNA. We also correlated data for the same genes derived from different methods in order to elucidate inter-assays differences. Finally using available patho-biologic data of the samples and hierarchical clustering methods, we verified the biological reliability of results. Both DASL® and QuantiGene® assays provided highly reproducible and biologically reliable data when compared with either whole genome and qPCR expression measurements, nevertheless we found differences related on sensibility and dynamic range.
Conclusion: These data, together with technical issues, such as amount of material required and number of measured genes, can provide guidance for the choice of the strategy to apply in studies involving gene expression analysis of FFPE samples.

PP108

Genes associated to development of distant metastases in node negative breast cancer patients

V. Cappelletti, M. Callari, L. De Cecco, P. Miodini, V. Musella, L. Sensi, M.L. Carcangiu, S. Veneroni, M.G. Daidone. *Fondazione IRCCS Istituto Nazionale Tumori, Italy*

Background: In the group of node-negative breast cancer patients patients, about 30% will develop distant metastasis. Adjuvant treatments used for high-risk patients are poorly target-oriented and are characterized by high toxicities. Linking the risk prediction to specific biological mechanisms would therefore improve the ability to eventually plan target-oriented treatments
Materials and Methods: 63 tumors from women with resectable primary node-negative breast cancer, who developed distant metastases within 5 years from surgery and 64 tumors from women free of distant metastases for at least 5 years, were subjected to whole genome profiling. Differentially expressed (DE) genes among patient groups were investigated by significance analysis of microarrays
Results: In the 104 women with ER+ tumors 113 DE probes corresponding to 101 genes were found between primary tumors from women who developed metastases compared to those from women disease-free (FDR = 0.34), while in the 23 ER− primary tumors 594 probes were DE in two subsets, with a FDR = 0.49. Interestingly, in ER+ samples, at the same FDR level (0.34) a higher number of DE probes was found by separate analysis according to metastatic site (629 DE probes for bone, 707 for lung and 656 for liver metastases).
Clusters of correlated genes ($r > 0.40$) were identified and interrogated for biological function Compared to primaries from metastasis-free women, ER+ primary tumors from patients developing distant metastases over-expressed a cluster of interferon-related genes (IFN). In the 19 tumors from women with bone metastases beside IFN gene also a set of immunoresponse-related genes was over-expressed while extracellular matrix/cell adhesion genes and morphogenesis-related genes were down-regulated. In women developing lung metastases (7 cases) IFN-related and immunoresponse genes behaved oppositely and were down-regulated compared to disease-free controls, suggesting a different molecular mechanism promoting distant spread according to the site of metastatization.
In the 23 ER− primaries, genes related to glucoronization, morphogenesis and immunoresponse were up-regulated in patients developing distant metastases.
Conclusion: Metastatic dissemination can be fostered by the ability of tumors to recruit immune cells promoting an inflammatory environment which favours distant spread. Definition of involved tumor features would allow 1) prediction of distant spread risk and 2) identification of treatment targets.

PP111

Examination of the expression profiles of PGIS and TXS in NSCLC: Regulation of tumor cell growth and invasive potential

M.-C. Cathcart[1], K. Gately[2], E. Kay[3], J.V. Reynolds[1], K.J. O'Byrne[2]. [1]*Trinity College Dublin, Ireland;* [2]*St. James's Hospital, Dublin, Ireland;* [3]*Beaumont Hospital, Dublin, Ireland*

Background: Prostacyclin Synthase (PGIS) and Thromboxane synthase (TXS) metabolize the cyclooxygenase product, prostaglandin H (2), into prostacyclin (PGI2) and thromboxane (TXA2) respectively. PGIS over-expression inhibits cancer growth in a murine model, while TXS over-expression has the opposite effects. TXS over-expression has been reported in a number of cancers and is associated with a poor prognosis. The aim of this study was to determine the individual roles of these enzymes in NSCLC.
Materials and Methods: Stable cell lines over-expressing PGIS and TXS were generated and their effect on tumor cell survival was examined (BrdU, FACS, Invasion Assay). PGIS and TXS expression were examined in human lung tumors and matched normal controls by western analysis and IHC. In a separate study, a 200-patient NSCLC TMA was generated and stained for TXS and PGIS expression. Staining intensity was correlated with clinical parameters and Kaplan-Meiers survival curves were constructed. Cell growth was examined in NSCLC cell lines following selective TXS inhibition. Apoptosis was assessed by High Content Screening (HCS) and validated by DNA laddering and Cell Death ELISA.
Results: Tumor cells over-expressing PGIS grew significantly slower than controls, were less invasive, and more sensitive to apoptosis following serum-starvation. In contrast, over-expression of TXS resulted in opposing effects. Examination of PGIS/TXS expression profiles revealed PGIS to be down-regulated/absent in tumor protein samples relative to normal, while TXS was up-regulated in tumors. TMA analysis revealed TXS expression to be significantly higher in adenocarcinoma tumor tissue, relative to squamous and in females, relative to males. A direct contrast in PGIS expression profile was observed, with significantly reduced expression in adenocarcinoma, relative to squamous and in females, relative to males. No correlation between TXS expression and patient survival was observed. Selective TXS inhibition significantly reduced tumor cell growth and increased apoptosis.
Conclusion: Overexpression of TXS increased proliferation and invasiveness of NSCLC cells, while PGIS overexpression had contrasting effects. Expression patterns of these enzymes are altered in NSCLC. The balance in PGIS/TXS expression may underlie the pathogenesis of NSCLC. While TXS does not appear to be prognostic, it may be a potential therapeutic target in NSCLC. In contrast, PGIS over-expression may be a novel strategy for chemoprevention studies.

PP86

Sister chromatid exchanges as marker of genomic instability in familial breast cancer patients

P. Chiarappa, M. Bruno, C. D'Amico, N. Muscaridola, R. Daprile, A. Paradiso, A. Mangia. *National Cancer Institute, Giovanni Paolo II, Italy*

Background: Cancer predisposition is correlated with spontaneous chromosomal instability. The aim of the present study was to determine whether there are an increased in number of SCE in peripheral blood lymphocytes of familial breast cancer patients compared to sporadic ones.
Materials and Methods: SCE were evaluated in 29 familial breast cancer patients with one first degree relative with a history of breast and/or ovarian