of VHL function increases the expression of several growth factors like VEGF, PDGF and TGF-a. The activity of these factors is associated with tumour angiogenesis, growth and progression. Multitarget kinase inhibitors such as Sunitinib and Sorafenib, focusing on the inhibition of the involved pathways, constitute the current gold standard in therapy. However, non-responders and side effects suggest that our knowledge about the affected signal networks in renal cancer is still incomplete.
The aim of this proteome project is to analyze human kinases (kinome) as major and druggable signaling components in renal cancer patients systematically. Both protein regulation and site-specific phosphorylation, signifying their activity status, were comparatively examined by quantitative peptide sequencing (LC-MS/MS): Tumour samples and "healthy" counterparts dissected from nephrectomies were used as starting material to affinity purify more then 150 kinases by chemical proteomics. iTRAQ^{TM} peptide labelling of the kinase-enriched fractions in combination with a novel statistical validation method allowed the detection of RCC-associated alterations. In addition to already known cancer-related proteins this approach suggests novel kinases that have to be considered for diagnosis and as potential drug targets.

178 Development of a novel PEG-DOX-E-[c(RGDfK)2] conjugate for avb3 integrin-targeted cancer therapy

D. Polyak[1], C. Ryppa[2], F. Kratz[2], R. Satchi-Fainaro[1]. [1]*Tel Aviv University, Physiology and Pharmacology, Tel Aviv, Israel*, [2]*Tumour Biology Center, Macromolecular Prodrugs, Freiburg, Germany*

Background: Targeting drugs that will affect selectively the tumour site is of great challenge and thus has become a critical issue while designing an anticancer drug. Doxorubicin (DOX) is extensively used in cancer therapy; however, it is cardiotoxic in cumulative doses and chemoresistance can evolve with its prolonged use. Conjugation of a chemotherapeutic agent with a water-soluble polymeric carrier prolongs its circulation time, promotes its accumulation at the tumour site due to the enhanced permeability and retention (EPR) effect and prevents the drug from extravasating into healthy tissues. We synthesized a PEG-DOX-E-[c(RGDfK)$_2$] conjugate which actively and selectively targets endothelial and tumour cells overexpressing $\alpha_v\beta_3$ integrin.
Methods: a_vb_3 cell surface expression was determined by flow cytometry. The fluorescent properties of doxorubicin were utilized to follow the cellular uptake of PEG-DOX-E-[c(RGDfK)$_2$]. The cytotoxicity profile of the conjugate was assessed by MTT assay. The ability of PEG-DOX-E-[c(RGDfK)$_2$] conjugate to overcome DOX-resistance was determined by cytotoxicity assay on M109 sensitive and resistant murine lung carcinoma cells. The anti-angiogenic properties of our conjugate were evaluated on human umbilical vein endothelial cells using cytotoxicity and adhesion to fibrinogen assays. Tumour specific accumulation of PEG-E-[c(RGDfK)$_2$] in mCherry-labeled mammary adenocarcinoma inoculated in mice was followed by non-invasive fluorescence imaging.
Results: The PEGylation of DOX and E-[c(RGDfK)$_2$] had resulted in a conjugate of 15 kDa in size. PEG-DOX-E-[c(RGDfK)$_2$] conjugate binds to U87-MG glioblastoma cells overexpressing a_vb_3 integrin, internalizes and demonstrates a similar cytotoxic effect as free DOX following incubation. PEG-DOX-E-[c(RGDfK)$_2$] conjugate overcomes resistance to DOX of M109R murine lung carcinoma cells. PEG-DOX-E-[c(RGDfK)$_2$] had an inhibitory effect of ~75% on HUVEC attachment to fibrinogen. Preliminary *in vivo* near-infrared studies revealed that a PEG-E-[c(RGDfK)$_2$]-cyanine conjugate preferentially accumulated in mCherry-labeled-DA3 murine mammary tumours.
Conclusions: Our results show a proof of principle for a selective delivery of DOX to endothelial and cancer cells overexpressing a_vb_3 integrin. By showing the advantages of our conjugate which accumulates selectively at the tumour site, we hope to warrant it as a novel targeted, anti-angiogenic and anticancer therapy.

179 Elevated expression of cyclooxygenase-2 is a negative prognostic factor for disease free survival and overall survival in patients with gastric carcinoma

A. Tamburini[1], M.C. Salandini[1], J. Nifosi[1], L. Albarello[2], C. Doglioni[2], C. Staudacher[1]. [1]*University Vita-Salute San Raffaele, Division of Molecular Oncology, Gastrointestinal Surgical Oncology Unit, Milan, Italy*, [2]*University Vita-Salute San Raffaele, Division of Pathology, Milan, Italy*

Background: Cyclooxygenases regulate the production of prostaglandins and play a role in tumour development and progression. We investigated the prognostic impact of expression of the cyclooxygenase (COX) isoform, COX-2, on disease-free survival and progression-free survival in patients with primary gastric adenocarcinoma (any pN any pT) without distant metastasis as well as the association between COX expression and other clinicopathologic parameters.
Patients and Methods: A cohort of 194 patients with gastric cancer (123 males, 87 women) without distant metastasis who underwent R0 gastric resection were enrolled in this study. Immunoistochemical immunoreactivity was assessed by the intensity of staining and percentage of positivity areas. Association between factors including clinico-pathological variables and COX-2 scores, were assessed by χ^2 and Student t test. Survival rates were calculated using Kaplan–Meier method and the difference between the groups were analyzed by log-rank test.
Results: A correlation between COX-2 expression, grading and advanced penetration dept (mean COX-2 expression 74% in early gastric cancer (EGC) versus 52% in non-EGC, $p = 0.0017$). There was an association between COX-2 expression and the presence of lymph-node metastasis ($p < 0.0001$, χ^2). We also observed a significant association between COX-2 expression and relapse of disease ($p = 0.05$ KM) but not with poor survival.
High COX-2 protein expression, serosal invasion (pT3-pT4), and presence of lymph-node metastasis are poor prognostic factors in patients with gastric carcinoma without distant metastasis. COX-2 expression in any percentage strongly correlates with lymph-node invasion and penetration dept, so it may indicate tumour aggressiveness.
Conclusions: The current data suggest that increased expression of COX-2 may play a role in the progression of primary gastric carcinoma. It remains to be investigated whether treatment with selective inhibitors of COX-2 may be an additional therapeutic option for patients with gastric carcinoma.

180 Biomarker discovery in plasma of breast cancer patients using microspot immunoassays

J. Sonntag[1], A. Jöcker[1], A. Schneeweiss[2], S. Wiemann[1], U. Korf[1]. [1]*German Cancer Research Center, Division of Molecular Genome Analysis, Heidelberg, Germany*, [2]*University of Heidelberg, Department of Gynecology and Obstetrics, Heidelberg, Germany*

The potential use of biomarkers in breast cancer includes aiding early diagnosis, determining prognosis and predicting response or resistance to different therapies. The ease with which blood can be sampled makes it a logical choice for biomarker applications.
Over the past few years different protein microarray platforms emerged as experimental tools for biomarker discovery. The microarray format allows for simultaneous determination of various parameters from a minute amount of sample within a single experiment. The experimental design of microspot immunoassays is based on antibody pairs specifically recognizing different epitopes of the analytes. One antibody is used to capture the analyte from the complex sample and the second antibody is used for detection.
Various transmembrane proteins are proteolytically released from the cell surface by a process known as ectodomain shedding both under normal and pathophysiological conditions. We have developed a microspot immunoassay for the evaluation of biomarker signatures focusing on the ectodomain shedding products of the ERBB1, ERBB2, and ERBB3 receptor. In addition, the ectodomain shedding product of the MET receptor is quantified as well. This 4-plex microspot immunoassay has been used to determine target protein concentrations in 100 plasma samples from breast cancer patients taken at primary diagnosis. The resulting quantitative data was compared with clinical data, e.g. lymph node status. This study gives an overview of baseline ectodomain shedding product levels in breast cancer patients at primary diagnosis and serves as a basis for a long term follow up study in these patients. Approval of the study was obtained from the local ethics committee at the University of Heidelberg.

181 Accuracy of castPCR-based KRAS testing on paraffin embedded tissues

H. Blons[1], D. Le Corre[1], J.C. Pagès[2], R. Tan[3], M. Joiner[4], R. Petraroli[3], C. Chen[3], P.L. Puig[1]. [1]*Bases Moléculaires de la Réponse Aux Xénobiotiques, Université Paris Descartes, Paris, France,* [2]*Faculty of Medicine, U966 Inserm, Tours, France,* [3]*Life Technologies, Foster City, USA,* [4]*LIife Technologies, Paris, France*

Background: Predicting targeted therapy sensitivity has become a part of the standard care of patients with solid tumours to optimize treatment options. Molecular marker testing for patient care implies that technologies move from the bench to the clinics. Testing methods have therefore to be properly validated and quality control procedures have to be established. Care samples can be heterogeneous, possibly due to poor quality and quantity. Using KRAS testing as an example, we report here the analytical performances of the competitive allele specific TaqMan PCR (castPCR) test developed by Applied Biosystems.
Material and Method: CastPCR assays were designed and manufactured by Applied Biosystems. CastPCR assays for seven KRAS mutations were tested on an ABI7900HT using Universal Genotyping Master Mix (Applied Biosystems, Foster City, USA). Eight mutated cell lines were initially used to validate the assays (H1573:p.G12A; H358:p.G12C; A427:p.G12D; LS123:p.G12S; SW620:p.G12V; Lovo:p.G13D; SW48:Wild Type; Tours: p.G12R). Tours is a cell line obtained after directed mutagenesis for G12R mutation. DNAs were extracted using QIAamp DNA Mini Kit (Qiagen, Courtaboeuf, France). Mutated DNAs were titrated in the wild type DNAs from 100% to 0.5%. Twenty-four anonymous tumours and 12 non-tumour tissues