indicate that K562/IMA-3 cells are more effectively sensitized by silencing the *STAT5a* compared to the sensitive K562 cells. Non-transfected K562/IMA-3 cells showed 1.85- and 3.46-fold increases in caspase-3 enzyme activation in the presence of 5- and 10 μM of imatinib, while siRNA-transfected counterparts have shown 11.21- and 20.17-fold increases.
Conclusion: Observing these significant responses to imatinib after transfection with single gene-specific siRNA might provide new opportunities for dealing with the frequent occurrence of resistance to chemotherapeutic agents in leukemia.

202 Identification and evaluation of novel breast cancer related biomarker proteins by antibody proteomics technology

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Background: The identification of biomarkers is a promising approach for the diagnosis and effective therapy of cancer. In particular, disease proteomics is a potentially useful method for identifying such biomarkers. However, very few biomarker proteins for drug development have been discovered using this approach. The main difficulty is to efficiently select potential biomarkers from the many candidate proteins identified by the proteomics approach. To circumvent this problem, we have developed "antibody proteomics technology" that can screen for biomarker proteins by isolating antibodies against each candidate in a rapid and comprehensive manner. Here, we applied "antibody proteomics technology" to breast cancer-related biomarker discovery and evaluated the utility of this novel technology.
Material and Methods: *2D-DIGE analysis:* Cell lysates from breast tumour cells (SKBR3) and established normal breast cells (184A1) were labeled with Cy3 or Cy5 and analyzed by 2D-DIGE according to the manufacture's protocol. Non-labeled samples were also loaded in a normal gel for MS analysis and a modified gel that can be solubilized by sodium periodide for antibody isolation. Proteins of interest were extracted from the gel and subjected to MS analysis and used as targets for the phage antibody library.
Isolation of monoclonal antibody: Protein samples extracted from the solubilized gel pieces were immobilized onto a nitrocellulose membrane. Using these proteins as targets, phages displaying scFv antibody, which have affinity to the targets, were selected from non-immune scFv phage libraries.
Tissue microarray (TMA) analysis: Expression profiles of candidate proteins were analyzed by using breast tumour TMAs stained with the isolated scFv-phages.
Results and Conclusions: By 2D-DIGE analysis, 18 candidate proteins over-expressed in SKBR3 cells were identified. Using an *in vitro* scFv-phage affinity selection procedure, monoclonal scFvs binding to each of the 18 candidate proteins were successfully isolated within a few weeks. TMA analysis then identified novel biomarker proteins over-expressed in breast tumours and correlated with lymph node metastasis. Our data demonstrates the utility of the antibody proteomics technology for discovering and validating tumour-related proteins in pharmaceutical proteomics. We are currently analyzing the functions of the identified proteins as potential diagnostic markers or therapeutic targets.

203 Targeting the transcriptional activity of sarcoma specific chimeric fusion proteins

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Background: Alveolar rhabdomyosarcoma (aRMS) is an aggressive childhood sarcoma characterized by expression of the fusion protein PAX3/FKHR which is thought to drive malignancy of this tumour. Downregulation of the fusion protein induces apoptosis in aRMS cells supporting the notion that their survival depends on continuous expression of PAX3/FKHR. Hence, it could be hypothesized that small molecular drugs might already exist for the treatment of aRMS, but strategies to prioritize them have been lacking. We are trying to identify small molecules capable to specifically modulate the transcriptional activity of chimaeric oncogenic transcription factors in sarcomas.
Material and Methods: We screened a small compound library (LOPAC 1280, Sigma) which covers 1280 different drug-like and well annotated compounds covering all major drug types. We used an endogenous cellular model, Rh4, which represents a PAX3/FKHR bearing aRMS with a transcription profile very similar to tumour biopsies. As a read-out system we simultaneously assessed cell viability together with a well established and highly sensitive luciferase reporter assay based on the AP2beta target gene promoter to monitor fusion protein activity. Cells were plated into 96 well plates and treated with the compunds at a final concentration of 5 μM during 24 hours. We set as cut-off a 65% reduction in luciferase activity.
Results: We have obtained a list of nearly 53 promising compounds affecting the activity of PAX3-FKHR. Among them there are different types of molecules with a broad role of functions such as kinase inhibitors, topoisomerase II inhibitors or compounds related to nitric oxide. We have chosen a final list of 11 compounds that we have tested using different concentrations (5, 1 and 0.5 μM) and time points (24, 48, 72 hours) and finally we have decided to focus our attention in two compounds: fenretinide (retinoic acid p-hydroxyanilide) and the kinase inhibitor IC261.
Interestingly, both fenretinide and IC261 reduced the mRNA expression level of PAX3-FKHR and some well known target genes such as AP2β, fibroblast growth factor receptor 2 (FGFR2) or fibroblast growth factor receptor 4 (FGFR4).
Then, we analyzed the effects of both compounds in additional rhabdomyosarcoma cell lines, both alveolar and embryonal, measuring again the cell viability and expression levels of PAX3-FKHR and its target genes. Both compounds were effective in all aRMS cell lines (Rh41, RMS13) but not in cells of embryonal origin (Ruch-2, RD) that lack PAX3-FKHR expression.
Conclusion: Unlike most other experimental strategies which reason that an increased understanding of the biology (target genes) would lead to identification of active compounds, our strategy is a reverse approach that has allowed us to identify compounds that are not tested yet for aRMS treatment such as fenretinide and IC261, and that then in turn are expected to help understanding the biology of sarcomas, identify pathways critical for aRMS progression and lead to the development of new therapeutic strategies.

204 Identification and pre-clinical validation of surrogate soluble biomarkers correlating with therapeutic response to met inhibition

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Serum proteins may function as early and dynamic surrogate biomarkers of molecular sensitivity to targeted inhibitors in selected cohorts of patients. In a search for a panel of potential soluble biomarkers of response to Met receptor inhibitors in sensitive tumours, we pursued an unbiased screening approach followed by experimental validation using diverse Met-addicted models, both *in vitro* and *in vivo*. Two independent and complementary technological platforms – (1) a large-scale cDNA microarray profiling and (2) a medium-scale protein array – have been applied to the Met-addicted cell line GTL16 treated with the selective inhibitor PHA-665752. The intersection of the two datasets enclosed two candidate molecules robustly down-modulated upon treatment, interleukin-8 (IL-8) and urokinase-type plasminogen activator receptor (uPAR). By employing available ELISA kits to test for the concentrations of IL-8 and uPAR in the supernatants of GTL16 cells upon PHA treatment, we observed a significant reduction in the levels of IL-8 and of uPAR. Moreover, we performed the same experiments in two cellular models of resistance to Met inhibitors: (1) A549 cells and (2) GTL16 cells infected with the constitutively active Ras^{G12V}. In both models, Met inhibition negligibly influenced secretion of the two molecules. We performed serial blood sampling at day 0 (pre-treatment), 3, and 10, to assay the concentration of IL-8 and uPAR in the plasma of nude mice injected subcutaneously with GTL16 cells. We could detect a clearcut reduction in IL-8 levels over basal values at 72 hs, that persisted until the 10^{th} day of treatment; conversely, established xenografts of GTL16 expressing the Ras^{G12V} 'resistance gene' did not feature a similar reduction in IL-8 levels. We further extended our fishing analysis to six other molecules significantly modulated either in the microarray dataset (stanniocalcin-1 and REG4) or in the protein array (IL-6, GROα, MIF, and MCP-1) and in order to generalize the model, we gauged expression and dosage of such molecules in a tissue-specific panel of Met-addicted cell lines (MKN45, HS746T, and SNU5). ELISA-based analysis of such molecules indicated that two of them (GROa and IL-6) displayed consistent rmodulation in culture supernatants of Met-addicted cells following Met inhibition. These soluble proteins may warrant further investigation as surrogate plasma biomarkers of response to anti-Met targeted therapies in drug-responsive tumours.

205 In vivo delivery of siRNA to tumours and their vasculature by novel dendritic nanocarriers

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Background: New targets for RNAi-based cancer therapy are constantly emerging. Nevertheless, *in vivo* delivery of siRNA remains a crucial issue for its therapeutic success. We propose to encapsulate the siRNA in a cationic carrier system, which can strongly improve its stability, cellular uptake and silencing efficacy. We developed novel polymerized dendrimer core shell structures to deliver siRNA *in vivo*. These water-soluble macromolecular carriers accumulate in the tumour environment due to the enhanced permeability and retention (EPR) effect and therefore, represent ideal delivery vehicles for antitumour biological agents.