immunohistochemistry in sections from paraffin-embedded slices. Epithelial cell death was also quantified using an ELISA based evaluation of different CK18 isoforms released to the medium during apoptosis or necrosis. Overall viability was analyzed in homogenized tissue slices by ATP quantification assay. In addition, we investigated the influence of erlotinib treatment on protein expression by 2D gel electrophoresis and mass spectrometry in tissue slices from lung metastases.
Results: The defined thickness of the tissue slices (200 μm) allowed a smooth diffusion of drugs, and antibodies. Fluorescent labelled taxol and antibodies were found to be distributed throughout the slice. As revealed by different read out systems cells remained viable and proliferated for at least 4 days within their tissue environment. The response to the anti-cancer drug taxol determined for 11 individual breast cancer samples turned out to be heterogeneous. One tumour sample was almost completely resistant to taxol whereas 6 tumours turned out to be highly sensitive. In lung metastases treated with erlotinib, initially 40 proteins were found to be changed significantly including proliferation and tumour associated factors.
Conclusions: We describe a tissue culture method combined with different read out systems for rapid assessment of drug efficacy, the simultaneous identification of different cell types, and proteomic analysis within individual cancer tissues. This method has significant potential for studying tumour responses to conventional anticancer drugs and novel treatment strategies in the complex environment of a primary cancer tissue *ex vivo*.

319 POSTER

Biochemical characterization of PXD101, a small molecule HDAC inhibitor, and a library of additional compounds on recombinant class I and II HDAC isoforms

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The human histone deacetylase (HDAC) family plays a key role in the control of gene expression through transcriptional regulation, and HDAC inhibitors (HDACi) are being clinically evaluated as potential anti-cancer therapeutics. The human HDAC family can be subdivided into three main classes: class I (HDAC1, 2, 3, 8, 11), class II (HDAC4, 5, 6, 7, 9, 10) and class III (sirtuins). A recent review (Minucci and Pelicci, Nature: 6:38, 2006) has summarized the importance of both class I and class II HDAC isoforms in cell functions affecting tumorigenesis. PXD101, a hydroxamic acid-derived, small molecule HDACi has growth inhibitory activity on a wide variety of human cancer cell lines and is in clinical trials for a number of indications, including multiple myeloma, T-cell lymphoma, AML, colorectal and ovarian cancers. The current study was designed to characterize the biochemical HDACi activity of PXD101 and a library of additional compounds against both a HeLa-derived whole cell extract containing a variety of HDACs, and a panel of individual HDAC isoforms.
cDNAs corresponding to eight human HDACs (HDAC1, 2, 3, 4, 6, 7, 8, 9) were expressed in a baculoviral system, and recombinant HDAC protein isoforms were purified. A Fluor de Lys™ (FDL) HDAC assay was optimized and used to assess the HDAC-inhibitory activity of a library containing over 100 compounds, including PXD101. A good correlation in the EC_{50} values obtained on HeLa extracts and individual HDACs1, 2, 3, 4, 6, 7 and 9 was noted. The HDAC8 isoform activities were lower in the screen and did not follow the trend of the EC_{50} values obtained on HeLa extracts. The data indicates a clear difference in activity between HDAC8 and the other class I and class II recombinant human HDAC isoforms tested in this study. The results of this analysis also demonstrated that PXD101 potently inhibits all isoforms tested (EC_{50}s ranging from 30–216 nM), thus establishing PXD101 as a pan HDACi. The findings that PXD101 is active on both class I and II HDAC isoforms known to be involved in tumorigenesis, and is well-tolerated in the clinic (de Bono *et al*. Proceedings of the AACR-NCI-EORTC International Conference, Abstract C88, 2005; Gimsing *et al*. Blood 106: 3337, 2005) support the continued clinical evaluation of this compound as an anti-cancer therapeutic.

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Identifying synthetic lethal candidate genes in DPC4 isogenic pancreatic cancer cell lines

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With a 5-year survival rate of less than 4% pancreatic cancer is the fourth leading cause of cancer death in the United States. The transforming growth factor beta (TGF-beta) signal transducer, DPC4 (Smad4) is deleted or otherwise inactive in approximately 55% of pancreatic cancers. An isogenic cell pair has been generated by vector-based re-expression of DPC4 in the BxPC3 human pancreatic cancer cell line, which normally exhibits a homozygous deletion of the DPC4 gene. To identify potential synthetic lethal partners of DPC4, we are applying the high-throughput screening of small interfering RNA (siRNA) libraries in the pair of DPC4 isogenic cell lines. Using a cell viability assay, genes targeted by siRNAs that result in a lethal phenotype in the DPC4− cell line but not in the DPC4+ cell line were identified. We hypothesize that the products of these genes may be involved in or associated with DPC4 signaling pathways or regulators of normal DPC4 function. Following extensive assay development and validation, we have evaluated differential sensitivity of ~8000 siRNAs against the two isogenic cell lines. There were 460 initial hits from the screen, which have been selected for re-confirmation and follow-up. Of these initial hits, several of them are involved in cancer-related signaling pathways such as cell-to-cell communication, cellular movement, cell cycle regulation and cell death. Our study has demonstrated that genome-wide siRNA screening is a powerful functional genomic tool that can be employed in the elucidation of signaling pathways and in identifying novel drug targets. We are vigorously pursuing these initial hits to devise a strategy for eliminating pancreatic cancer cells, which have deletions in DPC4.
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Mechanism of action analysis in cell-based screens using Gleevec and Velcade as examples

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Background: For a large scale secondary cellular screen compounds were tested in a panel comprising 36 adherent cell lines derived from non-hematologic human tumors (1–6 cell lines for each of 14 different tumor types) and 14 suspension cell lines derived from human hematologic malignancies (4 acute leukemias, 1 chronic leukemia, 2 NHLs and 7 myelomas). Gene expression (Affymetrix chip HG-U133, 34,000 genes) and protein expression profiles (bead suspension assays for total and phospho signal transduction proteins) are available for all non-hematologic cell lines.
Material and Methods: 4,000 to 10,000 adherent cells and 20,000 to 100,000 suspension cells were seeded in 96 well plates, compounds at 5 different concentrations were added 1 day later (all tests in triplicate) and left over for 4 days. The read-out was propidium iodide–based fluorescence, which is a measure of viable cell number. To obtain clues as to the test compounds mechanisms of action, their specific IC50 and IC70 activity profiles were compared with the profiles of more than 100 registered anticancer agents or compounds with known mechanisms of action.
Results: Gleevec, an inhibitor of Bcr-Abl, PDGFR and c-Kit, displayed mean IC70s of 48 and 24 μg/ml, respectively, in the 36 and 14 cell line panels. The most sensitive cell lines were the CML line K562 representing the bcr-abl translocation with an IC70 of 0.01 μg/ml followed by acute leukemia line HL60 (3 μg/ml), the renal cell cancer line RXF 393NL (8.3 μg/ml) and the CNS line CNXF 498NL (8.7 μg/ml). These 4 cell lines could represent indicator cell lines to detect novel compounds resembling Gleevec in their mechanism of action.
Velcade, a proteasome inhibitor, was generally more potent, exhibiting mean IC70 values of 9 ng/ml for the hematologic and 29 ng/ml for the non-hematologic cell line panel. It was most active in 4/7 myelomas, followed by 2/2 NHLs, and 4/5 leukemia cell lines. Among the non-hematologic cell lines, 2/4 breast lines, 1/2 colon and 1/2 bladder cell lines were most sensitive. The compare analysis revealed similarities to other proteasome inhibitors like Tyropeptin A (Spearman's rho = 0.63).
Conclusions: Gleevec represents a highly specific anti-cancer agent, and compounds with similar mechanisms of action can be identified based on their activity in sensitive indicator cell lines. The proteasome inhibitor Velcade has broader activity, and compounds with a similar mechansim of action can be identified by Compare Analysis.