315 POSTER

Advancing bioluminescence imaging technology for evaluating anticancer agents in MDA-MB-435 subcutaneous, mammary orthotopic and subrenal capsule tumor models

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In vivo bioluminescence imaging (BLI) has emerged as a sensitive and quantitative imaging modality gaining popularity due to the capacity to conduct non-invasive longitudinal studies monitoring tumorgenesis, metastasis, regression and response to anticancer agents. Diligent optimization of imaging parameters applied to relevant disease models is critical to realize the potential of BLI as a tool to reliably evaluate anticancer agents. Luciferase (Luc), a widely used optical reporter gene, stably integrated into tumor cells is replicated with every cell division enabling spatiotemporal analysis of tumor growth via BLI. Luc produces light in the presence of the substrate luciferin, oxygen and ATP; light photons are emitted and can be externally detected and quantified using sensitive light imaging systems. We describe the importance of luciferin kinetics as it relates to detection of peak bioluminescence signals and quantification of primary tumor burden and metastatic lesions arising from tumors of the same cellular origin but implanted in distinct locations in the mouse. MDA-MB-435-Luc mammary carcinoma cells were implanted subcutaneously (sc) in the flank, orthotopically in the mammary fat-pad or surgically in the subrenal capsules of mice. All models displayed high take rate (>90%) while the subrenal grafts showed a higher occurrence of lung metastasis (85%) compared to mammary fat-pad (20%) or sc (<5%). Using an IVIS 100 (Xenogen®), we collected multiple images between 5–30 minute intervals for an exposure time of 5–30 seconds to determine peak bioluminescent signals for each model. Results indicate the post-luciferin time interval to reach peak bioluminescence was variable depending on the model and tumor size. In the sc flank and mammary model the peak bioluminescence signal occurred within a narrow time interval and a strong correlation was demonstrated between the tumor mass and peak bioluminescence intensity. In contrast to the sc flank and mammary models, the time interval to reach peak bioluminescence intensity in the primary tumor and lung metastasis of the subrenal capsule model was highly variable spanning 5–30 minutes depending on the mouse. Capture of the peak bioluminescence intensity was essential to optimize signal to noise and accurately quantitate tumor burden. Failure to detect the peak bioluminescence signal may underestimate tumor burden and lead to incorrect evaluation of therapeutic response. Upon identifying the optimal imaging parameters for various MDA-MB-435-Luc models we evaluated the antitumor activity of Taxol® and AG-024322 (CDKi) and demonstrated the utility of BLI as a tool to reliably evaluate anticancer agents. Advancing BLI technology in relevant disease models creates an opportunity to improve preclinical evaluations of anticancer agents.

316 POSTER

Animal study management software: novel technology to optimize cancer research in vivo

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Efficacy and preliminary safety tests performed in animals for promising drug candidates represent a critical bottleneck in cancer research and form the basis for the pivotal decision to pursue full-scale preclinical development. Remarkably, most animal studies are still performed like they were 20 years ago, using a combination of paper scientific notebooks and electronic spreadsheets. These manual methods are highly inefficient, are prone to problems with data integrity, accuracy and accessibility. The inefficiencies intrinsic to a single screening study are multiplied with each additional study conducted and constitute a serious but avoidable problem for cancer research organizations; that of prolonged discovery time for new drugs. Data collection for animal studies commonly entails technicians measuring and recording results on paper, entering the data into spreadsheets, and later transferring the data into statistical software for further analysis. Spreadsheets do manage measurement data, but do not manage detailed information on study design, conditions, animal models or projects. A lack of consistent data formatting convention within spreadsheets makes the analysis and inter-study comparison quite challenging and time consuming. Results from completed studies are pasted into the research notebook, which is later microfiched and archived out of reach. The manual recording of data into notebooks and the transcription to spreadsheets, statistical programs and databases is prone to errors. Problems with data integrity and security due to accidental destruction, notebook and datasheet loss, file spreadsheet deletion or change, theft and intentional falsification have also been identified as serious concerns. Commercially available systems, such as Studylog Systems' Study Director®, IPA's Labcat and Tumor Tracker automate study processes such as study design, data collection, task management, data analysis, graphing, report generation, and enable enterprise-wide access to study information for current and archived studies. Animal study management software improves process efficiency, data integrity and security, and data accessibility. This software also increases the transparency of study data and processes, facilitating personnel oversight, IACUC compliance, and inter-study comparability. The advent of study automation technology provides the research enterprise with valuable new means to significantly decrease the time to discovery of novel therapies.

317 POSTER

Antitumour activity of new compounds from N-sulfonyluracil and benzothiazole group with hyperthermia on the growth of mouse tumours

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Aim: The purpose of this study is to investigate antitumour activity of newly synthesized compounds from N-sulfonyluracil and benzothiazole group. These types of compounds showed potent inhibitory activity on the growth of human tumour cell lines *in vitro*. In this study we have investigated antitumour activity of 5-bromo-1-(metanesulfonyl)uracil on the growth of transplantable mouse mammary carcinoma (TMMCa) *in vivo* and antitumour effect of newly synthesized benzothiazoles on the growth of mouse fibrosarcoma (FsaR), melanoma (B16-F10), and squamous cell carcinoma (SCCVII). Also some of these new agents have been applied with local hyperthermia (LTH).
Material and Methods: In this study we used a mouse mammary carcinoma and fibrosarcoma and melanoma. Tumour cells were injected into the mouse footpad of the right hind leg. Tumour bearing mice have been treated with new compounds as a single agent or in combination with hyperthermia (43.0ºC/60 min). The end point was tumour growth time (TGT). TGT is the time needed for tumour volume to grow five times over the treated volume measured by calliper and calculated by the formula $A \times B \times C \times \pi/6$. 5-bromo-1-(metanesulfonyl)uracil has been synthesized at Rudjer Boskovic Institute and benzothiazole have been synthesized at Faculty of Chemical Engineering and Technology, University of Zagreb.
Results: The obtained data show that examined 5-brom-1-(metansulfonyl)-uracil has suppressed tumour growth of TMMCa in comparison to control group. When 5-brom-1-(metanesulfonyl)uracil has been combined with local hyperthermia, antitumour activity of this derivative was enhanced. Benzothiazole compounds have shown good antitumour activity against melanoma B16 and fibrosarcoma as well as against SCCVII carcinoma.
Conclusion: The obtained data show that new antitumour compounds (*N*-sulfonyluracil and benzothiazole derivatives) can reduce tumour growth time in mice.

318 POSTER

Cancer tissue model to study anti cancer drug effects and to identify predictive markers

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Background: The current model systems to study anticancer drug sensitivity such as cell lines, isolated tumour cells and animal models only poorly predict clinical response. One reason is that the drug response of tumour cells is greatly influenced by their cellular and non-cellular environment. Therefore, model systems which allow studying drug effects on primary tumour cells within different compartments of an individual tumour *ex vivo* provide a great advance for research on response prediction or the investigation of efficacy of novel treatment strategies.
Material and Methods: We prepared 200 μm thick tissue slices from freshly excised tumour samples from primary breast cancer and lung metastases and cultivated them in the presence or absence of anticancer drugs for 4 days. To visualize viability, cell death, and expression of surface molecules in different compartments of non-fixed primary breast cancer tissues we established a method based on confocal laser scan microscopy using mitochondria- and DNA-selective dyes and fluorescent-conjugated antibodies. Proliferation and apoptosis was assessed by

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.

320 POSTER

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.
Supported by Program Project Grant P01CA109552.

321 POSTER

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.