analysed using MSD® technology. In addition, drug effects and targets within the tissue were evaluated using immunohistochemical staining against key phosphoproteins (e.g. pAkt, pMAPK, pmTOR) and numerous other cancer relevant molecules (e.g. p53 and Ki67).
Results: The model was validated using 28 colon, 32 NSCLC and 12 breast cancer specimens. The slices remained viable for at least 4 days and showed a good response to cytotoxic treatment in ATP and caspase 3/7 assay. Analysis of signalling pathways revealed individual differences in drug response, which was supported by IHC staining.
Conclusions: Deciding which drug should enter the clinical trial phase is one of the most critical and expensive parts in drug development. Thus, new models are urgently needed to improve the success rate of drugs in clinical trials. In this study we established a standardised protocol for the preparation of viable tumour tissue slices which allows the testing of anti-cancer agents in a preclinical model. The model was established for colon, NSCLC and breast cancer and tested with various anti-cancer agents used in standard therapy. The model both allowed evaluating target expression, functional drug-effects and inhibition/activation of specific pathways in parallel as well as determination of heterogeneity among patients. The use of such organoid cultures bears great potential for studying tumour responses to anticancer drugs because the complex environment of the primary cancer tissue is being maintained.

1014 POSTER DISCUSSION

Genomics-based selection and characterization of pre-clinical oncology testing models and prediction of patient response in clinical trials

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The current paradigm in cancer drug discovery is to target molecular lesions rather that histopathological cancer types. We used patterns of copy number abnormalities (CNAs) to identify genomic subgroups of several cancer types. This information was used to rationally select panels of pre-clinical testing models. We used high-resolution comparative genomic hybridization (CGH) to create a database of genomic profiles of tumors that includes hundreds of tumors and cells lines. A computational algorithm was developed to process gene copy number data to identify distinct groups within a cancer type and assign cell lines and xenografts to appropriate groups. The analysis was performed for NSCLC, SCLC, colorectal cancer, and melanoma, resulting in creation of panels of characterized pre-clinical models.
A proactive approach to genomic biomarker discovery involves copy number analysis of model systems with known sensitivity to drug candidates. Once a CNA is found that correlates with the sensitivity of the model system to the candidate compound, it can be tested as a predictor of drug response in clinical trials by using FISH. We have implemented this early biomarker discovery strategy for a number of our oncology drug candidates. For example, we applied integrative genomics to identify predictors of sensitivity of SCLC to Bcl-2 family inhibitors. Our CGH screen of SCLC cell lines followed by genome-wide analysis of aberrations has identified a novel gain on 18q21−23 that is associated with sensitivity to ABT-263, a first-in-class Bcl-2 family inhibitor. Our data suggest that the 18q21−23 copy number may be a clinically relevant predictor for sensitivity of SCLC to Bcl-2 antagonists. As the finding is being validated in clinical trials, we use circulating tumour cells (CTCs) from SCLC patients, as biopsies are rarely available. Preliminary results show increased Bcl-2 copy number in some patients.
In summary, our use of high-resolution copy number profiling in cancer has resulted in rational genomics-based selection of preclinical testing models and identification of a stratification marker for therapy with Bcl-2 inhibitors.

1015 POSTER DISCUSSION

Activity of the MAPK signalling cascade correlates to gemcitabine sensitivity in pancreatic cancer cells

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Introduction: Pancreatic cancer is a leading cause of cancer-related death in the world. Gemcitabine remains to be the gold standard of therapy. However, the effectiveness of this treatment has been hindered by increasing numbers of patients exhibiting resistance. Research has hinted at a relationship between the Ras/Raf/Mek/Erk pathway and chemoresistance, as this pathway induces a strong survival signal in cancer cells. The purpose of this study was to establish a molecular background of pancreatic cancer cell lines and to identify the molecular signature of gemcitabine.
Methods: PANC-1, MIA-PaCa-2 and BxPC-3 pancreatic cancer cells were seeded into 96 well plates at 5×10^3 cells/well and treated with gemcitabine (1 uM to 10 mM) for 48 h. IC50 of gemcitabine for each cell line was then determined using MTT or Alamar Blue assays. MAPK specific protein arrays from R&D Systems were used to screen cells pre- and post-gemcitabine treatment (10 uM, 48 h) to create a list of protein affected by gemcitabine treatment. In addition, Western Blot analysis was used to confirm the results of the protein arrays.
Results: MTT and Alamar Blue assays showed in order of gemcitabine sensitivity, PANC-1>BxPC-3>MIA-PaCa-2 (see table 1). Screening with protein arrays demonstrated that basal levels of MAPK related proteins differ between cell lines and this relates to gemcitabine sensitivity. Comparison of untreated cells and gemcitabine treated cells created a shortlist of proteins with greater than 20% change in expression for each cell line. Differences in expression levels in proteins from this shortlist were then quantified using Western Blot analysis. Results showed that gemcitabine resistant PANC-1 cells exhibit higher basal Erk activity than sensitive MIA-PaCa-2 cells. Currently work is underway using the MEK inhibitor U0126 to knock out Erk signalling. Preliminary results suggest a relationship between Erk activity and gemcitabine sensitivity.

	PANC-1	MIA-PaCa-2	BxPC-3
Kras	Mutant	Mutant	Wild type
IC50 (uM)	300	60	120
Untreated (%GAPDH)			
tErk	78.5±17.6	60.8±9.8	98.5±13.4
pErk	27.1±6.9	21.3±3.4	9.2±4.1
Gemcitabine (10 uM)			
tErk	83.4±9.1	60.7±17.8	53.2±11.0
pErk	45.6±17.4	36.0±5.6	12.9±1.8

Conclusions: Results show that Erk activity may be important in cellular resistance to gemcitabine and sensitivity may be increased through its inhibition. This suggests that combining gemcitabine with an inhibitor of Erk may enhance gemcitabine efficacy.

1016 POSTER DISCUSSION

Prognostic and predictive significance of BRAF mutation in patients with metastatic colorectal cancer treated with 5-fluorouracil-based 1st line chemotherapy

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Background: The genetic events underlying colorectal cancer (CRC) are well characterized and include common somatic mutations in the *APC*, *TP53* and *KRAS* genes, followed in frequency by *PIK3CA* and *BRAF* mutations. Activating mutations in the *BRAF* oncogene deregulate growth factor pathways, stimulate cell proliferation, and promote metastasis. As previously reported by our group, patients with metastatic CRC primary tumors harbor *BRAF* mutations have an increased risk of disease progression and death. In the present study the significance of *BRAF* mutations was evaluated in an independent group of patients with metastatic CRC.
Material and Methods: The *BRAF* (V600E) mutation was determined by allelic discrimination, using Real-Time PCR, in 150 primary tumors from patients treated for metastatic CRC at a single institution. The underlying *BRAF* mutation was correlated with time to tumor progression (TTP) and overall survival (OS).
Results: The median age of this patients' population was 64.5 years (range 23−81) and 63% were male. All patients received 5-FU-based 1st line chemotherapy, 33% in combination with oxaliplatin, 18% with irinotecan and 49% with both drugs; bevacizumab or cetuximab was added to chemotherapy in 22% and 7% of patients, respectively. *BRAF* mutations were present in 12 (8%) cases. Patients with *BRAF* mutated primary tumors had a median OS of 14 months compared to patients with wild-type primary tumors whose median OS was 30 months ($p < 0.0001$). In addition, TTP was 2.6 months for patients with *BRAF* mutated primary tumors whereas it was 10.3 months for patients with *BRAF* wild-type primary tumors ($p < 0.0001$). Cox regression analysis uncovered *BRAF* mutations as prognostic factor for decreased OS (Hazard Ratio [HR] 4.4, 95% CI 2.3−8.2). Patients with *BRAF*-mutant tumors had significantly lower TTP (HR 2.98, 95% CI 1.6−5.4) than those whose primary tumors carried only wild-type *BRAF*.

Conclusions: *BRAF* V600E mutation in the primary tumor marks a subgroup of patients with especially poor prognosis and indicates that this patients' population might justify foregoing approved treatments in favor of investigational therapy. The adverse significance of BRAF mutation should be used for patient selection and stratification in future clinical trials. These results underscore the potential of mutational profiling to help patients whose tumors have a different natural history or differential response to traditional therapies.

1017 POSTER DISCUSSION

Impact of sequence variants in CYP2C8 on paclitaxel clearance in ovarian cancer patients

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Background: Toxicity and effect of paclitaxel vary greatly between patients and remain a clinically relevant problem with regard to the handling of dose delay/reduction or termination of treatment. We investigated the notion that single nucleotide polymorphisms (SNPs) in *CYP2C8* could be partly responsible for this variation. Paclitaxel is mainly metabolized by CYP2C8; SNPs have been investigated in this context before but conclusions are still lacking. We present early results from a prospective study of paclitaxel clearance (CL) in 93 Caucasian females with ovarian cancer with regard to the *CYP2C8* *1b, *1c, *3 and *4 genotypes. Ten other genes are considered
Materials and Methods: All patients were diagnosed with primary ovarian/peritoneal cancer and received 175 mg/m^2 paclitaxel over 3 hrs plus carboplatin AUC5−6 q3w. All patients gave written and verbal consent. The study was approved by ethics commitees in Denmark and Sweden. Blood was sampled at 3, 5−8 and 18−24hrs after start of infusion. Total plasma paclitaxel was quantified by HPLC. CremophorEL (CrEL) was determined using Coomassie blue assay. CL of unbound paclitaxel was estimated using total concentrations, CrEL and other parameters in the model described by Henningsson et al.in 2003 using NONMEM VI. Genotyping were done using Pyrosequencing. Genotypes were in HW equilibrium, except *1b ($p = 0.01$).
Results: The PK model predicted the data well. The CL of unbound paclitaxel was lower for patients with the *CYP2C8*3* and **4* variants ($p < 0.05$). For the one patient carrying both *3 and *4 variant CL was 269.7 l/h.

CYP2C8 variant	N	Paclitaxel CL geometric mean (l/h)	95% c.i	P-value (T-test of log transformed CL)
Wt/Wt	49	395	370.3–421.4	
Wt/*1b + *1b/*1b (n = 1)	44	374.5	348.8–402.2	0.267
Wt/Wt	69	382.4	362.1–404	
Wt/*1c	24	393.1	355.4–434.8	0.617
Wt/Wt	74	394.7	375.3–415.3	
Wt/*3	19	350	310–395.3	0.041*
Wt/Wt	86	390.9	372.1–410.7	
Wt/*4	7	320.8	281.7–365.3	0.028*

Conclusions: This study implies reduced elimination of paclitaxel in Caucasian female patients with the *CYP2C8*3* and **4* genotypes. This confirms several *in vitro* studies and pilot studies but is different to Henningsson et al 2003 and Marsh et al 2006 which could be explained by differences in dose ranges, infusion times and/or related to gender. The finding is important in terms of understanding inter individual variability of paclitaxel pharmakokinetics and might in the future provide useful information for individualized chemotherapy.

1018 POSTER DISCUSSION

Trichostatin A and decitabine reverses resistance to docetaxel in human breast cancer cells

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Introduction: Resistance mechanisms of the chemotherapeutic docetaxel are poorly understood, however, epigenetic modifications may be important. Epigenetic events such as DNA methylation and histone deacetylation can alter gene expression due to gene silencing. Altered gene expression can be linked to drug resistance, therefore, we hypothesise that treatment with a DNA methylation or a histone deacetylation inhibitor may reverse docetaxel resistance in human breast cancer cells.
Methods: Docetaxel-resistant human breast cancer cells (MCF-7 and MDA-MB-231) were treated with either trichostatin A (TSA), a histone deacetylation inhibitor, or 5-aza-2′-deoxycytidine (decitabine), a DNA methylation inhibitor, or in combination. Global methylation and DNA methyltransferase (DNMT) enzyme activity was measured using an ELISA-based assay. Histone acetylation levels were measured by western blot analysis. Quantitative PCR was used to measure *DNMT* gene expression changes. Cell viability was measured using MTT assay.
Results: Decitabine did not alter response to docetaxel in either cell line even though global methylation, DNMT enzyme activity and *DNMT* gene expression were changed following treatment with decitabine. Histone H3 acetylation was increased after TSA treatment. Addition of 200 nM TSA increased sensitivity to docetaxel in MCF-7 resistant cells ($P = 0.007$) but, in contrast, increased resistance to docetaxel in MDA-MB-231 cells. Concurrent treatment with decitabine and TSA enhanced response to docetaxel in MCF-7 docetaxel-resistant cells (IC_{50} resistant cells: 18.1 ± 1.9 vs. IC_{50} resistant-treated cells: 5.5 ± 5.0; $P = 0.016$). Furthermore, docetaxel sensitivity was also increased in MDA-MB-231 cells (IC_{50} resistant cells: 43.3 ± 8.6 vs. IC_{50} resistant-treated cells: 22.4 ± 2.6; $P = 0.016$).
Conclusions: Decitabine alters the methylation machinery in both docetaxel-resistant cell lines, but response to docetaxel could not be enhanced using this DNA methylation inhibitor. Trichostatin A did increase sensitivity to docetaxel but only in MCF-7 cells suggesting a cell line specific effect using this histone deacetylation inhibitor. Treatment with both inhibitors, however, increased response to docetaxel in both docetaxel-resistant cell lines. These results suggest that combination of methylation and histone deacetylation inhibitors may offer better therapeutic potential than using single inhibitors to overcome drug resistance.

1019 POSTER DISCUSSION

Withaferin-A modulates Akt/FOXO3a/Par-4 axis and induces apoptosis in human prostate cancer cells

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Background: In advanced prostate cancer (PCa), the most common apoptotic genes, such as p53 and PTEN, are either mutated or deleted; such mutations render PCa resistant to current treatments. To overcome this therapeutic impasse, activation of other pro-apoptotic signaling pathways is necessary. The forkhead transcription factor class O (FOXO) family regulates a wide variety of cellular responses, several of which are related to important aspects of tumorigenesis, and activation of FOXO 3a results in either cell cycle arrest or apoptosis. Akt, which negatively regulates FOXO 3a, is highly expressed in androgen-independent prostate cancer (AIPC) cells and thereby blocks the pro-apoptotic function of FOXO 3a. Logically, overcoming this block might be an effective chemopreventive and/or chemotherapeutic strategy.
Materials and Methods: Differential expression of Akt/FOXO 3a/Par-4 expressions in AIPC cells were studied in human PCa cells. AIPC cells were exposed to WA was studied with transwell migration and Matrigel invasion assays. To study the effects on AKT/FOXO 3a/Par-4 signaling were determined by SDS-PAGE, Western blot; Par-4 expression was evaluated after overexpression Akt and FOXO 3a by RT-PCR. Knock down and over expression of FOXO 3a and Akt on Par-4 gene was studied using siRNA and plasmid transfections, respectively.
Results: We have identified a dietary molecule, Withaferin-A, that in AIPC cells concomitantly inhibits Akt phosphorylation and activates FOXO 3a by importing it into the nucleus to induce apoptosis. Similarly, activation and nuclear import of Par-4 induce apoptosis. Interestingly, over expression of Akt inhibits the activation of both FOXO 3a and Par-4, causing PCa to become resistant to WA treatment. Based on these observations, we hypothesize FOXO 3a and Par-4 signaling directly interacts, as inhibition of FOXO 3a downregulates Par-4, as well as WA-induced apoptosis in AIPC cells. Notably, inhibition of Par-4 does not alter WA-induced FOXO 3a activation. This suggests FOXO 3a is upstream of Par-4 signaling, and that FOXO 3a signaling is essential for WA-induced, Par-4-mediated apoptosis in AIPC cells. Our data reveal that over expression of FOXO 3a up-regulates Par-4 expression, and that FOXO 3a physically binds Par-4 and activates Par-4 at the promoter level.
Conclusions: Overall, our studies indicate that Par-4 is one of the target genes of FOXO 3a, and that activation of Par-4 signaling by WA leads to apoptosis in AIPC cells.