resulting in the inactivation of membrane associated endoglin (mEng) and the release of soluble endoglin (sEng) in the stroma. Both the reduction of mEng expression in SCC cells through the use of siRNA, and the expression of endoglin in SpCC cells, demonstrate that this protein attenuates the TGF-b_1/Smad2/3 signalling, and modulates cellular growth and invasiveness. Loss of mEng in SCC cells activates the TGF-b_1/Smad2/3 signalling, which promotes an epithelial-mesenchymal transition and a progression from SCC to SpCC. Loss of mEng also leads to the inhibition of cellular growth, both *in vitro* and *in vivo*.
Conclusions: The shedding of mEng is associated with progression from SCC to SpCC. Downregulation of mEng activates ALK5-Smad2/3 signaling allowing to cell growth inhibition and a SCC-SpCC conversion Downregulation of mEng function (by shedding) emerges as a critical event for progression to highly aggressive undifferentiated carcinomas. Membrane endoglin behaves as a suppressor of malignancy.

1010 POSTER DISCUSSION

Cloning of Spalax heparanase splice variants family and its effect on tumour growth and extracellular matrix degradation

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Background: Heparanase is an endoglycosidase that degrades heparan sulfate at the cell surface and in the extracellular matrix. Heparanase is expressed mainly by cancer cells, and its expression is correlated with increased tumor aggressiveness, metastasis, and angiogenesis. Blind subterranean rodents of the genus *Spalax* live all their life in underground tunnels, and they are adapted to tolerate severe hypoxia as low as 3% oxygen. *Spalax* possesses high blood vessel density in some of its tissues compared to above ground mammals. Moreover, heparanase is highly expressed in normal *Spalax* tissues, unlike its scarce expression in human. Recently we reported the cloning of two splice variants of *Spalax* heparanase; here we report the identification of additional three splice variants of this enzyme.
Methods: *Animals*: The animals used for cloning the splice variants of *Spalax* heparanase belong to *S. judaei*. *Gene cloning*: Cloning of *Spalax* heparanase splice variants, was performed utilizing *Spalax* kidney cDNA. *Spalax* specific primers around different exons were designed, and PCR reactions were performed using *Taq*DNA polymerase and kidney cDNA as a template. *Tumorigenicity studies*: U87, HEK293, and B16 melanoma cells were used for *in vitro* and *in vivo* studies. The experiments were approved by the Ethics Committee of the University of Haifa.
Results: We cloned a novel splice variants family of heparanase from *Spalax* which includes five members: Splice #7, splice #36, splice #12 splice #67 and splice #612. Splice #36 of *Spalax* heparanase functions as a dominant negative to the wild-type enzyme: it inhibits heparan sulfate degradation, glioma tumor growth, and melanoma cell metastasis. Interestingly, splice variant #7 enhances tumor growth. Splice #12 enhances tumor growth but to less extent than splice #7. These results indicate that alternative splicing of heparanase plays a pivotal role in the regulation of its function and malignant potential.
Conclusion: We cloned five splice variants family of heparanase that modulates the function of the wild type enzyme. Three splice variants of this family are reported here for the first time. The dominant negative effect of *Spalax* splice #36 on heparanase, and the resultant inhibition of tumor growth and metastasis *in vivo* could be utilized to develop anti-heparanase human recombinant splice #36 directed to inhibit tumor growth through inhibition of the heparanase enzyme. The functions of the other splice variants are currently under investigation.

1011 POSTER DISCUSSION

Upregulation of Fibulin-5 in tumor cells protects against metastasis formation in mouse model

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Dissemination of tumor cells and settlement in other organs are one of the biggest challenges to overcome in achieving successful treatment of cancer. Pre-metastatic niches and the criteria's for metastatic spread are under intense investigation and evidence emerges that the interplay between tumor and stromal cells that creates the tumor microenvironment is more important than previously thought.
We and others found that tumor cells and fibroblasts reciprocally activate each other, consequently leading to stimulation of tumor progression and metastasis formation. Co-injection of VMR mouse mammary carcinoma cells (VMR) with immortalized mouse embryonic fibroblasts (MEF) into mice showed increased ability of VMR cells to colonize lungs and liver in an experimental metastatic assay. Expression profiling of MEFs, treated with conditioned media from VMR tumor cells showed modulation of expression of several genes. Genes encoding secreted and cell surface proteins were chosen for further analysis. We confirmed downregulation of four genes by qPCR analysis of MEFs grown both in 2D and 3D cell culture conditions, making them candidates for metastasis-suppressor genes. The effect of one of the genes, namely Fibulin-5 (FBLN-5), on the ability of VMR cells to form metastases was analyzed in *in vivo* assays.
FBLN-5, previously known as DANCE and EVEC, is a secreted extracellular matrix protein that functions as a scaffold for elastin fiber assembly and as a ligand for integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_9\beta_1$. Treatment of fibroblasts with proinflammatory cytokines abolished FBLN-5 mRNA expression, suggesting its involvement in inflammatory responses.
Overexpression of FBLN5 in VMR tumor cells lead to decreased adhesion to fibronectin. Moreover, VMR/FBLN-5 cells exhibit decreased ability to colonize lung and liver in an experimental metastasis assay. We therefore suggest that FBLN-5 indeed could act as a metastasis-suppressor gene.

1012 POSTER DISCUSSION

Liver-specific homing ligands of colorectal cancer

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Background: Cancer metastasis begins with cancer cell evasion from the primary site and ends ultimately with seeding to a distant organ site. It has been established that endothelial surface molecules on the vascular bed of metastatic target tissues contribute to organ specific spread of cancer. We are studying the metastatic process at the molecular level to target molecules critical for organ-specific metastasis of cancer.
Materials and Methods: In previous work we have identified a set of ligands by in vivo phage display selection of a colon cancer cell line metastasizing to liver. In order to evaluate the differential expression of the metastasis ligands, mRNAs from various tissues and cancer cell lines were analyzed by qRT-PCR.
For functional analysis, the metastasis ligands were cloned and expressed as MBP-fusion proteins. We analyzed the activity of the purified ligands by monitoring the phosphorylation of various key molecules of the signal transduction cascade in endothelial cells.
In in vivo assays we monitored by real-time fluorescence imaging the organ homing of Quantum-dot labeled protein in mice. In a metastasis model in mice we utilized the purified metastasis ligands to block of liver specific metastasis of colon cancer cells.
Results: We identified ligands with a yet unknown function in metastasis. We performed an extensive cluster analysis of the expression pattern of the ligands among various cancer and normal tissues and cell lines. Subsets of the metastasis ligands are higher expressed in more aggressive cancer and interestingly in bone marrow progenitor cells while others are expressed in differentiated cells.
With signal transduction assays we showed for a subset of ligands that MAPK and JNK signaling is upregulated.
Real-time in vivo imaging showed that the metastatic ligands home specifically to the liver in mice. In the metastasis model the metastatic spread of tumor cells was blocked.
Conclusions: We found that cancer cells express an overlapping set of tissue-targeting genes suggesting a similar function. We assigned a novel function to already known genes as tissue-specific homing ligands. We anticipate using such homing proteins as targets to visualize and eradicate occult tumor metastases.

1013 POSTER DISCUSSION

Cultivated cancer tissue slices as a meaningful preclinical model for evaluation of drug responses

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Background: The aim of this study was to develop a preclinical model of solid tumours which allows detailed drug testing in a natural environment to identify promising indications for clinical trials, prioritise drugs, support dose finding and to individualise therapy.
Materials and Methods: 400 µm tissue slices were prepared from freshly resected colon, NSCLC and breast cancer tumours. The slices were cultivated and treated with various anti-cancer agents, e.g. FOLFOX, oxaliplatin, camptothecin and gemcitabine, for up to 4 days. Functional drug effects on viability and apoptosis were measured via ATP and caspase 3/7 assays. Inhibition or activation of specific cell signalling pathways was

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*.