Results: IGF-IR expression ($p = 0.02$) and decreased IGFBP-3 ($p = 0.009$) were independent predictors of sensitivity to F in NSCLC cell lines. These pharmacologically defined groups matched 2 of 3 phenotypes identified by tissue and plasma marker profiling of NSCLC pts: Epithelial (differentiated) and EMT. A third phenotype, mesenchymal (undifferentiated), did not appear responsive to F. The epithelial high E-cadherin-expressing phenotype included most (73%) SqCC tumors. These tumors exhibited high levels of IGF-IR ($p = 0.05$), low vimentin levels, low free (unbound to IGFBPs) plasma IGF-I (fIGF-I), and an association between IGF-2 and its inhibitor, the IGF-2R ($p = 0.02$). Mesenchymal-like NSCLC was represented by LC/NOS that expressed the highest levels of vimentin ($p < 0.001$) and low receptor and ligand levels. The transitional EMT phenotype was observed in the majority of AD pts (63%) who had high plasma fIGF-I levels ($p = 0.06$). fIGF-I correlated directly with vimentin ($R = 0.475$, $p = 0.03$) and inversely with E-cadherin ($R = -0.524$, $p = 0.02$), indicating ligand-driven EMT, and it was predictive of F clinical benefit. Median PFS were 2.73 and 6.53 months for chemotherapy alone and chemotherapy with F 20 mg/kg, respectively, in pts with high fIGF-I levels ($p = 0.001$) while no significant treatment effect of F was observed in the low ($\leqslant$0.54 ng/mL) fIGF-I group.
Conclusions: High IGF-IR expression characterizes SqCC while IGF-I driven EMT is a key element in the biology of AD NSCLC. Both IGF-IR and fIGF-I levels may contribute to the identification of NSCLC pts who could benefit from F therapy.

1007 ORAL

Myelosuppression and kinase selectivity of multikinase angiogenesis inhibitors

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Background: Vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors often inhibit other kinases, besides VEGFRs, which may contribute to their adverse event profiles. Myelosuppression has been observed with several multikinase angiogenesis inhibitors in clinical studies, although the frequency and severity varies among the different agents. The present study evaluated differences in kinase selectivity of pazopanib, sorafenib and sunitinib and their effects on ligand-induced human bone marrow colony formation unit (CFU).
Methods: Kinase selectivity of pazopanib, sorafenib and sunitinib was evaluated using Upstate kinase profiler against 242 kinases at 0.3 and 10 mM. Ki^{app} was determined against key tyrosine kinases for all 3 compounds. Cellular potency against VEGFR2, PDGFRb, c-Kit and Flt-3 was measured using receptor autophosphorylation assay. Inhibition of CFUs in the presence of GM-CSF, SCF and Flt-3 ligand was evaluated and correlated with their kinase selectivity profile.
Results: In the Upstate kinase profiler assay, sunitinib inhibited 49 additional kinases, besides VEGFR, PDGFR and c-Kit, at IC_{50} within 10-fold of VEGFR2, whereas pazopanib and sorafenib inhibited 7 and 10 additional kinases, respectively. Sunitinib was more potent against Flt-3 compared to VEGFR2 in both enzyme and cellular assays. Pazopanib was 25 to 100-fold less active against Flt-3 compared to VEGFR2 in enzyme and cellular assays. Sunitinib inhibited the human CFUs induced by SCF and/or Flt-3 ligand at 7 to 16-fold lower IC_{50} than that required for inhibition of VEGFR2 autophosphorylation in endothelial cells. Pazopanib and sorafenib had >10-fold higher IC_{50} in the CFU assays compared to VEGFR-2 autophosphorylation.
Conclusion: Sunitinib inhibits c-Kit and Flt3 tyrosine kinases at potency $\geqslant$ to VEGFR2, whereas sorafenib has similar potency against the 3 kinases and pazopanib is less potent against Flt3 compared to VEGFR2 and c-Kit. Sunitinib inhibits proliferation of bone marrow cells in the presence of SCF and Flt-3 ligand more potently than VEGF-induced VEGFR2 phosphorylation in endothelial cells. These results provide a potential explanation for the observed differences in myelosuppression observed with various multikinase angiogenesis inhibitors in the clinic.

Poster discussion presentations
(Wed, 23 Sep, 17:00–18:00)
Basic Science/Translational research

1008 POSTER DISCUSSION

Mesenchymal Stem Cell (MSC) secretion of TGFβ and VEGF stimulates Epithelial to Mesenchymal Transition (EMT) in breast cancer cell lines

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Background: Adult Mesenchymal Stem Cells (hMSCs) are bone marrow-derived multipotent cells that have the ability to self renew and differentiate into multiple cell types including bone and cartilage. Chemokines and growth factors secreted by MSCs have been reported to have a significant effect on tumor growth and development. The aim of this study was to examine the effect of MSC secreted factors on breast cancer cell proliferation and gene expression, and to potentially identify the factors mediating these effects.
Materials and Methods: MSCs were harvested from healthy volunteers and grown in a six-well plate format for collection of conditioned medium, containing all factors secreted by the cells. Indirect co-culture was established by culturing breast cancer cell lines (T47D, MDA-MB-231, SK-BR-3) in MSC conditioned medium. Cell proliferation was assessed at 72hrs using an Apoglow® assay and cells were harvested for analysis of gene expression by RQ-PCR. Factors potentially mediating observed changes in gene expression were identified by repeating the experiments in the presence of antibodies targeting Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor b-1 (TGFb-1).
Results: Following indirect co-culture with MSCs, all three breast cancer cell lines displayed a downregulation of proliferation, with the greatest decrease seen in the T47D cells. Analysis of gene expression revealed a significant increase in expression of a panel of genes associated with Epithelial to Mesenchymal Transition (EMT) in both T47D and SK-BR-3 cell lines. In both the SK-BR-3 and T47D cell lines there was significant upregulation in expression of the mesenchymal marker Vimentin (range 158–276 fold), the anti-apoptotic transcription factor Snail (range 4–7 fold) and N-Cadherin (range 9–32 fold). Inclusion of an antibody to VEGF in the MSC- conditioned media significantly reduced the change in Vimentin expression in both cell lines. MSC secreted TGFb-1 was also shown to play a role in upregulation of N-Cadherin expression in the SK-BR-3 cell line.
Conclusion: Mesenchymal stem cells have a distinct paracrine effect on breast cancer epithelial cells, which is mediated at least in part by VEGF and TGFb-1. These factors play an important role in the metastatic cascade and may represent potential therapeutic targets to inhibit MSC-breast cancer interactions.

1009 POSTER DISCUSSION

A role for auxiliary TGF-beta receptor endoglin as a modulator of tumor progression

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Background: We and others have provided evidence for a direct role of endoglin in malignant progression. Thus, reduction of endoglin expression in endoglin heterozygous ($Eng^{+/-}$) mice had a double effect on two-stage chemical skin carcinogenesis, by inhibiting the early appearance of benign tumors (papillomas), but increasing progression to spindle cell carcinomas (SpCC).
Materials and Methods: Swiss albino mice were used for induction of tumors by initiation with DMBA and promotion with TPA for 15 weeks. Endoglin expression has been checked by qRT-PCR, Western-blot and immunohystochemistry. Luciferase reporter genes have been used to study TGFbeta pathway status. Cell growth assays "in vitro", and "in vivo" to study tumorigenicity in immunodeficiency mice
Results: Our finding that endoglin is expressed both in epidermal basal keratinocytes and in their appendages (hair follicles and sweat glands), led us to study the expression of endoglin during the different stages of chemical mouse skin carcinogenesis: benign papilloma, squamous cell carcinoma (SCC), and spindle cell carcinoma (SpCC). Endoglin undergoes a proteolitic cleavage (shedding) during the SCC to SpCC progression,

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