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

N.J. Nasser[1], A. Avivi[2], I. Vlodavsky[3], E. Nevo[2]. [1]*Shaare Zedek Medical Center, Oncology Institute, Jerusalem, Israel;* [2]*University of Haifa, Institute of Evolution, Haifa, Israel;* [3]*Technion, Cancer and Vascular Cancer Center, Haifa, Israel*

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

H. Møller[1], U. Ralfkjær[1], N. Cremers[2], J. Sleeman[2], P. Guldberg[1], E. Lukanidin[1], N. Ambartsumian[1]. [1]*Danish Cancer Society, Molecular Cancer Biology, Copenhagen, Denmark;* [2]*University of Heidelberg and Mannheim, Centre for Biomedicine and Medical Technology, Mannheim, Germany*

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

M. Schmidt[1], S. Flunkert[1], J. Mazarati[1], A. Wellstein[1]. [1]*Georgetown University, Oncology, Washington DC, USA*

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

I. Schonn[1], A. Sprüssel[1], J. Krüger[1], J. Oehlmann[1], C. Rosenbrock[1], J. Spangenberg[1], H. Juhl[1], K. David[1]. [1]*Indivumed GmbH, Cell Culture Research, Hamburg, Germany*

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