#6124 Cyclin D1 amplification is uncommon in squamous cell carcinoma of the thymus. Takeshi Fujii, Midori Kikuchi, Yasunori Sohara, Ken Saito, and Akira Tanaka. *Departments of Pathology and Thoracic Surgery, Jichi Medical School, Tochigi, Japan.*

Among thymic epithelial neoplasms, thymoma represents essentially a functioning neoplasm derived of thymic epithelial cells with an uncertain biological aggressiveness, whereas thymic carcinoma is a rare but clearly malignant thymic epithelial neoplasm, a majority of which are squamous cell carcinomas. Amplification of chromosome 11q13 is a frequent event in squamous cell carcinogenesis of the head and neck, esophagus and skin. We examined the cyclin D1 gene (*CCND1*) amplification status in 12 squamous cell carcinomas of the thymus. Fluorescence in situ hybridization (FISH), using a specific probe for the *CCND1* locus together with a reference probe for the chromosome 11 centromeric region, was performed on formalin-fixed paraffin-embedded tissue sections from 12 patients with thymic carcinomas. *CCND1* numerical aberration was identified in none of 12 thymic carcinomas. Immunohistochemical detection using anti-cyclin D1 rabbit antisera demonstrated sporadic cyclin D1 overexpression in 11 of 12 thymic carcinomas. Our data suggest that, unlike squamous cell carcinomas of the other anatomic sites, *CCND1* amplification is uncommon in squamous cell carcinogenesis of the thymus.

#6127 Combined approach to identify EWS/ETS direct target genes. J Daniel Ozeran, Habib Hamidi, and Chris Denny. *David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Many tumor-associated chromosomal abnormalities result in the expression of chimeric fusion proteins that promote oncogenesis by acting as aberrant transcription factors. Identifying direct target genes that are transcriptionally modulated by these oncogenic fusion proteins is a major challenge. We are using a combined approach of microarray expression analysis and chromatin immunoprecipitation (ChIP) to find direct target genes of EWS/ETS fusion proteins found in human Ewing's family tumors. Microarray expression analysis is used to identify a core set of target genes that are modulated by three structurally distinct EWS/ETS fusions in murine NIH 3T3 cells. A modified ChIP procedure is then used to purify and clone genomic fragments that are physically bound by epitope-tagged EWS/FLI1 in NIH 3T3 cells. Comparison of clone nucleotide sequences to public murine genome databases allows precise mapping of the genomic location of each clone. In this way large scale cross comparisons of in vivo EWS/FLI1 DNA binding sites and EWS/ETS transcriptionally modulated genes can be made. Through this strategy, the subset of genes that are transcriptionally modulated by EWS/FLI1 as a direct result of in vivo physical genomic binding, can be identified.

#6130 The influence of NEP and ECE-1 metalloproteinase expression and stromal-epithelial interactions on prostate cancer progression. Louise A. Dawson, Anthony J. Turner, Norman J. Maitland, and Badar A. Usmani. *University of Leeds, Leeds, UK and University of York, York, UK.*

Changes in stromal-epithelial interactions in the developing tumour have been reported to contribute to cancer progression and metastasis. Prostate adenocarcinoma is a highly invasive tumour with one in five men currently developing invasive prostate cancer (PC). Small bioactive peptides such as endothelin-1 (ET-1) can exert an autocrine (epithelial) or paracrine (stromal) influence on the developing PC. Active ET-1 is generated by endothelin-converting-enzyme (ECE). ECE has homology to Neutral Endopeptidase (NEP) which cleaves and inactivates mitogenic peptides including ET-1, in non-metastatic PC cells. In previous studies we have shown elevated levels of ECE-1 within the stroma adjacent to tumor epithelia in primary prostate adenocarcinomas. In this study we have investigated the interaction between metastatic tumor epithelial cells which lack NEP and stromal cells (STO) expressing ECE-1, using Matrigel invasion chambers. The cells utilised in this study include androgen-sensitive LNCaP, androgen-independent PC-3, Du145 and novel PNT-1a, PNT2-C2 and P4E6 prostate cell lines. Data show that PC cell invasion through matrigel is increased in the presence of ECE-1 expressing STO cells. SM-19712, a specific inhibitor of endogenous ECE-1 activity, reduced PC-3 invasion by 70%. Inactivating mitogenic peptides by addition of rNEP to PC-3 and Du145 cells also reduced invasion by 50% and 20% respectively, No NEP effects were observed in the presence of thiorphan (NEP inhibitor). Supplementation of defined media with bradykinin and ET-1 increased PC-3 invasion by 40% and 50% respectively. These studies reveal that stromal/epithelial interactions can influence the invasive ability of PC cells as a consequence of their NEP and ECE-1 activity. Studies are being extended to include pre-malignant and malignant prostate stroma and epithelia.

#6131 Orthotopic prostate tumors produce high levels of viable blood-borne cancer cells in contrast to subcutaneous tumors. Anna B. Glinskii, Yelena A. Ivanova, Robert M. Hoffman, and Gennadi V. Glinsky. *Sidney Kimmel Cancer Center, San Diego, CA and AntiCancer, Inc., San Diego, CA.*

Orthotopic PC-3 human prostate carcinoma models, in contrast to subcutaneous (sc) models, exhibit extensive invasive and metastatic growth. To determine whether the increased malignancy of orthotopic tumors is sufficient to produce viable circulating PC-3 cells, we isolated blood-borne PC-3 cells in nude mice bearing orthotopic or sc green fluorescent protein (GFP)-expressing PC-3 tumors. FACS analysis of fluorescent cells recovered from the blood of tumor-bearing animals showed that mice with orthotopic GFP-PC-3 tumors have a substantially higher circulatory load of cells with high green fluorescence than sc tumors. GFP-PC-3 cells survive in the circulatory system in the orthotopic models, since viable cultures of these cells could be grown and expanded in vitro after isolation by fluorescent cell sorting or by an attachment-based separation. We successfully recovered and expanded in culture viable blood-borné PC-3 cells from 75% of mice with orthotopic tumors. In contrast, we failed to recover viable blood-borne PC-3 cells from mice bearing sc tumors in multiple independent experiments. The results suggest that the orthotopic microenvironment allows selection of tumor cells capable of entering and surviving in the circulatory system.

#6132 Over-expression of erbB2 enhances ethanol-stimulated intracellular signaling and invasion of human mammary epithelial and breast cancer cells. Cuiling Ma, Hong Lin, Stephen S. Leonard, Xianglin Shi, Jianping Ye, and Jia Luo. *West Virginia University School of Medicine, Morgantown, WV, National Institute for Occupational Safety and Health, Morgantown, WV, and Louisiana State University, Baton Rouge, LA.*

Both epidemiological and experimental studies indicate that ethanol is a tumor promoter and may promote metastasis of breast cancer. However, the molecular mechanisms underlying ethanol-mediated tumor promotion remain unknown. Over-expression of ErbB proteins in breast cancer patients is generally associated with poor prognosis. The ErbB proteins are a family of receptor kinases that include four closely related members: epidermal growth factor receptor (EGFR/ErbB1), ErbB2/neu, ErbB3, and ErbB4. Particularly, ErbB2 plays a pivotal role in ErbB-mediated activities. Here we demonstrated that amplification of ErbB2 expression sensitized a cellular response to ethanol. Human breast cancer cells or mammary epithelial cells with a high expression of ErbB2 exhibited an enhanced response to ethanol-stimulated cell invasion. On the other hand, ethanol-mediated cell proliferation was similar between the cells over-expressing ErbB2 and the cells with normal expression levels. In the cells over-expressing ErbB2, ethanol was more effective in the activation of JNKs and p38 MAPK as well as the induction of reactive oxygen species (ROS) than the cell with normal ErbB2 expression. Blockage of either JNKs or p38 MAPK activation eliminated ethanol-mediated cell invasion. In contrast, inhibition of hydrogen peroxide formation by catalase had little effect on ethanol-induced cell invasion. These results indicated that ethanol-induced cell invasion was mediated by JNKs and p38 MAPK, but was independent of ROS formation. Our study suggests that over-expression of ErbB2 may augment ethanol-elicited signaling and promote ethanol-stimulated tumor metastasis.

#6133 A red fluorescent protein-expressing orthotopic metastatic model of human prostate cancer. Meng Yang, Ping Jiang, Norio Yamamoto, Lingna Li, A.R. Moossa, and Robert M. Hoffman. *AntiCancer, Inc., San Diego, CA and University of California at San Diego, San Diego, CA.*

We report here a fluorescent spontaneous metastatic model of human prostate cancer developed by surgical orthotopic implantation (SOI) and visualized by red fluorescent protein (RFP) expression. Hu-