#2987 Putative tumor suppressor genes on chromosome 11q13 for cervical cancer. Rita Chakrabarti, Thomas Simmen, Mysore S. Veena, Kayvan Zainabadi, Esther L. Chen, Eric J. Stanbridge, J. Leslie Redpath, Gary Thomas, and Eri S. Srivatsan. *Dept of Surgery, VAGLAHS/ David Geffen School of Medicine at UCLA, Los Angeles, CA, Vollum Institute, Oregon Health Science University, Portland, OR, Department of Microbiology and Molecular Genetics, Irvine, CA, and Department of Radiation Oncology, Irvine, CA.*

In earlier studies, we have demonstrated that the loss of a putative tumor suppressor gene (TSG) on chromosome 11q13 was associated with cervical cancer. Extensive molecular genetic studies with 36 tumors and corresponding normal tissues localized the minimal deletion to a 300Kb interval between markers D11S4908 and D11S5023. Additionally, deletion studies have also shown homozygous deletion of 5.7Kb of chromosome 11q13 sequences in HeLa cells (a cervical carcinoma cell line), two HeLa cell derived tumorigenic hybrids and a primary tumor. Genscan/Grail analysis of 400Kb genomic sequence overlapping the homozygous deletion showed at least 11 known genes including: Splicesome Associated Protein (SAP-145), a Cytosolic Sorter Protein (PACS-1), Breast Cancer Metastasis Suppressor (BRMS-1), mapping to the region. In addition, number of hypothetical genes and a perfect 120bp exon (52160) mapped in proximity to the homozygous deletion. Normal tissue Northern blots with the exon-52160 probe showed presence of multiple transcripts, varying in size from 0.75Kb to 5.0kb. High-level expression of transcripts was observed in heart, smooth muscle, pancreas and salivary gland. We are currently performing RACE (Rapid Amplification of cDNA Ends) to isolate full-length cDNAs representing the exon-52160. Equal expression of SAP145 and BRMS1 is observed in both the non-tumorigenic and tumorigenic hybrid cell lines. However, western blotting for PACS-1, a 114KD protein with 24 exons and spanning the homozygous deletion, shows a differential expression. PACS-1 has a higher expression in normal fibroblast and non-tumorigenic hybrid cell lines. However, HeLa cells and the tumorigenic hybrid cell lines show reduced or absence of protein expression. Thus, there is a direct correlation between the PACS1 protein levels and the suppression of tumorigenic phenotype. Expression of PACS1 in primary tumors will be investigated.

#R2988 Identification and evaluation of candidate tumor suppressor genes at chromosome 22q13.31 implicated in the development of sporadic human breast and colorectal cancer. Cameron Neil Johnstone. *University of Pennsylvania, Philadelphia, PA.*

One mechanism contributing to malignant transformation is functional inactivation of tumor suppressor genes (TSGs). TSGs are often inactivated in cancer cells via point mutation of one allele and loss of the second allele by chromosomal deletion. Thus, studies of allelic imbalance (or LOH) have been used to identify TSG-containing loci. Using a PCR-based analysis of dinucleotide repeat sequences, we previously identified a minimal region of LOH at 22q13.31 in 53% of sporadic breast tumors (1), and 22% of sporadic colorectal tumors analyzed (2). We hypothesize that a critical TSG for both breast and colorectal cancer is located within this short interval of approximately 1MB and are taking a candidate gene approach as well as a cDNA microarray approach to its identification. EST-mapping and exon prediction strategies were used to identify fourteen putative genes within the region. Three candidate TSGs (ARHGAP8, PP610 and PARVG) were selected for comprehensive analysis on the basis of homology to and/or the expression patterns of, known or putative TSGs. We confirmed their expression in mammary gland and colon and determined the transcription start sites of ARHGAP8 and PP610. Several alternatively spliced transcripts of each gene were identified in mammary gland and colon, many of which have potentially important implications for the function(s) of the encoded proteins. The coding exons of ARHGAP8, PP610 and PARVG were assessed for somatic mutation in tumors using single strand conformation polymorphism (SSCP) analysis. No tumor-specific mutations were found in any gene. AF and PP610 possess CpG-islands in their 5' regions indicating t could be potentially downregulated in tumor cells by hypermeth Hence, the expression levels of these two genes were determ paired RNA samples from tumor tissue and adjacent norma using quantitative PCR. Functionally, we demonstrated that ARI is a novel broad-specificity RHOGTPase-activating protein for RHOA, RAC1 and CDC42 in vitro. The ability of ARHGAP8 expr to inhibit RHOA-mediated actin stress fiber formation was also evaluated in vivo. Additionally, in an unbiased approach, we manufactured cDNA microarrays containing ESTs representing each of the fourteen putative genes within the 1MB region of deletion and are examining their relative expression levels in paired colorectal tumor/normal colon and breast tumor/normal mammary gland. In summary, LOH analyses have led to the identification of a minimal region of deletion on chromosome 22q13.31 in breast and colorectal tumors. We have characterized the structures of candidate TSGs in the region and assessed them for involvement in breast and colorectal tumors. 1 Castells A. et al., Cancer Res. (2000) 60:2836-2839 2 Castells A. et al., Gastroenterology (1999) 117:831-837

#2989 Dlc-1, a new tumor suppressor gene for human non-small-cell lung carcinomas as well as other common kinds of cancer. Bao-Zhu Yuan, Amy M. Jefferson, Kimberly T. Baldwin, and Steven H. Reynolds. *National Institute for Occupational Safety and Health, Morgantown, WV.*

DLC-1 (Deleted in Liver Cancer) was originally isolated from a liver cancer tissue as a candidate tumor suppressor gene. It is localized on human chromosome 8p22, a region frequently deleted in several common kinds of human cancer including liver, breast and lung cancers. One of three well-characterized domains in DLC-1 gene is the GTPase Activating Protein (GAP) domain, which can inactivate RhoA, a member of Ras oncogene superfamily. In previous studies, down-regulation of DLC-1 was found in 28% of liver, 70% of breast, 70% of colon and in 50% of prostate tumor cell lines. Genomic deletion of DLC-1 was observed in 40% of primary liver and breast cancers, and in 90% of liver cancer cell lines. DLC-1 is also a hypermethylated gene. DNA hypermethylation in the promoter region of DLC-1 was found in liver, breast, colon and prostate cancer cell lines and is directly associated with the down-regulation of the gene. Cell growth inhibition by DLC-1 in an in vitro cell proliferation assay was observed in both liver and breast cancer cell lines. Complete suppression of tumorigenicity was observed in breast cancer cells injected into nude mice after DLC-1 gene transfection. Our new studies of DLC-1 on human non-small cell lung carcinoma (NSCLC) cell lines suggest that DLC-1 is also a bona fide tumor suppressor gene for lung cancer. Decreased or no expression of DLC-1 gene was found by RT-PCR in 11 out of 19 NSCLC cell lines. Of these 11 cell lines, nine showed re-expression of the gene after treatment with 5-aza-2'-deoxycytidine, a DNA demethylation agent. Promoter DNA hypermethylation was detected by Southern blot analysis in 80% of human NSCLC cell lines showing down-regulation of the DLC-1. Significant in vitro growth inhibition was observed in four NSCLC cell lines after transfection with the DLC-1 gene a an in vitro cell proliferation assay. To date, in an in vivo nude mice tumorigenicity assay, complete suppression of tumor growth was observed in two of three NSCLC cell lines transfected with DLC-1. The combined data suggests that DLC-1 may be a new important tumor suppressor gene for several kinds of human cancer that is frequently targeted and inactivated either by genomic deletion, as in human liver and breast cancers, or by DNA hypermethylation, as in human lung cancer.

#2990 Identification and characterization of two novel brain tumor suppressors. Victoria Robb, Wen Li, Katherine Lee, Philippe Gascard, Narla Mohandas, Arie Perry, and David Gutmann. *Washington University, St. Louis, MO, Lawrence Berkeley National Laboratory, Berkeley, CA, and New York Blood Center, New York, NY.*

Meningioma is the second most common tumor of the central nervous system, however the genetic events underlying its pathogenesis are largely unknown. The most frequent genetic alteration detected in meningioma is chromosome 22q loss of heterozygosity (LOH) with inactivation of the neurofibromatosis 2 (*NF2*) tumor suppressor gene. The *NF2* gene product, [illegible] f the Protein 4.1 superprotein, Ezrin, Radixin, ave demonstrated *NF2* sporadic meningiomas r in meningioma cells. bers of the Protein 4.1 as tumor suppressors)% of sporadic meningtion (FISH) and immuntain three highly con-