#R1197 Reciprocal translocations in mucoepidermoid carcinoma. Giovanni Tonon, Kristen Stover, Raluca Yonescu, Anna Roschke, Frederic J. Kaye, and Ilan R. Kirsch. *Genetics Branch, National Cancer Institute, Bethesda, MD.*

Mucoepidermoid carcinoma is the most common human malignant salivary gland tumor and can arise from both major salivary glands and minor salivary glands including sites within the pulmonary tracheobronchial tree. We performed Comparative Genomic Hybridization (CGH) and Spectral Karyotyping (SKY) on two tumor cell lines that were generated from tumors located in either the parotid gland or the lungs. In both cell lines, CGH showed a gain at the chromosomal band 7p21 and SKY demonstrated the presence of the previously reported balanced translocation t(11;19)(q21;p12). Multiple chromosomal rearrangements were also present in both cell lines, including four reciprocal translocations in cell line H292 (t(1;16), t(6;8)x2 and t(11;19)), and four reciprocal translocations in cell line H3118 (t(1;7), t(3;15), t(7;15), t(11;19)). A review of the literature confirmed the presence of a pattern predominated by reciprocal translocations in other reported cases of mucoepidermoid carcinomas analyzed with standard G-banding techniques, as well as distinct benign salivary gland tumors like pleomorphic adenomas and Warthin's tumor. Among solid tumors, only some pediatric sarcomas present a similar cytogenetic feature with frequent reciprocal chromosomal rearrangements. In addition to the shared characteristic t(11;19), fluorescence in situ hybridization with BAC clones demonstrated the presence of translocations involving the same chromosomal bands among the two cell lines. In particular, one partner of two distinct translocations in the two cell lines presented a breakpoint in an interval of less than 3.5 megabases, at 5p15. The involvement of similar chromosomal bands in breakpoints in these two cell lines suggests that this additional region may be selected or predisposed to chromosomal rearrangements in this tumor type. The presence of multiple reciprocal translocations in selected benign and malignant salivary gland tumors may also suggest a mechanism within mucous/serous glands mediating chromosomal rearrangements.

#1198 Increased gene copy number may alter E2F1 activity in melanoma growth. Mark A. Nelson, Anthony Beas, Anne Christine Goulet, David T. Lowry, Steven H. Reynolds, Amy M. Jefferson, Jamie R. Senft, and Linda Sargent. *Department of Pathology, The University of Arizona, Tucson, AZ and Centers for Disease Control, National Institute for Occupational Safety and Health, Morgantown, WV.*

The identification of recurring translocations and unique chromosome break points in melanoma will aid in the identification of the genes that are important in the neoplastic process. Previous studies by our group identified translocations der(12)t(12;20) in malignant melanoma cells. The transcription factor E2F1 maps to 20q11. Deregulated E2F transcriptional activity has been associated with the autonomous growth of melanoma cells, but the molecular basis has not yet been elucidated. To this end, we investigated E2F1 gene copy number and structure in nine different early passage human melanoma cell lines. Fluorescent in situ hybridization analysis using a specific E2F1 probe indicated increased E2F1 gene copies in several melanoma cell lines compared to normal melanocytes. The FISH observations were confirmed by comparative genomic hybridization array to BAC clones and Southern Blot analysis. In addition, Western blot analysis demonstrated increased E2F1 and DP-1 protein levels in 8 out of 9 melanoma cells compared to normal melanocytes. These data suggests that the release of E2F activity by elevated E2F1 gene copy numbers may play a functional role in melanoma growth. (Supported by CA 70145).

#1199 Frequent alterations of β-catenin gene in gastric cancers with microsatellite instability. Jiang Wang, Tara Shepherd, Andrew Lowy, Amy Noffsinger, Grant Stemmermann, and Cecelia Fenoglio-Preiser. *University of Cincinnati College of Medicine, Cincinnati, OH.*

Gastric cancer is one of the commonest malignances in the world, but the genetic mechanisms of gastric carcinogenesis are not well understood. Recent studies have shown that microsatellite instability (MSI) is a characteristic of this cancer. To better understand the molecular mechanism of gastric cancer with MSI, we analyzed the alterations of beta-catenin, an important component of Wnt pathway. Laser capture microdissection (LCM) samples from 118 gastric cancers were analyzed for ten MSI markers using fluorescence-labeled primers after amplification by PCR. The expression of beta-catenin was evaluated by immunostaining for these samples and PCR products of exon 3 of this gene were directly sequenced. Tumors were classified as follows: 20 (17%) high-frequency MSI (MSI-H), 27 (23%) low-frequency MSI (MSI-L), and 71 (60%) microsatellite stable (MSS). Abnormal beta-catenin nuclear accumulation was detected in 75 tumors, which is more commonly in MSI-H (85%) and MSI-L (78%) tumors than in MSS samples (52%) ($P=0.017$ and 0.038 respectively). 16 mutations were identified in 104 informative samples. MSI-H samples harbored more beta-catenin mutations (33%) than MSS samples (9%) ($P=0.041$). There is no mutation difference between MSI-L (23%) and MSS ($P=0.164$) or MSI-H ($P=0.708$). Our results suggest that MSI may involve in the activation of the Wnt signaling pathway and play an important role important role in gastric tumorigenesis.

#1200 Aurora2 amplification and chromosomal instability in human pancreatic cancer cell lines. Jijiang Zhu, James L. Abbruzzese, Julie Izzo, Walter N. Hittelman, and Donghui Li. *University of Texas M.D. Anderson Cancer Center, Houston, TX.*

Aurora2 (STK15/BTAK) is a mitotic kinase that is localized to centrosome and plays an important role in maintaining genomic integrity by ensuring the formation of bipolar spindles and equal segregation of the duplicated chromosomes during the mitosis. Overexpression of Aurora2 had been related to centrosome amplification, aneuploidy and cell transformation. Cytogenetic and molecular studies showed that a high proportion of human pancreatic cancers had chromosome abnormalities. We have previously shown that Aurora2 was overexpressed in pancreatic carcinoma cell lines. The current study is aimed to explore the association between Aurora2 amplification and chromosomal instability as well as centrosome abnormality in pancreatic cancer. Aurora2 amplification and chromosome 9 and 17 instabilities were examined by fluorescent in situ hybridization in 12 human pancreatic cancer cell lines. Centrosome abnormalities were examined by immunofluorescence using anti-pericentrin or anti-γ-tubulin antibodies. Abnormal mitosis (multipolar mitosis) was detected by examining the immunofluorescent staining of spindle using an anti-α-tubulin antibody. The percentage of cells with more than 3 signals was defined as Aurora2 amplification index (AAI). The average number of Aurora2 signals per cell (ANASPC) was calculated. Chromosomal instability was defined as the percentage of cells with non-modal numbers of signals. Abnormal centrosomes were defined as more than 3 centrosomes per cell or large patchy aggregate signals. Both AAI and ANASPC were significantly correlated to instability of chromosome 9 ($P = 0.01$ and 0.014, respectively) but not chromosome 17. ANASPC was correlated with centrosome abnormality as detected by anti-γ-tubulin antibody ($P = 0.042$). Centrosome abnormality detected by anti-pericentrin antibody was significantly correlated with abnormal mitosis ($P = 0.02$). But centrosome abnormalities showed no significant correlation with chromosomal instabilities. These observations suggest that Aurora2 amplification may cause centrosome abnormalities and abnormal mitosis, which in turn lead to chromosome gains or loss and subsequent chromosomal instability.

#1202 CGH-based microarrays provide for a genomic fingerprint of prostate cancer. Timothy Mark Lane, Dan Berney, John Strefford, Bryan Young, and R. Tim Oliver. *Department of Medical Oncology, St Bartholomews Hospital, London, UK.*

It has been estimated that between 5-10 deletions of tumour suppressor genes are required before malignant transformation takes place and that a variable number of genetic gains are acquired before metastatic potential is achieved. Defining the pattern of loss and gain is essential if new and informative genetic markers of disease progression are to be defined. The application of microarray technology to the investigation of genomic copy number change in the form of CGH-based microarrays has revolutionised the molecular and cytogentic investigation of malignancy. A series of 50 paraffin-embedded prostate cancer specimens were identified ranging the pathological spectrum from high grade prostatic intraepithelial neoplasia (HGPIN) through a variety of gleeson scores to hormone resistant and metastatic disease. Following histopathological review, laser capture microscopy was used to micro-dissect tumour specimens. Sample DNA and reference controls underwent random primer labelling with Cy3 and Cy5 fluorophores and were co-hybridised onto arrays containing a series of nearly 300 genes previously implicated in tumorigenesis. Following counter-stain-