343 The Association of Cytoplasmic Overexpression of Cycillin D1 and Tumor Metastasis in Hepatocellular Carcinoma (HCC). Y. Wang4, M. C. Wu4, D. Xie4, D. J. Tang4, X. Y. Guan1. 1Clinical Oncology, UNI Hong Kong, Hong Kong, China; 2Department of Pathology, Sun Yat-sen University, Guangzhou, China; 3Eastern Hepatobiliary Surgery Hospital, The Second Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; 4Department of Hepatobiliary Surgery, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China.

Hepatocellular Carcinoma (HCC) is one of the worldwide most common malignant tumors with poor prognosis. In the present study, a marker chromosome containing a homozygously missing region (HSR) in a recently established metastatic HCC cell line (H4-M) was characterized by comparative genomic hybridization and chromosome microdissection. The result showed that the HSR was composed of DNA sequence from 11q13 and amplification of cycillin D1 (CCND1) in H4-M was confirmed by fluorescence in situ hybridization using a cosmid clone containing CCND1 gene. Amplification and overexpression of CCND1 in H4-M has been demonstrated by Southern blot, Northern blot, and Western blot analysis. Immunohistochemical staining showed that the overexpression of CCND1 was located in cytoplasm in H4-M. Further study in a tissue microarray with 339 HCC samples showed that cytoplasmic overexpression of CCND1 was significantly higher in HCC metastasis (115/36 cases, 32%) than that in HCC without metastasis (23/186 cases, 12%) (P=0.001). This finding strongly suggested that the cytoplasmic overexpression of CCND1 may play an important role in the metastasis of HCC.

344 Genome-wide search for homozygous deletions in oral cancer. H. Kayaahara1, H. Yamagata2, T. Miyosaki3, M. Asa-Ochii4, J. Nakura2, I. Kondoh2, T. Miki2, H. Hamakawa1, 1Dept Oral and Maxillofacial Surgery, Ehime Univ Sch Medicine, Onsen- gun, Etsu, Japan; 2J callable Hygiene, Ehime Med Sch, Ehime, Japan; 3Dept Geriatric Medicine, Ehime Univ Sch Medicine, Ehime, Japan.

To date, in head and neck cancer including oral squamous cell carcinoma (OSCC), loss of heterozygosity (LOH) has been identified on chromosomes 3q, 3p, 4q, 7q, 9q, 9p, 10q, 11q, 13q, 14q, 17b, 18p and 22q. These findings suggest that some of these regions might contain tumor suppressor gene(s). In this study, we tested the presence of homozygous deletions (LOH) in OSCC using 120 microsatellite markers and the allelic loss data were analyzed by Genotyper software. The deletion was found in the following regions on 3p, 3q, 4q, 5q, 7q, 9q, 9p, and 10q.

345 Study of chromosomal abnormalities in esophageal squamous cell carcinoma by comparative genomic hybridization. L. Wei2, X. Liu1, W. S. Wang1, J. S. Sham1, J. Guan. Clinical Oncology, The University of Hong Kong, Hong Kong, China.

Esophageal carcinoma is the nine most common cancer in the human worldwide. In China, its incidence is particularly high compared to the western countries. Squamous cell carcinoma is the dominant histological type found in the Asian populations. In this study, comparative genomic hybridization was used to screen for the genomic alterations among 60 primary esophageal squamous cell carcinoma cases globally. Chromosomes 3, 3q, 4, 4q, 7, 7q, 9, 9q, 12, 13, 15, 15q, 21, 22, and Y were found to be commonly involved in this malignancy of esophageal squamous cell carcinoma. 7q, 7q31-q32.3, 15q, 16q22-q23.3, and Y lost in copy number.

346 Molecular FISH markers for metastasis in ductal breast carcinoma. H. Zhao, J. G. Johnson2, B. Klinger, B. Vikram, P. M. Acharya. Albert Einstein College of medicine, Bronx, NY.

The objective of this project is to construct a panel of molecular genetic markers for detecting those 10% of breast cancer patients with negative lymph nodes, so that they could be treated more aggressively. Representational Difference Analysis was used to compare the DNA of cells from archival normal tissue or primary ductal tumor with that of the metastatic lymph node of the same patient in order to isolate those sequences that were lost in the course of tumor metastasis. The tumor cells were recovered by laser capture microdissection. We isolated 11 sequences that are candidates for metastasis associated genes (MAGs) because they were lost in metastatic cells. To-date three of these 11 sequences were used to screen normal, primary and metastatic cell DNA samples. MAGS-XI was found to be lost in the metastatic cells of 3 out of the 5 tumors. MAGS-IX was found to be lost in metastases from 2 out of 5 primary tumors, and MAGS-IV was found to be lost in 3 out of 4 primary tumors which may have important clinical implications. The significance of our findings is that 11 sequences out of 243 seem to be potential MAGs. Presently we are isolating partial and/or full-length sequences of these MAGS to use as fluorescent in situ hybridization (FISH) probes to screen DNA samples. A 2kb sized MAGS-IX has been generated and localized to the 8q21 region of human chromosome 8 number 10 by FISH. Screening of MAGS-IX as fish probe in the primary tumor was in 1 out of 3 breast tumor and metastases to lungs indicated that MAGS-IX was located on the long arm of chromosome 10 where the PTEN gene is located. Use of FISH should help in the identification of the PTEN gene as a potential metastasis marker. MAGS-IX is as a potential marker in that it could possibly be used as a FISH probe to identify primaries that are prone to develop metastasis.

347 MLH1 exon 3 deletion in cDNA associated with 213_215delAGAA: Probable exon splicing enhancer mutation as a cause of HNPCC. G. Chong2, A. Quellec1, L. LeMieux3, I. Thiffault1, E. MacKinnon2, W. F. Powell1, 1Department of Human Genetics; 2Department of Medicine; 3Department of Pathology; 4Program in Cancer Genetics, Mcgill University, Montreal, QC, Canada; 5Diagnostic Medicine Department, SMBD-Jewish General Hospital, Montreal, QC, Canada; 6Medical Genetics Service,University of Sherbrooke Medical Centre, Sherbrooke, QC, Canada.

We describe a new mutation in MLH1 in an Amsterdam criteria fulfilling HNPCC kindred. The proband is a 53 yr old woman who was diagnosed with colon cancer at age 49. Her brother was diagnosed with rectal cancer at 30 and died at age 48. Her son died of colon cancer at 28. IHC showed loss of MLH1 protein in the two available colon cancers. RT-PCR analysis of her cDNA revealed a spliced product, which on sequencing was found to be caused by the entire in-frame deletion of exon 3. Sequencing of the genomic DNA did not detect any splice site variant which might have explained the loss of exon 3. Wang L, et al, 2008. A 3 bp deletion at nt 213, predicted to result in ΔE71, was detected. The same deletion was also seen in her son from whom. These findings suggest that the exon 3 deletion has resulted from the 213_215delAGAA (77%). The relevance around the mutation is purine-rich (AAAGAAGTA), and as these have been associated with exon splicing enhancers (ESEs), we postulate that 213_215delAGAA has removed an ESE for exon 3. An in-frame deletion in exon 7 of SMN has been previously associated with aberrant splicing of this exon 3 and is thought to be disease-causing. Similar ESEs (AAAGAAGTA) have also been identified in exon E3Δ1Galactosidase (exon 3). As the significance of our mutation may not have been appreciated without examination of both DNA and RNA this case highlights the importance of multi-modal molecular screening for mutations in mismatch repair genes.

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