Title	The association of cytoplasmic overexpression of cyclin d1 and tumor metastasis in Hepatocellular Carcinoma
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Molecular characterization of a constitutional t(5;6)(q21;q21) in a patient with bilateral Wilms' tumor. *C.D. Day[1], S-I. Matsui[1], P. Liang[1], N.J. Nowak[1], S. Jani-Sait[2], J.K. Cowell[1], M.J. Higgins[1].* 1) Dept Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY; 2) Dept Cytogenetics, Roswell Park Cancer Inst, Buffalo, NY.

Wilms' tumor, one of the most common pediatric tumors, is believed to arise from persistent metanephric blastema cells that fail to differentiate. Although the*WT1* gene (11p13) was identified more than a decade ago, it remains the only tumor suppressor gene known to be involved in Wilms tumor; furthermore, *WT1* is mutated in less than 10% of cases. Analysis of chromosomal rearrangements, particularly translocations, has been extremely useful in the identification of genes involved in cancer. Our lab has been investigating a balanced constitutional translocation in a patient with bilateral Wilms' tumor. The breakpoints of this t(5;6)(q21;q21) have been previously mapped between *D5S495* and *D5S433* on chromosome 5 and between *D6S301* and *D6S447* on chromosome 6, genetic distances of 4 cM and 3 cM, respectively (Hoban et al., J Med. Genet. 34:343-345, 1997). Using BAC clones selected from the interval between these flanking markers as FISH probes, we have narrowed the breakpoint region to approximately 100 kb by identifying BACs from both chromosomes 5 and 6 that span the translocation breakpoints. The derivative chromosomes have also been segregated into somatic cell hybrids to facilitate molecular analysis of the chromosome rearrangements. Three candidate transcripts, a known gene, an EST, and a predicted gene, map within the breakpoint regions. These genes are being assessed for changes in structure and/or expression resulting from the translocation. Analysis of our large Wilms tumor tissue collection and other tumor types will establish the involvement of one or more of these genes in human cancers. Funded by NCI/NIH CA63333 to MJH.

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The Association of Cytoplasmic Overexpression of Cyclin D1 and Tumor Metastasis in Hepatocellular Carcinoma. *L. Hu[1], J.M. Wen[2], W.S. Wang[3], J.S.T. Sham[1], Y. Wang[4], M.C. Wu[4], D. Xie[1], D.J. Tang[1], X.Y. Guan[1].* 1) Clinical Oncology, Univ Hong Kong, Hong Kong, China; 2) Department of Pathology, Sun Yat-sen University, Guangzhou, China; 3) Department of Hepatobilliary Surgery, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; 4) Eastern Hepatobilliary Surgery Hospital, The Second Military Medical University, Shanghai, 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 homogeneously staining 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 cyclin D1 (CCND1) in H4-M was confirmed by fluorescence in situ hybridization (FISH) using a BAC clone containing CCND1 gene. Amplification and overexpression of CCND1in H4-M has been demonstrated by Southern blot, Northern blot, and Western blot analyses. Immunohistochemical staining showed that the overexpression of CCND1 was located in cytoplasm in H4-M. Further study using a tissue microarray with 320 HCC samples showed that cytoplasmic overexpression of CCND1 was significantly higher in HCC with metastasis (19/56 cases, 34%) 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.

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Genome-wide search for homozygous deletions in oral cancer. *H. Kayahara[1], H. Yamagata[2, 3], T. Miyoshi[1], M. Abe-Ochi[3], J. Nakura[3], I. Kondo[2], T. Miki[3], H. Hamakawa[1].* 1) Dept Oral and Maxillofacial Surgery, Ehime Univ Sch Medicine, Onsen-gun, Ehime, Japan; 2) Dept Hygiene, Ehime Univ Sch Medicine, Ehime, Japan; 3) Dept 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 2q, 3p, 4q, 7q, 8p, 9p, 10q, 11q, 13q, 14q, 17p, 18q and 22q. These findings suggest that some of these regions may contain a tumor suppressor gene. However, the most studies failed to isolate the candidate genes, except for DPC4 in colon cancer and PTEN in breast cancer that came from the mapping of homozygous deletions. To identify the putative tumor suppressor gene locus, we analyzed 6 cell lines from OSCC with 811 microsatellite markers (ABI PRISM Linkage Mapping Sets LMS-HD5) covering the entire chromosome except Y for allelic changes. Multiplex PCR products were electrophoresed on an ABI PRISM 3100 Genetic Analyzer and the fluorescent signals from the different sized alleles were recorded and analyzed by Genotyper and GeneScan. Homozygous deletions were found in 38/811 markers (4.7%) on average. Among them, 26 markers were screened for further studies in 12 OSCC cell lines. We identified three homozygously deleted regions in 12 cell lines. The candidate gene loci were as follows: D6S292 (6q23.2), D10S192 (10q24.32), and D18S68 (18q22.1). At present, no gene deletions have been identified in the centromeric and telomeric boundary of these loci.

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Study of chromosomal abnormalities in esophageal squamous cell carcinoma by comparative genomic hybridization. *A.L.S. Tai, D.L.W. Kwong, J.S.T. Sham, X.Y. Guan.* Clinical Oncology, The University of Hong Kong, Hong Kong, China.

Esophageal carcinoma ranks among the nine most common cancer worldwide. In China, its incident rate is particularly high compare 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. Chromosomal aberrations were detected in 52 cases. The frequent chromosomal gains were detected in 3q (67.3%), 8q (57.7%), 5p (51.9%), 7q (28.8%), 15q (28.8%), 20q (28.8%), 20p (21.1%), 1q (26.9%), 7p (26.9%), 2p (23.1%) and 12p (23.1%), where the chromosomal losses involved 3p (46.2%), 4q (26.9%), 4p (23.1%), 3q (19.2%), 9p (17.3%) and 13 (15.4%). High copy number amplifications were found in 3q and 8q among 10 and 8 cases, respectively, with minimum overlapping regions of 3q26.1-26.2 and 8q24.1-24.2. Interphase-FISH, using BAC clones was used to study the expression of eIF-5A2 and myc. Amplification of both eIF-5A2 and myc was found. In summary, genomic changes are common in esophageal squamous cell carcinoma.

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

The objective of this project is to construct a panel of molecular genetic markers for detecting those 13% 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 lost in 1 out of 3 tumors. RH mapping and homology search results indicated that MAGS-IX was located on the long arm of chromosome 10 where the PTEN, a known metastasis suppressor gene is also located. To determine if MAGS-IX is perhaps a part of the PTEN gene we PCR screened the above mentioned five tumor cell DNA samples and a breast carcinoma cell line, HCC-1937, which has homozygous loss of the PTEN gene. The results indicated that MAGS-IX is a novel gene sequence. Presently we are isolating partial and/or full-length sequences of these MAGS to use as fluorescence in situ hybridization (FISH) probes to screen a larger number of tumor samples. A 2Kb sized MAGS-IX has been generated and localized to the q21 region of human chromosome number 10 by FISH. Screening of MAGS-IX as fish probe in the primary tumor tissue sections of a breast carcinoma which metastasized to lymph nodes showed nuclei with signals indicating normal, heterozygous and homozygous losses of MAGS-IX in the ratio of 11:13:1 respectively. We conclude that the MAGS-IX could possibly be used as a FISH probe to identify primaries that are prone to develop metastasis.

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***MLH1* exon 3 deletion in cDNA associated with 213_215delAGA: Probable exon splicing enhancer mutation as a cause of HNPCC.** *G. Chong[1,2,5], G. Ouellette[6], B. Lemieux[6], V. Marcus[3], I. Thiffault[1], E. MacNamara[2,3,5], W. Foulkes[1,4].* 1) Department of Human Genetics; 2) Department of Medicine; 3) Department of Pathology; 4) Program in Cancer Genetics, McGill University, Montreal, QC, Canada; 5) Diagnostic Medicine Department, SMBD-Jewish General Hospital, Montreal, QC, Canada; 6) Medical Genetics Service,University of Sherbrooke Medical Centre, Sherbrooke, QC, Canada.

We describe a new mutation in *MLH1* in an Amsterdam criteria I-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 39 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. PTT revealed an abnormal truncated protein. RT-PCR analysis of her cDNA revealed a shortened 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 deletion of exon 3 in the cDNA. However, a 3 bp deletion at nt 213, predicted to result in ΔE71, was detected. The same deletion was found in the tumor from her son, with LOH. These findings suggest that the exon 3 deletion has resulted from the 213_215delAGA genomic alteration. The sequence around the mutation is purine-rich (AAAGAAGAT), and as these have been associated with exon splicing enhancers (ESEs), we postulate that 213_215delAGA 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[1] and is thought to be disease-causing. Similar ESEs (-AAGAAGA-) have also been identified in the genes for human fibronectin (exon ED1)[2] and calcitonin (exon 3)[3]. As the significance of our mutation would not have been appreciated without examination of both DNA and RNA, this finding illustrates one benefit of multi-modal molecular screening for mutations in mismatch repair genes.

References **1.** Cartegni L. et al. Nature Rev Genetics (2002) 3:285-298. **2.** Lavigueur A. et al. Genes Dev (1993) 7:2405-2417. **3.** Yeakley M. et al. PNAS USA (1996) 43:7582-7587.