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Tumor classification using phylogenetic methods on expression data. *A.A. Schaffer[1], R. Desper[1], J. Khan[2].* 1) NCBI/NIH/DHHS, Bethesda, MD; 2) NCI/NIH/DHHS, Gaithersburg, MD.

Tumor classification is a well-studied problem in bioinformatics. Developments in the field of DNA chip design have now made it possible to measure the expression levels of thousands of genes in sample tissue from healthy cell lines or tumors. A number of studies have examined the problem of tumor classification: using the data from a DNA chip to accurately classify an unknown tumor. This problem is of high interest whether one seeks to differentiate cancerous tissue from healthy tissue, or whether one seeks to accurately classify unknown tumors according to a diagnostic or prognostic category. Our work applies phylogentic methods to this problem. We impose a metric on a set of tumors as a function of their gene expression levels, and we seek to infer a tree structure from the distance data, using tree fitting methods borrowed from the field of phylogenetics. Phylogenetic methods provide a simple way of imposing a hierarchical relationship on the data, with branch lengths in a phylogeny representing a degree of separation. We demonstrate the flexibility and robustness of the phylogenetic method with regard to resampling methods such as jackknifing and with regard to noise perturbation of the real data. Using our methods on a published data set of 87 tissues, comprised mostly of small, round, blue-cell tumors (SRBCTs),we fit the 87 samples to a pseudo-phylogenetic tree, which neatly separated into 4 major clusters corresponding exactly to the four groups of tumors: nuroblastomas, rhabdomyorsarcomas, Burkitt's lymphomas, and Ewing's sarcomas. We also tested our methods on a published data set of 22 breast tumors. The resulting tree separated tumors with BRCA1 mutations from those with BRCA2 mutations, with sporadic tumors separated from both other groups and from each other.

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Differential reduction of qkI isoforms in human glioma cell lines. *L. Ku, Y. Feng.* Emory University, Department of Pharmacology, Atlanta, GA b.

Cytogenetic alterations at 6q25-26 has been reported to associate with a variety of human malignancies, including gliomas. However, which gene(s) at this locus may play a role as potential tumor suppressor mains unclear. The human quaking gene (Hqk) has been recently mapped to 6q25-26, which encodes a selective RNA-binding protein QKI, a member of the signal transduction activators of RNA (STAR). Three major isoforms of QKI are derived from alternative splicing of the qkI primary transcript, which are named QKI-5, 6 and7 based on the length of the corresponding mRNA. The role of QKI has been implicated in cell growth, differentiation, as well as apoptosis. In the brain, all QKI isoforms are expressed in various types of glial cells but absent in neurons. We analyzed qkI mRNA expression in 23 glioma cell lines. Our preliminary result indicated that around one third of the tumor lines showed significant reduction of the total qkI transcripts. Interestingly, qkI-7, the isoform that can act as a potent apoptosis inducer, was preferentially reduced. Moreover, qkI-6, the isoform that promotes glia differentiation was diminished in most of the glioma lines analyzed. In contrast, qkI-5, the embryonic predominant isoform remains normal in all the glioma cell lines analyzed. These results suggest that abnormalities of qkI alternative splicing may affect glial differentiation and apoptosis, potentially in turn contributes to glioma tumorigenesis.

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Increased chromosomal instability at common fragile sites in Seckel syndrome. *S.G. Durkin, A.M. Casper, T.W. Glover.* Department of Human Genetics, University of Michigan, Ann Arbor, MI.

The partial perturbation of DNA replication induces the expression of common fragile sites. These sites are detected as gaps and breaks on metaphase chromosomes under conditions of replicative stress, such as from aphidicolin treatment or folate deficiency. Rather than arising by mutation, common fragile sites are a constant component of chromosome structure. These sites are often rearranged in tumor cells, and thus are important for understanding chromosomal instability observed in cancer. We have previously shown that ATR, a gene critical to S phase and G2/M checkpoint signaling in response to stalled replication forks, is crucial for the maintenance of chromosomal stability at common fragile sites. Cells lacking ATR are non-viable, making the effects of ATR deficiency challenging to study. Recently, however, a subgroup of patients with Seckel sydrome was reported by O'Driscoll et al. (Nat. Genet. 33:497-501, 2003) to have a mutation in ATR. Seckel syndrome is a heterogeneous disorder characterized by severe dwarfism, mental retardation, microcephaly, and in some cases, chromosome instability, hematological disorders and leukemia. Two Pakistani families with Seckel syndrome were found to have a silent mutation in ATR that resulted in use of cryptic splice donor sites in exon 9, leading to a frameshift. Homozygotes for this hypomorphic allele produce greatly reduced levels of correctly spliced message. We hypothesized that cells from Seckel syndrome patients would have increased instability at common fragile sites. We obtained lymphoblastoid cell lines from two affected and three unaffected members of these families. ATR deficiency in all affected individuals was confirmed by western blots. Following treatment with aphidicolin, cells homozygous for the hypomorphic allele showed a ~3 fold increase in total gaps and breaks, and in breaks at specific fragile sites, compared to unaffected controls. These results are consistent with our previous findings that ATR deficiency results in increased fragile site instability and suggest that the Seckel syndrome hypomorphic mutation provides a model for studying the biological effects of fragile site instability.

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The Association of Up-regulation of FGF3 and Hepatocellular Carcinoma Metastasis and Recurrence. *X-Y. Guan[1], L. Hu[1], D. Xie[1], J.S. Sham[1], J-M. Wen[2], W-S. Wang[3].* 1) Department Clinical Oncology, University Hong Kong, Hong Kong, China; 2) Department Pathology, Sun Yat-Sen University, Guangzhou, China; 3) Department Surgery, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China.

Hepatocellular Carcinoma (HCC) is one of the most common malignancies worldwide with poor prognosis. The poor prognosis of HCC has been associated with the tumor metastasis and recurrence. Therefore, it is imperative to completely understand the molecular mechanisms involved in the tumor metastasis in HCC. 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 chromosome microdissection. The result showed that the HSR was composed of DNA sequence from 11q13. Amplification and overexpression status of CCND1-FGF19-FGF4-FGF3 gene cluster in 11q13 in H4-M was analyzed by Southern blot and Northern blot hybridizations. Amplification and overexpression of CCND1 and FGF3 were detected. The association of overexpression of FGF3 and HCC metastasis as well as recurrence was studied using a tissue microarray composed of 60 pairs of primary and matched metastatic HCCs and 30 pairs of primary and matched recurrent HCCs. The results showed that the overexpression frequency of FGF3 was significantly higher in metastatic HCC (19/40, 47.5%) than that in primary HCC (3/40, 7.5%). Similarly, the frequency of FGF3 overexpression was significantly higher in recurrent HCC (9/21, 42.86%) than that in primary HCC (1/21, 4.8%). Our results strongly suggested that up-regulation of FGF3 may play an important role in metastasis and recurrence of HCC.

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Altered Notch signaling resulting from expression of a WAMTP1-MAML2 gene fusion in mucoepidermoid carcinomas. *F. Enlund[1], A. Behboudi[1], Y. Andren[1], C. Öberg[2], U. Lendahl[2], J. Mark[1], G. Stenman[1].* 1) Lundberg Laboratory of Cancer Research, Dept ofPathology, Univ. Gothenburg, Sweden; 2) Dept of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden.

Chromosome translocations in neoplasia commonly result in fusion genes that may encode either novel fusion proteins or normal, but ectopically expressed, proteins. Here we report the cloning of a novel fusion gene in a common type of salivary and bronchial gland tumor, mucoepidermoid carcinoma (MEC). The fusion, which results from a t(11;19)(q21-22;p13) translocation, creates a chimeric gene in which exon 1 of a novel gene of unknown function, designated WAMTP, is linked to exons 2-5 of the recently identified Mastermind-like Notch coactivator MAML2. In the fusion protein, the N-terminal basic domain of MAML2, which is required for binding to intracellular Notch (ICD), is replaced by an unrelated N-terminal sequence from WAMTP1. Northern blot analyses revealed that the expression of WAMTP1 is restricted to certain fetal and adult tissues while MAML2 is expressed in most tissues. The fusion protein was found to colocalize with both MAML2 and Notch1 ICD to nuclear granules. Analysis of Notch target genes revealed altered expression of several genes in fusion positive MECs compared to normal salivary gland tissue and MECs lacking the fusion. These findings suggest that altered Notch signaling plays an important role in the genesis of neoplasms of salivary and bronchial gland origin.

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Differential Expression of a Gonadoblastoma Candidate Gene in Gonadoblastoma, Testis and Prostate Cancer Suggests a Role of the Y Chromosome in Human Oncogenesis. *Y.-F.C. Lau, H. Lau, Y. Li, L.G. Komuves.* Dept Medicine/ VA Med Ctr/ Univ California, San Francisco, CA.

Gonadoblastoma on the Y chromosome (GBY) is an oncogenic locus that predisposes the dysgenetic gonads of XY sex-reversed females to tumorigenesis at high frequency. Recent completion of the human Y chromosome sequencing revealed the testis-specific protein Y-encoded (TSPY) gene as the only functional gene in the GBY critical region. TSPY is 2.8-kb in size and is repeated tandemly in 20.5-kb units. TSPY shares significant homology to various cyclin B binding proteins, including oncoprotein SET, some of which possess cell cycle modulating functions. TSPY is expressed at high levels in both gonadoblastoma and testicular seminoma. To further evaluate the role of TSPY in other male-specific cancers, we had examined its expression in 10 cases of prostate cancer using both in situ hybridization and immunohistochemistry. Our results demonstrated that TSPY was expressed at low levels in normal epithelial cells and benign prostatic hyperplasia, but at elevated levels in tumor cells of prostate cancer at various degrees of malignancy. Sequence analysis of RT-PCR products obtained from both prostatic and testicular tissues using primers flanking the ORF revealed a complex pattern of RNA processing of the TSPY transcripts involving cryptic intron splicing and/ or intron skipping. All TSPY transcripts maintain the same ORF and encode a variety of polymorphic or shortened versions of TSPY with diverging properties ranging from 18.1 to 35.1 kDa in size and pI from 5 to 9. The abbreviated TSPY transcripts were more abundant in prostatic cancer than testis samples. GST pull-down assays demonstrated that all TSPY proteins were capable of binding to cyclin B. The binding domain was mapped to their carboxyl termini that share the most significant homology to the oncoprotein SET. Their preferential expression in cancer, diverse biochemical properties and conserved cyclin B binding function suggest that the TSPY gene/variants may be involved in the multi-step prostatic oncogenesis beside its putative oncogenic or tumor-promoting role in gonadoblastoma and testicular seminoma.