northern blotting and (4) comparative genomic analysis with distant vertebrate species such as *Fugu rubripes* and *Tetraodon nigroviridis*. This transcript-mapping strategy has identified 32 potential transcription units, including 2 known genes, 5 new genes, 9 Unigene entries and 16 other expressed sequence tag clusters. The region also contains five pseudogenes. The map should facilitate subsequent efforts to characterize the candidate genes. This study illustrates how the integration of genome-based approaches facilitates the identification of genes in a large interval.

Sjögren, Helene [31]

Fusion of the NH_2-terminal domain of the bHLH protein TCF12 to TEC in extraskeletal myxoid chondrosarcoma with translocation t(9; 15)(q22; q21)

Helene Sjögren, Barbro Wedell, Jeanne M. Meis Kindblom, Lars-Gunnar Kindblom & Göran Stenman

Sahlgrenska University Hospital, Gula Stråket 8, SE-413 45 Gothenburg Bohuslän, Sweden

Extraskeletal myxoid chondrosarcomas (EMC) are characterized by recurrent t(9; 22) or t(9; 17) translocations resulting in fusions of the NH_2-terminal transactivation domains of EWS or TAF2N to the entire TEC protein. We report an EMC with a new translocation, t(9; 15)(q22; q21), and a third type of TEC-containing fusion gene. The chimeric transcript encodes a protein in which the first 108 amino acids of the NH_2 terminus of the basic helix-loop-helix (bHLH) protein TCF12 is linked to the entire TEC protein. The translocation separates the NH_2-terminal domain of TCF12 from the bHLH domain as well as from a potential leucine zipper domain located immediately downstream of the breakpoint. These results demonstrate that the NH_2-terminal transactivation domains of EWS or TAF2N are not essential for the oncogenic properties of fusion proteins in EMC, and that EWS or TAF2N may be replaced by a similar domain from a bHLH protein that presumably endows the fusion protein with similar functions.

Sjögren, Helene [32]

Fusion of the NH_2-terminal domain of the bHLH protein TCF12 to TEC in extraskeletal myxoid chondrosarcoma with translocation t(9; 15)(q22; q21)

Helene Sjögren, Barbro Wedell, Jeanne M. Meis Kindblom, Lars-Gunnar Kindblom & Göran Stenman

Sahlgrenska University Hospital, Gula Stråket 8, SE-413 45 Gothenburg Bohuslän, Sweden

Extraskeletal myxoid chondrosarcomas (EMC) are characterized by recurrent t(9; 22) or t(9; 17) translocations resulting in fusions of the NH_2-terminal transactivation domains of EWS or TAF2N to the entire TEC protein. We report an EMC with a new translocation, t(9; 15)(q22; q21), and a third type of TEC-containing fusion gene. The chimeric transcript encodes a protein in which the first 108 amino acids of the NH_2 terminus of the basic helix-loop-helix (bHLH) protein TCF12 is linked to the entire TEC protein. The translocation separates the NH_2-terminal domain of TCF12 from the bHLH domain as well as from a potential leucine zipper domain located immediately downstream of the breakpoint. These results demonstrate that the NH_2-terminal transactivation domains of EWS or TAF2N are not essential for the oncogenic properties of fusion proteins in EMC, and that EWS or TAF2N may be replaced by a similar domain from a bHLH protein that presumably endows the fusion protein with similar functions.

Smith, David I. [33]

Comprehensive analysis of genetic alterations in ovarian cancer

Viji Shridhar[1], Ajay Pandita[1], John Lee[2], Steve Iturria[1], Julie Staub[1], Raji Avula[1], Ami Sen[2], Eric Calhoun[1], Fergus Couch[1], David James [1], Lynn Hartmann[1], Jim Lillie [2] & David Smith [1]

[1]*Mayo Foundation, Rochester, Minnesota, USA*[2]*Millennium Predictive Medicine*

Ovarian cancer is the leading cause of death from gynecological malignancies among women in the United States. The 5-year survival for the patients with late stage tumors is 20%, compared to 50–90% in early stage tumors. The aim of this study is to use state-of-the-art molecular technologies to better understand the biology of ovarian cancer. We used cDNA microarrays to distinguish the variation in gene expression of approximately 20,000 genes among 10 early stage (stage I/II) and 10 late stage (stage III/IV) ovarian tumors against 5 pooled normal ovarian epithelial cell brushings. Subtracted cDNA libraries of several of these tumors versus normal ovarian epithelial cell brushings were generated to identify additional genes not present on the cDNA microarrays. Calculation of average fold induction (both up and down) revealed no statistically significant increase in the number of genes showing differential expression in the late stage tumors compared to early stage, and the differentially expressed genes in both the early and late stage tumors were very similar. The loss of expression of 20 of 30 top candidate down-regulated genes was confirmed in a panel of both early and late stage tumors (15 each) by semi-quantitative RT-PCR. To complement the gene expression profiles obtained, DNA from 35 ovarian tumors of various stages/grades were used for comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) studies. Gains were commonly observed on chromosomes 1, 8, 17, 19 and 20, whereas losses were mainly observed on chromosomes 4q, 5q, 13q, and 18q. 13q14.1 and 19q13.4 were two regions that showed more loss in early stage than late stage tumors. Through these analyses we are developing a molecular signature for ovarian cancer and identifying important genes involved in early stage ovarian carcinogenesis.

Sood, Raman [34]

Use of experimentally constructed haplotypes in gene mapping studies of hereditary cancers

E. Gillanders[1], J.A. Douglas[2], S.B. Gruber[3], H. Yan[4], B. Vogelstein[4], R. Sood[1], J. Carpten[1], T. Dennis[1], M. Boehnke[2] & J.M. Trent[1]

[1]*Cancer Genetics Branch, National Human Genome Research Institute, Bethesda, Maryland, USA*
[2]*Department of Biostatistics, University of Michigan, Ann Arbor, Michigan, USA*
[3]*Department of Epidemiology and Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA*
[4]*Howard Hughes Medical Institute and Johns Hopkins Oncology Center, Baltimore, Maryland, USA*

Conversion provides several advantages for gene mapping projects of complex diseases such as cancer. The approach takes advantage of selective retention of a subset of human chromosomes within somatic cell hybrids, isolating single copies of all

desired human chromosome pairs. This strategy functions both to simplify mutation detection and to allow unambiguous phase information to be determined. Constructing haplotypes on the basis of conventional genotype and pedigree data is challenging, particularly for diseases with a late age of onset, such as cancer.

The homologue retained in a particular hybrid is identified by conventional genotyping of a few markers per chromosome. These "haploid" hybrids can then be used to increase the sensitivity of traditional mutation analysis, because the disease-causing chromosome will not be accompanied by the normal wild-type allele. Haplotypes may also be determined simply by genotyping each haploid hybrid. We have typed DNA from 100 hybrids to examine chromosomal retention patterns, test the feasibility of conversion for analysis of whole genomes, and evaluate assumptions regarding appropriate experimental design.

We have investigated the theoretical efficiency of using haplotypes compared with conventional genotypes in linkage and linkage disequilibrium studies. In the linkage disequilibrium setting, we determined the Fisher information (with respect to haplotype frequency) provided by unrelated individuals. For haplotypes with two single-nucleotide polymorphisms, conversion provides 5–45% more information per subject than standard genotyping, depending on true haplotype frequencies; for haplotypes with five single-nucleotide polymorphisms, improvement ranges from 20% to 92%. Since Fisher information is inversely related to sample size, conversion requires up to 12.5 times fewer subjects than standard genotyping to obtain the same information. We are currently completing similar calculations in the linkage analysis setting.

The extra cost associated with conversion includes hybrid construction and characterization, and duplicate genotyping. However, the increased information per subject reduces overall recruitment and phenotyping costs, which tend to exceed those for genotyping. The efficiency of using conversion to construct haplotypes will probably increase even further as automated methods of genotyping continue to improve.

Sood, Raman [35]

Molecular cloning and expression analysis of human and mouse homologs of a new transcriptional regulator as a candidate tumor suppresor gene

Raman Sood[1], Izabela Makalowska[2], Pamela Pollock[1], Christiane Robbins[1], Suzie Chen[3] & Jeffrey Trent[1]

[1]*Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA*
[2]*Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA*
[3]*Rutgers University, Piscataway, New Jersey, USA*

We report the cloning and detailed characterization of a new transcript containing two SNF2_N domains and one PHD domain. The SNF2_N domain is often seen in proteins involved in such processes as transcription regulation (for example, SNF2, MOT1), DNA repair (for example, ERCC6, RAD16), DNA recombination (for example, RAD 54), and chromatin unwinding (for example, ISWI). The PHD domain is a C4HC3 zinc-finger-like motif found in nuclear proteins thought to be involved in chromatin-mediated transcriptional regulation. Shotgun sequence analysis of a mouse BAC clone identified several exons with homologies to a variety of repair protein genes in the database. Inter-exon polymerase chain reaction followed by 5′ rapid amplification of cloned ends and identification of expressed sequence tags for the 3′ end led to the cloning of a 7,225-base-pair cDNA with an open reading frame consisting of 4,848 base pairs. The human homologue was cloned by polymerase chain reaction between the expressed sequence tags identified by a BLAST search of the mouse sequence, followed by 5′ rapid amplification of cloned ends. The coding sequence of the two genes is 83% identical at the amino acid level. Northern blot analysis shows a transcript of approximately 7.5 kilobases in all tissues examined with both human and mouse complementary DNAs as a probe. We have mapped the human cDNA to 6q23, a region reported to contain a tumor suppressor locus by several laboratories, including our own. We are currently generating antibodies, and we will present preliminary functional studies.

Sorensen, Poul H.B. [36]

Ewing tumor cells activate distinct signaling pathways in monolayer versus anchorage-independent cultures

Elizabeth R. Lawlor[1], Jerian F. Lim[1], Lydia A. Taylor[2], Chris Carthy[2] & Poul H.B. Sorensen[1,2]

[1]*Departments of Pathology and Pediatrics, Children's and Women's Hospital of British Columbia, Vancouver, British Columbia, Canada*
[2]*Inphogene Biocom, Inc., Burnaby, British Columbia, Canada*

The Ewing tumor (ET) family of childhood malignancies express translocation-associated EWS-ETS chimeric oncoproteins that function as aberrant transcription factors. These molecules are thought to facilitate passage through the cell cycle, contributing to increased cell proliferation. We have been comparing signaling pathways activated in ET cell lines grown in conventional monolayers with those in anchorage-independent spheroid cultures. As expected, we found that monolayer ET cells show serum-dependent activation of ERK 1/2 and p38 MAP kinases. Serum also activates PI3 kinase-dependent PKB/AKT, involved in cell survival, and induces high levels of cyclin D1/D2 protein expression. However, when the same cells are grown in spheroid cultures, there is dramatic serum-independent activation of ERK 1/2, p38, and PKB/AKT, but paradoxically cyclin D1 and D2 expression is virtually undetectable. Fluorescence-activated cell sorting analysis indicates that at least 30% of spheroid cells are cycling. This pattern of serum-independent MAP kinase activation and cyclin D1/D2 suppression can be recapitulated in monolayer cells by treatment with PI3 kinase inhibitors. To further understand changes in immediate and early gene expression that may underlie these findings, we subjected monolayer and spheroid ET cells (with and without PI3 kinase inhibitors) to gene expression profiling using Affymetrix U95A gene chips (containing over 12,000 annotated human gene sequences). The results of these experiments are currently being analyzed to identify distinct expression profiles characteristic of monolayer versus spheroid ET cells. Morphological and ultrastructural analysis of the latter indicate many similarities with primary ETs, suggesting that spheroid cultures may be a better model to study the biology of these lesions.

Sotiriou, Christos [37]

Core biopsy versus surgical tumor specimens for microarray analysis of gene expression profiles

Christos Sotiriou[1], Khanna Chand[2], David Petersen[1], Amir A. Jazaeri[1] & Edison T. Liu[1]

[1]*Medicine Branch, Division of Clinical Sciences, National Cancer Institute, National Institutes of Health, Gaithersburg, Maryland, USA*
[2]*Pediatric Oncology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA*

We compare the utility of core biopsy samples with surgical tumor specimens as starting material for microarray analysis. Two human tumor xenografts, Ewing sarcoma and neuroblastoma grown in Beige-Scid mice, served as source materials for this study. Six 16g-needle core biopsies and an excision biopsy were performed on each xenograft. Three of the six core biopsies were processed separately and the