tive gene expression intensities, ratios or both are measured in order to quantify the gene expression level relative to its reference sample. Our early reports discussed a systematic data extraction algorithm in which a unique method of extracting gene expression intensities and ratios along with an adaptive ratio confidence interval, measurement qualities of gene expression ratios and intensities were presented. In many methods of gene expression data analysis, only expression ratios or normalized intensities are employed because of insufficient assessment at the individual data points (clones). Common practice dictates that data derived with poor measurement quality—such as expression ratios derived from weak reference expression levels or noise-corrupted measurements—shall not be used in the analysis. We present an automatic decision-making process for various algorithms, such as gene expression clustering and classification, in which gene expression ratios and intensities are chosen to participate in the analysis according to their measurement quality, expression signal-to-noise ratio relative to the reference channel and other parameters derived from cDNA microarray image analysis software.

Chen, Zhong [36]

Molecular profiling of metastatic tumor progression of a murine squamous cell carcinoma by differential display and cDNA microarray reveals dysregulated expression of genes related to the nuclear factor-κb signal pathway

Gang Dong[1], Elena Loukinova[1], Zhong Chen[1], Lisa Gangi[2], Edison Liu[2] & Carter Van Waes[1]

[1]*National Institutes of Health, Bethesda, Maryland, USA*[2]*National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA*

To identify changes in gene expression associated with tumor progression and metastasis in vivo, we investigated differential gene expression in a metastatic squamous carcinoma model established in syngeneic mice, including a tumorigenic line PAM 212, and metastatic sublines derived from PAM 212 tumors, using mRNA differential display (DD) and cDNA microarrays. Using DD, seventy-two candidate cDNAs were detected, and thirty-four cDNAs were confirmed to be differentially expressed by northern blotting analysis. Global mRNA expression profiles were generated using an NCI mouse Oncochip composed of four thousand elements representing known genes and ESTs, plus 57 of the candidate cDNAs detected by DD to facilitate data validation. Clustering analysis of array results from metastatic cell lines and tumors identified a subset of genes that exhibited increased expression in the metastases, revealed that 22 unique clones are highly homologous to previously identified genes, and nine novel cDNAs. Strikingly, 10/22 of the genes identified have been associated with activation of the Nuclear Factor-kB signal transduction pathway. One of the genes identified, Gro-1, was recently confirmed to promote tumor growth, metastasis and angiogenesis of SCC in vivo in a separate report. These results demonstrate that early response pathway components and down stream genes related to NF-κB are expressed with metastatic tumor progression. Functional genomic approaches may promote a better understanding of the repertoire of related genes and molecular pathways involved in tumor progression and metastasis.

Chodosh, Lewis [37]

Functional analysis of mammary development using oligonucleotide microarrays

Stephen Master, Jennifer Hartman, Alexander Stoddard, Elizabeth Keiper, Susan Moody, Celina D'Cruz & Lewis Chodosh

University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

Epidemiological and animal studies clearly demonstrate that reproductive events such as puberty, pregnancy and parity play a critical role in the determination of breast cancer risk. We have carried out messenger RNA expression profiling using high-density synthetic oligonucleotide microarrays to identify genes that are differentially expressed between various developmental stages of the murine mammary gland. The use of DNA microarrays to study vertebrate development presents unique analytical challenges compared with expression profiling of homogeneous cell populations. These challenges include accounting for the impact of complex changes in the abundance of multiple cell types on gene expression profiles, as well as identifying functionally relevant patterns of gene expression in the absence of detailed prior knowledge either of the developmental system or of the genes expressed. In order to address these challenges, we have developed a general approach that permits the unbiased identification of biologically relevant patterns of gene expression by identifying statistically significant associations between clustered gene expression patterns and functional gene categories. We have tested the applicability of this approach by analyzing the expression of approximately 5,500 genes during 13 stages of murine mammary gland development. Our findings confirm the utility of this method by demonstrating the ready identification of cellular processes and pathways of known importance in mammary development, as well as shifts in the relative abundance of different cell types within the gland. This approach permitted the identification of genetic pathways with previously unsuspected patterns of developmental regulation, including those involved in fatty acid metabolism, angiogenesis and extracellular matrix synthesis. Our results demonstrate the ability of this analytical approach to suggest new hypotheses regarding mammary development and indicate that this approach will be broadly applicable to the study of complex tissues.

Chuang, Y. Eric [38]

Effects of p53 in human lymphoblast cells following ionizing radiation using 7K cDNA microarrays

Y. Eric Chuang[1], Howard Liber[2], Louis Staudt[1] & James Mitchell[1]

[1]*Division of Clinical Sciences, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA*
[2]*Department of Radiation Oncology, Massachusetts General Hospital, Boston, Massachusetts, USA*

Genomic instability is a characteristic of many human cancers. It has been well documented, utilizing endpoints such as karyotypic instability and gene amplification, that alterations in the tumor suppressor p53 are related to genomic instability at the chromosomal level. The p53 protein has been implicated in multiple cellular responses related to DNA damage, including apoptosis, cell cycle control and DNA replication, repair and transcription. Alterations in any of these processes could be related to increased genomic instability. We previously compared radiation-induced mutagenicity among human B lymphoblast cell lines with dif-

ferent p53 status, including p53-null cells (NH32), cells with wild-type p53 (TK6) and cells with mutated p53 (WTK1). The results showed that at the *TK1* locus p53-null cells had equivalent background mutation frequencies and were approximately as mutable as TK6, whereas WTK1 were much more sensitive to spontaneously arising and radiation-induced mutation. These results indicated that the lack of wild-type p53 does not lead to increased mutability. In this study, to explore further how p53 is involved in regulating mutational processes, we used 7K complementary DNA microarrays to compare the patterns of gene expression between TK6 and NH32 cells following irradiation. Total RNA was extracted 3, 6, and 24 h after irradiation with 10-Gy X-rays. Our preliminary results indicated that irradiation resulted in more genes being upregulated than downregulated in human lymphoblast cells regardless of their p53 status. Furthermore, cluster analyses of gene expression profiles in TK6 and NH32 revealed different patterns. In TK6 radiation-induced p53-related responses showed a rapid induction (higher at 3 and 6 h after irradiation than at 24 h), whereas in NH32 radiation-induced p53-unrelated responses showed different kinetics (higher at 3 and 24 h after irradiation than at 6 h).

Chung, L. Ping. [39]

Allelic imbalance in lung cancers of nonsmokers in Hong Kong

Maria P. Wong[1], L. Ping Chung[1], W.K. Lam[2], S.W. Chiu[3] & K. H.Fu[4]

[1]*Department of Pathology, University of Hong Kong, Hong Kong*
[2]*Department of Medicine, University of Hong Kong, Hong Kong*
[3]*Cardiothoraxic Unit, Grantham Hospital, Hong Kong*
[4]*Department of Pathology, Grantham Hospital, Hong Kong*

Lung cancer is a common malignancy in Hong Kong. The incidence in males is ranked medium, but that in females is among the highest in the world. More than 60% of female patients are lifelong nonsmokers, implying that carcinogenic mechanisms other than cigarette smoking may be involved in the development of lung cancers in female nonsmokers. To identify the candidate tumor suppressor genes involved, we screened 50 commonly deleted regions of all the chromosomal arms for loss of heterozygosity of microsatellite markers in 41 samples of cancerous lung tissue from nonsmokers. We found frequent allelic loss of 50–62.5% in the chromosomal regions 1q21–31, 3p14.2, 7q31, 8p21, 10q26, 13q12.3, 16q24, 17p13.1–13.3, 17q13.3, 18q23 and 19p13. Comparison with 40 lung cancers from smokers using the same markers showed a similar range of loss of heterozygosity frequency in the two populations. We found that some regions commonly deleted in smokers (for example, 4q32, 6q27, 9q21 and 11q23) were statistically less frequently deleted in nonsmokers, but we found no region frequently deleted in nonsmokers but not smokers. Our data on cancers from smokers indicate that smoking induces widespread genomic damage, leading to extensive chromosomal loss of long segments of DNA. Cancers from nonsmokers exhibit more targeted damage, with fewer and shorter segments of DNA loss. The deleted regions in cancers of nonsmokers might represent the essential complement of genetic material that must be lost for lung cancers to develop.

Collins, Colin [40]

Comprehensive sequence analysis of a human 20q13.2 cancer amplicon

Colin Collins[1], Stanislav Volik[1], David Kowbel[1], Meredith Wernick[1], Trevor Hawkins[2], Paul Predki[2], Wen-Lin Kuo[1] & Joe Gray[1]

[1]*University of California San Francisco Cancer Center, San Francisco, California, USA*
[2]*Department of Energy Joint Genome Institute, Walnut Creek, California, USA*

Amplification of 20q13.2 occurs in breast and other cancers and is associated with aggressive tumor behavior. We report the first sequence and comprehensive biological characterization of a tumor amplicon. Array-based comparative genomic hybridization resolved the 1.2-megabase amplicon into a pair of recurrent peaks. The proximal amplicon encodes the putative Zn-finger transcription factor ZNF217, a candidate oncogene recently shown to immortalize human mammary epithelial cells. Analysis of the genomic sequence for genes, repetitive elements, CpG islands, and gene expression revealed six previously discovered genes (*ZNF217, ZNF218, PIC1-like, NABC1, CYP24,* and *NABC2*) and four new genes (*PFDN4, NABC3, NABC4* and *NABC5*). *ZNF217* is the only protein-coding gene in the 160-Kb proximal amplicon peak. *CYP24* and *PFDN4* map in the distal amplicon. *PFDN4* is overexpressed in tumors in which it is amplified and in some in which it is not. Amplicon breakpoints cluster in regions of very high repeat content flanking *ZNF217* and *PFDN4.* A 14-Kb duplication, of a class associated with unstable chromosome regions, maps close to *ZNF217.* This duplicon is approximately 97% identical to a 14-Kb element on chromosome 22q13 and encodes one of three CpG islands in the amplicon and NABC3. We cloned and sequenced the syntenic region of mouse chromosome 2, revealing numerous homologies. These correspond to conserved exons and noncoding elements believed to be regulatory or structural in nature. We will report on these findings, which clearly demonstrate the power of comparative sequence analysis for cancer biology.

Coombes, K.R. [41]

Identifying and quantifying sources of variation in high-density cDNA microarray data using ^{33}P-labeled probes

K.R. Coombes[1,2,3], K.A. Baggerly[1,3], L.V. Abruzzo[4], W.E. Highsmith[5], T. Krogmann[5] & D.N. Stivers[1,3]

[1]*Department of Biostatistics, University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA*
[2]*Department of Biomathematics, University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA*
[3]*Cancer Genomics Program, University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA*
[4]*Department of Hematopathology, University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA*
[5]*Department of Pathology, University of Maryland at Baltimore, Baltimore, Maryland, USA*

A microarray experiment involves several steps, including spotting complementary DNA, extracting RNA, labeling the probe, hybridizing, scanning and analyzing images. Each step introduces variability, confounding our ability to obtain accurate estimates of the biological differences between samples. We ran repeated experiments using high-density cDNA microarray membranes (Research Genetics