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

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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

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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

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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

GeneFilters GF200) and a ^{33}P-labeled probe. Total RNA was extracted from a high-grade B-cell Burkitt lymphoma cell line (GA-10). We estimated the components of variation attributable to (1) image analysis (2) exposure time to phosphorimager screens (3) differences in membranes (4) reuse of membranes and (5) differences in probes prepared from multiple RNA extractions. We assessed variation qualitatively using a clustering algorithm and quantitatively using a version of ANOVA adapted to multivariate microarray data. The largest contribution to variation (44% of the total variation) came from reusing membranes. Differences in membranes, exposure time, and probe preparation each contributed about 15%. Image analysis contributed only 0.3%. Microarray results are generally reproducible, but each step in the process contributes some variability. The largest effects are intrinsic to the biological material (the cDNA spotted on the membrane and the RNA extracted from the sample). Much of the effect of reusing membranes is attributable to increasing levels of background radiation, and this effect can be reduced by using a given array no more than four times. The effects of exposure time (which are partly attributable to variation in the scanning process) can be minimized by using the same exposure time for all experiments.

Cornwell, Paul [42]

Differences in transcript profiles between hepatocarcinogen-sensitive and -resistant mice as a basis for understanding chemical induction of mouse liver tumors

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The B6C3F1 mouse strain, a cross between the hepatocarcinogen-resistant C57Bl/6J (B6) strain and the hepatocarcinogen-susceptible C3H/HeJ strain (C3H), is commonly used to assess human carcinogen risk. Recently mapped hepatocarcinogen sensitivity (*hcs*) loci probably influence the molecular mechanisms underlying hepatocarcinogen resistance and susceptibility. We propose that these loci affect the expression of genes whose products play important roles in DNA repair and hepatocyte growth control. Hepatic transcript profiles of B6 and C3H mice, obtained using the Clontech Atlas mouse 1.2-II array (covering a total of approximately 1,200 genes), showed twofold or greater differences in approximately 8% of the genes assayed. Approximately 88% of the altered genes showed increased expression in the C3H mice, whereas the other 12% were repressed. These genes are involved in a wide range of cellular activities, including the control of apoptosis and cellular proliferation. We are initiating transcript profile studies of chemically induced tumors to determine the role of these genes in hepatocarcinogenesis. Preliminary data from WY-14,643-induced tumors revealed significantly altered expression of peroxisome proliferator-activated receptor-γ and interferon-γ signaling pathway genes compared with surrounding (noncancerous) and control (untreated) tissue. Characterization of differences in gene expression in resistant versus susceptible mouse strains and of how these differences are manifested when cells are exposed to a chemical carcinogen will lead to a better understanding of the mechanisms of hepatocarcinogenesis in mice. This knowledge will help to determine the relevance of the mouse liver tumor response for human risk assessments.

Couch, Fergus [43]

Expression analysis of breast cancer progression

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We have used complementary DNA expression array and subtractive suppressive hybridization techniques to identify molecular alterations associated with the progression of breast cancer. Arrays representing up to 40,000 different transcripts were profiled against a large panel of clinical samples carefully selected by stage, histological type and clinical outcome. Samples included 6 normal breast epithelial cell, 8 ductal carcinoma *in situ,* 5 infiltrating lobular carcinoma and 28 infiltrating ductal carcinoma tumor samples. In addition, 11 cDNA subtraction libraries were generated from various comparisons of tumor versus normal samples and from comparisons of aggressive versus indolent tumor samples. Analysis of over 50,000 successful sequencing lanes from the subtraction libraries identified up to 6,000 unique transcripts expressed in breast cancer versus normal breast epithelium or in aggressive breast tumors versus indolent tumors. These 6,000 transcripts were assembled on custom arrays and have been hybridized to the same panel of breast samples. Analysis of the library and array data has led to the identification of several genes up- and down-regulated in breast cancer. A subset of these genes may represent new markers for breast cancer screening and prognosis as well as potential targets for future drug therapies.

Cravatt, Benjamin [44]

Chemical strategies for the global analysis of protein function: Profiling hydrolytic enzymes in cancer

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The molecular features specific to metastatic carcinomas that support their invasive behavior are complex and ill defined. Although one generally accepted notion assigns hydrolytic enzymes a central role in promoting the aggressive properties of metastatic tumors, the actual functions played by individual proteases and esterases in cancer remain elusive. To understand better how hydrolases and their endogenous inhibitors affect cancer, we are using a new chemical strategy, activity-based protein profiling. This method allows us to monitor simultaneously and directly the catalytic activities of numerous serine hydrolases from whole-cell, tissue and fluid samples. We have initiated a program to compare the serine hydrolase activity profiles of estrogen-receptor-positive and -negative human breast cancer cell lines. In breast carcinomas, a strong inverse correlation exists between estrogen receptor expression and several metastatic phenotypes, including cell invasiveness and motility. When this finding is coupled with the observation that serine protease inhibitors suppress estrogen-receptor-negative tumor cell migration and invasion, an intriguing model emerges in which serine hydrolases play a central role in mediating the aggressive behavior of these cells. Nonetheless, the identities of the participating enzymes remain unknown. Using activity-based protein profiling, we have identified several serine hydrolase activities that vary dramatically among these breast cancer lines. We anticipate that such studies will identify functional changes in key serine hydrolases involved in promoting or retarding tumorigenesis. These enzymes should in turn serve as both markers for cancer progression and targets for pharmaceutical efforts aimed at treating this disease.