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.