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genes on a microarray. Thirty genes were upregulated and eighty downregulated by fourfold or more over background. To confirm subtraction efficiency, we picked approximately 700 clones from the C225-upregulated subtraction library; spotted their inserts, amplified by the polymerase chain reaction, on glass slides and hybridized them with the subtracted cDNAs. The majority of the clones exhibit high intensity when hybridized with the upregulated cDNAs and low intensity when hybridized with the downregulated cDNAs. The differential expression of these clones is now being confirmed by quantitative polymerase chain reaction with revserse transcription or northern blot analysis, and their function will be evaluated using cell differentiation and motility assays.

Onay, U. Venus [68]

Identifying the role of single-nucleotide polymorphisms in breast cancer risk using microarray and mass spectrometry technologies

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A considerable proportion of the familial aggregation of breast cancer is probably due to alleles of low penetrance. Although their contribution to risk is relatively small, they can contribute to a large proportion of breast cancer cases in the population, because the risk-conferring alleles of these genes are common. The candidate gene approach is one of the most logical and practical strategies to identify these risk-enhancing, low-penetrant variants. A major obstacle in investigating the risk associated with multiple candidate genes has been the lack of large-scale genotyping technologies. Recently developed microarray and matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) mass spectrometry technologies are ideally suited to the problem of high-throughput genotyping. We are relying on the power of these techniques to carry out a population-based breast cancer case-control study for 32 single-nucleotide polymorphisms (SNPs). We selected these SNPs from genes of crucial pathways that are frequently perturbed in cancers, such as those governing the cell cycle, carcinogen metabolism and the immune response pathways. We are currently developing both microarray and MALDI-TOF mass spectrometry techniques so that both can use the primer extension method to detect the allelic variants of SNPs. Both techniques are used in combination on a panel of cases involving several SNPs, to assess specificity and reproducibility. The combination of both methods will be a powerful tool for genotyping a large number of SNPs in a large number of cases. Advances in our knowledge of disease etiology will significantly expand our abilities to design strategies for the prevention of breast cancer development and the inhibition of its progression.

Orr, Michael [69]

Discovery of 830 candidate therapeutic targets and diagnostic markers for breast cancer using oligonucleotide microarray technology

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To identify differentially expressed genes in infiltrating ductal carcinoma (IDC) of the breast, we measured the gene expression profiles of 15 IDC and 13 normal human breast tissues, using the Affymetrix GeneChip array platform for simultaneous analysis of over 60,000 genes. Fold-change comparison between normal and IDC breast tissue samples revealed 830 genes that were statistically over- or under-expressed by threefold or greater in the IDC samples. We identified 286 overexpressed genes and 544 underexpressed genes. Furthermore, the 830 genes were evaluated for tissue-specific expression by E-northern analysis of 28 different normal tissues, revealing tissue-specific candidate targets. We performed further analysis utilizing principal component analysis or hierarchical clustering with 5,467 prefiltered genes to determine if gene expression profiles could be used to distinguish between IDC and normal breast tissue samples. Both PCA and HCA indicated two distinct populations for the normal and tumor-derived samples, except for two samples, based on gene expression patterns. One aberrant tumor sample was explained by its histology, which showed only partial (<20%) involvement by malignant cells. We also employed cluster analysis to evaluate differences in samples at the single-gene level. Examination of a particular cluster of overexpressed genes displayed a multitude of differentially expressed sequence tags along with genes for topoisomerase II, cyclin B, CDC2, KI-67, and thymidine kinase, suggesting some functional similarities. Our studies provide a rational basis for additional analysis of differentially expressed genes in breast cancer, which may lead to the identification of therapeutic targets and diagnostic markers.

Parodi, Silvio [70]

Innovative leads for antineoplastic drugs suggested by a more intimate familiarity with structural motifs of oncoproteins

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Proteins, although structurally unsuitable for their own replication, are the structures that translate an abstract DNA sequence into real action, making possible catalysis, signaling and supramolecular morphologies. Protein targets have most often been recognized by starting from antineoplastic effects initially observed at the level of an organism or a cell and working in a reductionist manner. However, no oncoproteins have been identified as cancer chemotherapy targets through this

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downward approach; it has yielded only targets at low levels within the replication machinery, never at the level of proliferation control and signaling proteins. Drug-like molecules with molecular weights around 500 seem to work well as inhibitors of thyrosine kinases, docking in their ATP pocket; however, molecules of this kind are perhaps too compact for selective interference at the level of cross-talks between the domains of two signaling proteins. Rapid evolution has recently taken place in possibilities for internalizing amphipathic molecules of molecular weights of several thousand. Under these conditions it becomes possible to conceive peptidomimetic drugs of several dozen amino-acid-like units, and these are much better suited for such selective interference. Small, dedicated peptidomimetic combinatorial libraries, based on a natural protein motif involved in the cross-talk of interest and on computer modeling, are natural allies in this game. A recently developed peptidomimetic lead capable of interfering with Myc activity[1,2] will be discussed within the framework of this new strategy for developing antineoplastic drugs.

1. Pescarolo, M.P. *et al. Cancer Res.* **58**, 3654–3659 (1998).
2. Pescarolo, M.P. *et al. FASEB J. Expr.* January 2001.

Paules, Richard S. [1]

ATM-dependent responses to DNA-damaging agents

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Ataxia telangiectasia is an autosomal recessive disorder characterized by neuronal degeneration, telangiectasias, acute cancer predisposition and hypersensitivity to ionizing radiation (IR). The gene defective in this disorder, *ATM* (for AT-mutated), encodes a protein, pATM, that has been found to have IR-inducible kinase activity. Cells from individuals with AT exhibit severely attenuated cell cycle checkpoint function in response to IR exposure and are hypersensitive to IR-induced killing. It has been suggested that pATM acts as part of a complex that senses DNA damage and in particular DNA double-strand breaks; it has also been speculated that pATM participates in response to oxidative damage. We have been studying pATM-dependent cellular responses to various DNA-damaging agents. As part of this effort, we are investigating global gene expression changes following exposures to IR in both lymphoblast and fibroblast cells from multiple individuals with either normal or defective pATM function. We are comparing gene expression changes in both normal and pATM-deficient cells from one cell type that is predisposed to undergo apoptosis (lymphoblasts) with those in cells predisposed to undergo a prolonged cell cycle arrest (fibroblasts) following IR exposure. In addition, we are comparing these responses to IR in normal and pATM-deficient fibroblasts with responses to exposure to ultraviolet light in the 000- to 000-nm range and reactive oxygen species. These analyses are being performed using National Institute of Environmental Health Sciences Human ToxChips, with approximately 2,000 known complementary DNA clones, as well as with Human Discovery Chips, a collection of approximately 12,000 known and anonymous cDNA

Pedersen, Tanja X. [2]

Profiling changes in keratinocyte gene expression during wound reepithelialization by laser capture microdissection combined with cDNA array analysis

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Incisional wounding of the skin triggers a series of dramatic events in the epidermal keratinocytes that are located immediately adjacent to the margins of the wound, ultimately leading to complete reepithelialization of the wound by these cells. These events include hyperproliferation of basal keratinocytes, dissolution of cell–cell adhesions, detachment from the basement membrane, lateral migration into the wounded area, invasion and proteolytic degradation of the provisional matrix of the wound bed. In many aspects, the healing response resembles the phenotypic events observed during squamous carcinoma progression, in which normal keratinocytes undergo a malignant conversion to acquire a proliferative, migratory and invasive phenotype. To elucidate the molecular mechanisms underlying the transformation of keratinocytes to a migratory and proteolytic phenotype, we initiated a study of global changes in keratinocyte gene expression during mouse incisional skin wound healing. We used laser capture microdissection, which allows the procurement of pure cell populations from heterogeneous histological samples, to isolate wound keratinocytes that actively migrate through and degrade the provisional matrix of full-thickness incisional mouse skin wounds. For comparison, we isolated nonmigrating keratinocytes distal to the wound edge, as well as keratinocytes from mock-wounded mice, in a similar manner. We isolated total RNA (11–18 ng) from approximately 5,000 keratinocytes and generated complex complementary DNA probes by reverse transcription of the messenger RNA fraction. We then screened cDNA expression arrays to identify the expression pattern of 1,176 mouse genes in the three populations of keratinocytes. Results of the expression studies will be presented and discussed.

Perry, Mary Ellen [3]

Conditional inactivation of *Mdm2*

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The *mdm2* oncogene encodes p90MDM2, a critical negative regulator of the p53 tumor suppressor protein. The early embryonic lethality of mice of *Mdm2* null genotype precludes an evaluation of MDM2's role in regulating p53 in adult tissues. It is critical to understand the mechanisms regulating levels and activities of p53, because loss of p53 function leads to tumorigenesis whereas high levels of active p53 can stimulate apoptosis. The ability of p53 to regulate the expression of its own inhibitor, p90MDM2, has led to the suggestion that p53 controls its own activity through a negative feedback loop with *Mdm2*. This feedback loop is con-

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