um of healthy rats. We collected cells lining the anterior nasal passages, as confirmed by light microscopy, into 200 µl of Trizol reagent by controlled intranasal instillation. We performed gene expression analysis using Clontech complementary DNA Rat Atlas 1.2 arrays (approximately 1,200 genes) and verified selected transcripts using the polymerase chain reaction with reverse transcription (Taqman). The percentages of genes within specific average expression ranges, on the basis of phosphorimager signal intensities, were 1.4% at 3,000–45,000, 3.3% at 1,000–3,000, 30.1% at 100–1,000, and 65.2% below 100. The most highly expressed genes included those involved in phase I (for example, cytochrome Ps) and phase II (for example, glutathione *S*-transferases) xenobiotic metabolism, bioenergetics (for example, cytochrome oxidase), osmotic balance (for example $(Na^+ + K^+)$ATPase) and epithelial ionic homeostasis (for example, ion channels). These findings are consistent with normal nasal epithelial functions. Such baseline data will contribute to a further understanding of the functions of this region of the respiratory tract and facilitate the interpretation of treatment- or disease-related responses by the nasal epithelial transcriptome.

Holzmann, Klaus [12]

Kinetics of expression analysis during *myc*-mediated apoptosis

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Malignant transformation *in vivo* as well as *in vitro* requires the activation of oncogenes concomitant with the inactivation of tumor suppressor genes. Although data on the activities and functions of single oncogenes such as c-*myc* and c-Ha-*ras* are abundant, less information is available on their synergistic action. We found that primary rat embryo cell lines transformed by c-*myc* and c-Ha-*ras* were prone to apoptosis after inhibition of MEK, a downstream kinase of the main Ras signaling pathway. Addition of the MEK1 inhibitor U0126 to a series of *myc/ras*-expressing cell lines uniformly resulted in unphosphorylated ERK1 and ERK2, morphological alterations and cell cycle arrest, and apoptosis occurred to various extents. The MR-6 cell line apoptosed most promptly after inhibition of MEK1. We analyzed in detail the kinetics of gene regulation of MR-6 cells during apoptosis by means of complementary DNA arrays for the expression of 1,200 characterized rat genes. A twofold increase in expression or reduction of expression below 50% was chosen as the threshold. More than 100 genes were found to be regulated during the early stage of apoptosis induction. One hour after addition of U0126, numerous growth factor receptor genes were induced. By 3 h, G1- or S-phase-specific genes, genes of the AP-1 transcription factor complex and genes coding for cell-surface antigens were upregulated. By 6 h, when MR-6 cells started to apoptose, the expression of most of them returned to baseline values. Data obtained with cDNA arrays were confirmed by northern and western blot analysis. The expression pattern and kinetics of gene regulation in U0126-treated MR-6 cells were compared with those of the MR-2 cell line, in which inhibition of MEK1 resulted in G1 arrest but not cell death. We conclude that *myc*-mediated apoptosis is accompanied by a transient wave of upregulation of a complex array of genes. At least some of them might represent an abortive attempt by cells to avert cell death.

Huang, Kuan-Chun [13]

Global RNA expression analysis of primary and recurrent ovarian tumors

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Many antineoplastic agents fail because of intrinsic or acquired resistance developed by the cancer cells; drug resistance is therefore a major obstacle to successful chemotherapy of human cancers, including ovarian cancer. Understanding the mechanisms by which drug resistance arises and identification of the molecular factors affecting new drug target development is of paramount importance. In a study to compare the global RNA expression profiles of primary and recurrent ovarian tumors from the same patient by means of oligonucleotide microarray analysis, we have identified *XIST* (inactive X [Xi] chromosome-specific transcripts) as the most differentially expressed gene that was downregulated in the recurrent tumor. The *XIST* gene resides within the X inactivation center and is unique in being expressed exclusively from the inactive X chromosome. *XIST* is both necessary and sufficient for X inactivation. Preliminary studies of the cell line derived from the recurrent tumor showed that the line is resistant to the antineoplastic agent taxol, one of the anticancer drugs included in the treatment of the patients under study. The recurrent cell line has at least one copy of *XIST* in the genome, although the gene is not expressed. Further characterization of *XIST* expression in a panel of ten ovarian cell lines and six breast cancer cell lines showed that the expression levels of *XIST* correlate significantly with taxol sensitivity. These data indicate a possible correlation between X inactivation and taxol resistance in ovarian cancer. We have launched a detailed mechanistic study to characterize the underlying mechanism.

Huang, Tim Hui-Ming [14]

Dissecting complex genetic and epigenetic alterations in cancer genomes using CpG island microarrays

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We describe a high-throughput approach for investigating both copy number alterations and aberrant methylation of CpG islands in human cancer. The technique involves generation of a microarray panel of 5,150 short GC-rich tags (0.2–2 kilobases) derived from a CpG island genomic library. This DNA chip was used to study a patient with invasive breast cancer. We first restricted tumor and normal DNA from the patient with a four-base frequent cutter known to preserve larger GC-rich CpG island fragments. The digests were ligated to linkers for the polymerase chain reaction in low-amplification cycles, allowing for semiquantitative analysis in subsequent microarray hybridization. We co-hybridized amplicons from test and reference samples in a two-color fluorescence system, similar to that of the comparative genomic hybridization. The differential signal intensities observed in the microarray panel reflect copy number alterations of CpG islands in the tumor DNA. For methylation analysis, we further digested the ligated DNA with methylation-sensitive restriction enzymes and amplified it using the linker polymerase chain reaction. The amplified products may therefore contain different pools of DNA fragments owing to the differential methylation status of tumor relative to the normal control. We detected these methylation differences in subse-

quent hybridization using the same microarray panel. The results show that the overall changes in this tumor amount to about 16% of the CpG islands analyzed, with 8.2% of these loci showing changes in copy number, 6.1% showing changes in methylation status and 1.6% showing both types of changes. We have further categorized these genetic and epigenetic changes into eight distinct classes. Selected clones from each class have been confirmed by Southern hybridization. This study is the first such detailed analysis of complex molecular alterations in a single tumor, and it demonstrates the potential utility of this CpG island microarray for tumor classification.

Huminiecki, Lukasz [15]

In silico cloning of new endothelial-specific genes

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We present a powerful new approach for comparative expression analysis combining two data-mining strategies followed by experimental verification.The endothelium plays a pivotal role in many physiological and pathological processes and is known to be an exceptionally active transcriptional site. To advance our understanding of endothelial cell biology and to elucidate potential pharmaceutical targets, we have developed a new database screening approach to permit identification of new endothelial-cell-specific genes. We screened the UniGene index using high-stringency BLAST against a pool of endothelial and a pool of non-endothelial expressed sequence tags constructed from cell-type-specific db expressed sequence tag libraries. UniGene clusters with matches in the endothelial pool and no matches in the non-endothelial pool were selected. We then combined this approach with Serial Analysis of Gene Expression–library subtraction and the polymerase chain reaction with reverse transcription to further examine clusters of interest. We identified and labeled four new genes: those coding for endothelial-cell-specific molecules (ECSM) 1–3 and magic roundabout (similar to the axon guidance protein roundabout).

Ignatovich, Irina A. [16]

Suicide effect of herpesvirus thymidine kinase gene delivered into human hepatoma cells in complex with oligopeptides

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The delivery of the suicide herpes simplex virus type 1 thymidine kinase (TK) gene into tumor cells followed by therapy with synthetic guanylic nucleotide analogues, such as gancyclovir and acyclovir, is one approach to cancer treatment. The most effective strategy for suicide gene therapy is virus-mediated TK gene transfer, using mainly recombinant adenoviruses or retroviruses. The main disadvantages of this strategy include immunogenicity, toxicity (for adenoviral vectors) and the risk of side effects (for retroviral vectors). An alternative approach involves the use of nonviral gene delivery systems, such as molecular conjugates, cationic oligopeptides and cationic lipids. Although these are less effective than viral vectors, they lack the above-mentioned disadvantages and may be used repeatedly. To evaluate the possibility of *in vivo* nonviral delivery of the herpesvirus TK gene into mammalian tumors, we have studied the antitumor effect of this gene after its transfer into human hepatoma (HepG2) cells in complex with cationic oligopeptides, followed by acyclovir treatment. The herpesvirus TK gene was under the control of the mouse ribosomal L32 protein promoter, which is active only in proliferating cells. We used a gene transfer system based on the cationic peptide YKAK8WK, which is capable of forming compact complexes with plasmid DNA, and the amphipatic peptide JTS-1, a pH-dependent, endosome-destabilizing agent. TK-gene-transduced cells were cultivated in growth medium containing acyclovir (50 μg ml^{-1}). The percentage of dead cells was estimated after 48 h by propidium iodine staining. The apoptotic death of cells was simultaneously confirmed by gel electrophoresis detection of oligonucleosomic DNA fragments. The amount of dead cells among the HepG2 cells transfected by suicide TK genes was four- to fivefold more than the amount among control HepG2 cells grown at the same concentration of acyclovir in medium. These results demonstrate the possibility of using cationic oligopeptides as suicide TK gene carriers in gene therapeutic treatments of human tumors.

Inoue, Norimitsu [17]

Determination of the breakpoints of a chromosomal translocation found in a patient with paroxysmal nocturnal hemoglobinuria

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Paroxysmal nocturnal hemoglobinuria is an acquired hematopoietic stem cell disorder that causes clonal expansion of glycosylphosphatidylinositol (GPI) anchor–deficient cells. A somatic mutation of *PIG-A* that encodes a subunit of the enzyme complex in GPI anchor synthesis results in deficiency of GPI-anchored proteins on the surface of the blood cell. The mechanisms of clonal expansion of abnormal hematopoietic stem cells are unclear. We found a patient with the disorder having chromosomal abnormality 46XX, t(12; 12)(q13; q15) in all cells bearing a mutation of *PIG-A* and only in those cells, suggesting that the translocation may be causally related to clonal expansion. We mapped the breakpoints to clone genes that may be involved in the pathogenesis of this disease. Because the patient did not have GPI anchor–deficient lymphocytes, it was difficult to establish GPI anchor–deficient cell lines. We constructed somatic cell hybrids by fusing GPI anchor–deficient monocytes to mouse myeloma cells deficient in hypoxanthine phosphoribosyltransferase and selected hybrids carrying chromosome 12 by monitoring the expression of human CD9. We chose hybrids carrying chromosome 12 with deletion or duplication of the q13–q15 region by analyzing microsatellite markers. We mapped the breakpoints between WI-9630 and CHLC.ATA29H01 in q13 and between D12S355 and sts-N34486 in q15 using the hybrids carrying the deleted chromosome 12. We designed new sequence tagged site markers around D12S355 and fine-mapped the breakpoint in q15 within a distance of 2 kilobase pairs. The breakpoint in q13 was determined by the inverse polymerase chain reaction using primers near the breakpoint in q15.