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-