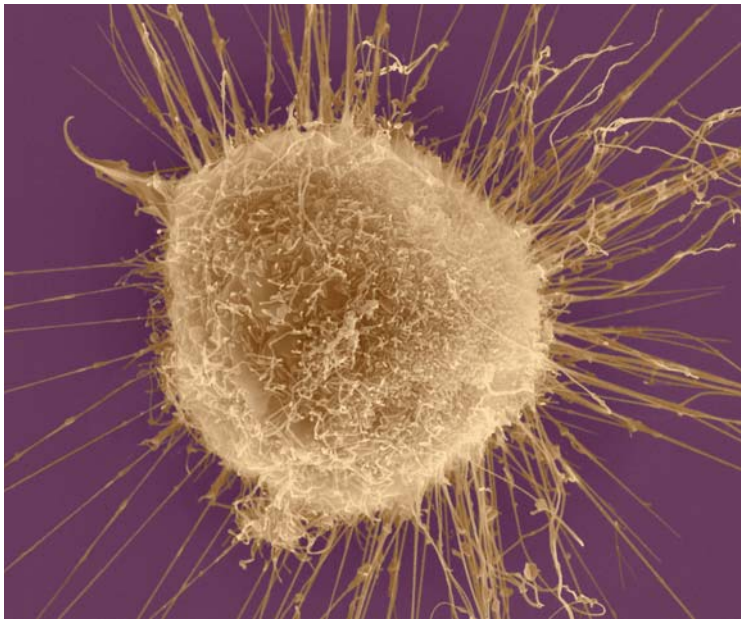
Template for Toxicants

Gene Expression Varies by Cell Type

Gene expression profiling shows that cells generally respond to toxicant stress by repressing genes that guide cell growth and inducing those that govern DNA repair and other protective functions. However, the specific genes repressed or induced vary, depending on the cell type and—according to research presented in this issue—the toxicant to which the cells are exposed [***EHP* 112:1607–1613**]. Melissa Troester of the University of North Carolina at Chapel Hill and colleagues note that this study demonstrates the utility of microarrays in predictive toxicology.



Profiles in chemistry. New research examining chemotherapeutic agents applied to breast cancer cells shows how known gene expression profiles may be used to predict the mechanism of action of other drugs.

The current study builds upon previous research showing that separate breast cancer cell lines have distinctive responses to two different chemotherapeutic agents, doxorubicin (DOX) and 5-fluorouracil (5FU). Because DOX and 5FU have different mechanisms of action, the researchers hypothesized that cells treated with one compound would express a different transcription profile compared with cells treated with the other. In establishing support for this hypothesis, the researchers were also able to demonstrate that a profile of expressed genes could serve as a template to predict the mechanism of action for a third cancer drug, etoposide (ETOP).

The researchers cultured four breast cell lines for their experiments—two each of basal-like and luminal epithelium—and determined comparable toxic concentrations for DOX, 5FU, and ETOP at 36 hours' exposure. Next, cell cultures were treated at these concentrations for 12, 24, or 36 hours in order to identify genes that were consistently expressed over time. At the end of the treatment periods, mRNA was extracted from the cells, pooled according to treatment and cell line, and used to create labeled complementary DNA samples. These samples were hybridized to microarrays representing 22,000 genes.

Microarray analysis identified which genes had been up- or down-regulated and revealed unique patterns of gene expression in response to DOX and 5FU in each cell type as well as each cell line. In general, luminal epithelial cells responded by regulating a large number of genes—974 in one line, 883 in the other. Basal-like epithelial cells regulated fewer genes (76 and 193) and also exhibited significant differences in gene expression over time. The cells exhibited a distinctly different profile at the 12-hour time point as compared with the 24- and 36-hour points. The difference was great enough that the DOX-treated samples clustered with 5FU-treated samples at 12 hours but not at 24 or 36 hours. This temporal shift blurred the lines between profiles and affected the accuracy of predictions.

Further investigation pinpointed 100 genes that could be used to differentiate between DOX- and 5FU-treated samples. This list of genes provided the basis for the final evaluation—testing whether the mechanism of action for ETOP could be accurately classified based upon the genes expressed following exposure. Because ETOP acts by a mechanism similar to that of DOX, it was expected that the gene set expressed by ETOP-treated cells would more closely resemble that of DOX-treated cells as compared to 5FU-treated cells.

Indeed, the mechanism of action for ETOP was predicted with 100% accuracy. When the researchers included cell type in the predictive model, the accuracy dropped to 75%, due in part to the temporal variability in gene expression in the basal-like cell lines.

With regard to the identity of regulated genes, published reports corroborate this toxicant-specific expression. For example, DOX has previously been shown to impair cellular respiration; the current research reveals that DOX alters mitochondrial gene expression, which provides a plausible explanation for the documented impairment. The findings also show several unanticipated changes in gene expression. For example, 5FU treatment induced the genes *ID1* and *ID3*, an effect that has not been previously noted. Knowledge of Id proteins is incomplete, and the researchers suggest that their pathways warrant attention as potential targets for therapeutic treatments.

Many toxicogenomics studies are providing expression data for toxicants that have known mechanisms of action, with the eventual goal of inferring mechanisms of action for novel compounds. Based on the success of their own mechanistic analysis, Troester and colleagues contend that this is feasible. **–Julia R. Barrett**

How E_2 Induces Uterine Effects

Transcription Coordinates Cascade

The rodent uterotrophic assay, a standard method for assessing a compound's estrogenicity, offers a model for phenotypic anchoring, or linking changes in gene expression to specific pathologic changes. Typically, when an immature rodent uterus is exposed to the endogenous estrogen 17β-estradiol (E_2), it undergoes cell proliferation and differentiation that can be measured through weighing and histological analysis. The uterine changes triggered by estrogens are directed by numerous genes, but little has been known about the molecular events involved and how they relate to observable physical change. A wealth of detail is now provided through research by Jonathan Moggs of Syngenta's Central Toxicology Laboratory in the United Kingdom and colleagues [***EHP* 112:1589–1606**]. According to the team's findings, E_2 induces a highly coordinated transcriptional program that orchestrates a cascade of cellular events related to uterine growth.

Dennis Kunkel Microscopy

The scientists' findings are based on a standard rodent uterotrophic assay. Female mice were given a single E_2 or control injection at approximately 3 weeks of age and then euthanized at specified time intervals (1, 2, 4, 8, 24, 48, or 72 hours). After the animals' uteri were weighed, samples were taken for histological analysis, and remaining tissue was subjected to RNA extraction for microarray analysis.

The researchers confirmed the physical events of this typical assay. Uterine weights began to change rapidly after the E_2 injections. A significant increase was seen by 4 hours, with maximum weight gain reached at 24–72 hours. Cellular changes were also rapid. By 4 hours after injection, the stromal endometrium had thickened due to water uptake; cell growth and proliferation were apparent between 8 and 24 hours.

Total RNA was isolated from the pooled uteri for each treatment group, and labeled complementary RNAs were constructed and hybridized to microarrays to yield 42 data sets. Analysis of gene expression led to the identification of 3,538 E_2-responsive genes. Further analysis allowed the grouping of these genes into coregulated clusters and the identification of the predominant gene functions associated with each cluster. Finally, by comparing gene expression and changes in uterine weight and histology with regard to time, the scientists were able to anchor changes in gene expression to changes in uterine characteristics.

These new microarray data reveal that the interaction of an exogenous estrogen with estrogen receptors initiates a highly coordinated molecular cascade that drives uterine growth and cell differentiation. The molecular program begins with the induction of genes that regulate transcription and signal transduction. It continues with the regulation of genes involved in protein biosynthesis, cell proliferation, and epithelial cell differentiation. Other gene functions are interwoven into the program, including the direction of fluid uptake and coordination of cell division.

With regard to time, changes in gene expression and uterine characteristics fell into four distinct phases. In the first phase, covering the first 4 hours after injection, E_2 rapidly induced transcriptional regulators and signaling components for a multitude of pathways, including those responsible for regulating fluid influx. The second phase, 4–8 hours after injection, was characterized by induction of genes needed for mRNA and protein synthesis, but no changes in physical uterine characteristics. During the third phase, occurring 8–24 hours after injection, uterine weight doubled, and cells entered the replication cycle, while genes controlling chromosome regulation and cell cycle were under active regulation. Finally, in the fourth phase, 24–72 hours following E_2 exposure, the genes being induced were those involved in uterine cell differentiation and defense responses.

The researchers write that their findings provide a basis for understanding the mechanisms by which other estrogenic compounds, including environmental chemicals, induce their effects. Also, the large number of E_2-responsive genes that they identified provides an array of potential marker genes that could be useful in short-term estrogenicity assays. Finally, the scientists note that their work provides a paradigm for understanding the mechanisms of action for estrogen as well as other nuclear receptors. **–Julia R. Barrett**