Tox/Path Team Takes On Differential Gene Expression

Toxicology and pathology are critical elements in toxicogenomics studies. The National Center for Toxicogenomics (NCT) has established a Tox/Path team that includes both NCT scientists and toxicologists and pathologists from the National Toxicology Program (NTP). The Tox/Path team advises the NCT by formulating research questions, designing studies, and mining databases for information. NTP members of the Tox/Path team also bring their toxicogenomics experience to bear on study design and assessment of proposed NTP toxicogenomics evaluations.

One of the goals of the NCT is to determine whether phenotypic alterations can be associated with differential gene expression (DGE) changes. To help meet this goal, the Tox/Path team has designed a series of studies to elicit different responses within the liver to determine whether DGE can distinguish specific pathological processes.

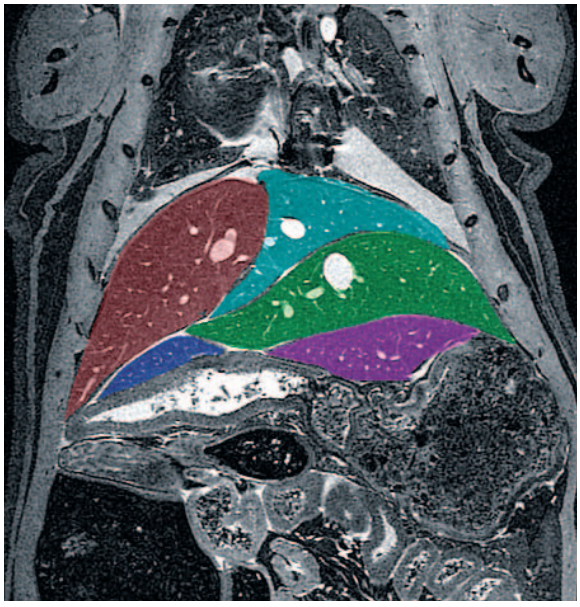
Correlating Phenotypic Alterations with DGE Changes

Initial Tox/Path liver studies focused on acetaminophen, a compound that causes centrilobular hepatic necrosis. Acetaminophen has been widely studied both because of its importance as a drug for humans (misuse of acetaminophen is the most common reason for admission to emergency rooms with acute liver toxicity) and because it exerts a specific regional acute centrilobular (zone 3) necrosis in the liver.

Pathological evaluations awaiting publication have revealed that acetaminophen-induced hepatic necrosis is not uniformly distributed throughout the liver. Further study has revealed differences in the extent of lesions among the liver lobules. The Tox/Path team has designed studies to evaluate the distribution of lesions throughout the liver. These studies will use magnetic resonance imaging to obtain a three-dimensional view of the liver. This technology may also allow the researchers to follow the development of lesions using noninvasive techniques, and possibly to correlate data obtained by noninvasive techniques with the development of lesions.

In addition to the acetaminophen studies, a second compound under study is the industrial chemical carbon tetrachloride, a known liver carcinogen also known to cause acute hepatic centrilobular necrosis. Comparison of acetaminophen and carbon tetrachloride may help to identify DGE changes that are specific to centrilobular hepatic necrosis and possibly differentiate between pathways to toxicity.

Allyl alcohol, also a large-scale industrial chemical, causes a different form of liver toxicity: acute hepatic periportal (zone 1) necrosis. Allyl alcohol will be contrasted with acetaminophen and carbon tetrachloride to further probe variable genetic pathways to toxicity. Other chemicals that target specific subpopulations in the liver such as biliary epithelial or endothelial cells are under consideration for study.



Looking at livers. The Tox/Path group is using rat liver studies to elucidate whether phenotypic alterations can be associated with differential gene expression changes.

One issue in associating phenotypic alterations with DGE changes involves histological sampling relative to sampling for gene expression. In some cancer studies, a frozen-tissue histological analysis is performed on each sample before is it subjected to RNA isolation for gene expression. Although this provides a direct morphological diagnosis for each DGE sample, such sampling is too time-consuming and expensive for most toxicogenomics studies. The Tox/Path team is exploring means for taking histological samples immediately adjacent to the samples taken for DGE analysis to ensure the least amount of variance in the tissue samples used for different assays.

Julie Foley, a researcher in the Laboratory of Experimental Pathology, is investigating yet another sampling technology: laser-capture microscopy coupled with RNA amplification for gene expression. This method would allow regional sampling of the liver, for example of centrilobular hepatocytes versus periportal hepatocytes. Comparing such samples is critical for hepatic toxicants where the lesions appear regionally. Laser-capture microscopy would also allow NCT researchers to target specific cell populations within the liver, taking DGE from the tissue to the cellular level.

Controlling the Variables

There are many parameters that may affect toxicogenomics study results, and the experimental details are crucial to DGE interpretation. For example, the composition of the test animal diet and circadian rhythms can profoundly affect gene expression. Due to their nocturnal nature, rodents will naturally eat during the night and sleep during the day, resulting in diurnal differences in liver glycogen and glutathione content that affect metabolism and toxicity of compounds. This circadian cycle has profound effects on DGE in the liver.

NCT protocols have been designed to control for time of dosing, light/dark cycles, feeding schedules, time of tissue collection, and other factors that may influence DGE. Where the palatability of the feed may induce changes in the time or amount of feed consumed, appropriate controls are included. The decision to fast animals overnight prior to morning dosing is questionable for DGE studies, because fasting is a powerful stressor. Room temperature, humidity, number of animals per cage, and even the person conducting the experiment have all been suggested to contribute to differences in transcription. The Tox/Path team is still considering how to control for these variables.

Chemical and toxicokinetic parameters are also important to toxicogenomics studies. Toxicokinetic data help in the selection of time points depending on peak chemical or metabolite concentration in the target tissue (time points are often selected when the chemical can be expected to be cleared from the tissue). Understanding when pathological lesions may appear and the development of the pathological response to injury is also important in determining the time intervals for sampling for DGE.

The route of exposure is critical because it can influence serum and tissue concentration levels, and also affects the

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kinetics of distribution and elimination of the compound. Intravenous and intraperitoneal exposure results in faster and higher plasma and tissue concentrations than oral gavage, dietary, or drinking water exposure. Unlike oral exposure, in which the liver is exposed primarily through the first pass portal venous blood, intravenous and intraperitoneal routes expose the liver by arterial perfusion. Enzymes within the stomach, the intestine, and the cells of the intestinal wall may modify many chemicals. Selection of the route and duration of exposure to match expected human exposure is the choice for most studies.

Variables in the animal model also may influence toxicogenomics studies. Strain differences between commercially available rodents may, in some cases, reflect differences in metabolic rates. Murine viruses and pathogenic bacteria confound experiments, so there is an emphasis on specific pathogen-free rodent sources and an active sentinel animal program.

Selection of the appropriate indicators of toxicity is highly important. If the purpose of the toxicogenomics study is to evaluate the gene changes during progression of a toxic effect (such as necrosis or apoptosis), then adequate documentation of that altered phenotype is critical. For many experiments, the standard battery of clinical chemistry and histopathology assays is sufficient to document the desired phenotypic changes. However, hematological evaluations may be necessary if alterations in blood cell number or composition are suspected. Special histopathological or immunohistochemistry stains may be needed to document apoptosis, increased cell proliferation, or other end points. Subtle changes that occur at low doses may require ultrastructure analysis. For subtle changes, immersion fixation of tissue samples for ultrastructure analysis may not be adequate, and a special study with fixation by liver perfusion may be necessary to avoid artifacts of fixation.

When the goal of the study is to translate the results to clinical practice, selection of surrogate tissues should be considered. Blood sampling for DGE, which offers the advantage of multiple sampling over time with minimal distress to the animal, is feasible, although not fully validated. Proteomic analysis of serum or plasma may also be performed on these samples, and may prove to be as useful as DGE analysis. Extensive validation is still required at the present time. However, the value of being able to follow gene changes in an easily acquired human sample makes the effort to develop blood sampling worthwhile.

Challenge and Promise

One challenge for correlating pathology diagnoses with DGE analysis involves standardizing pathology terminology. Especially with acute toxicities, multiple morphological diagnoses can be accurate and convey essentially the same information, and yet use different terms. To address this communication problem, the NCT is utilizing the NTP approach whereby each study has a study pathologist, a reviewing pathologist, and a pathology panel (known as a pathology working group) to ensure consistency and uniformity of diagnoses within and across studies. Tox/Path researcher Dave Malarkey is also working with the International Life Sciences Institute on an effort to standardize pathology terminology for toxicogenomics studies.

Another means of informing this process may be to include a description of the diagnostic process in the published study results and in databases. For example, with acute acetaminophen exposure, the process is acute hepatic centrilobular necrosis and repair; however, specific morphological diagnoses vary with time and dose.

Another challenge for toxicogenomics studies lies in dealing with the rapidly evolving technology, the extensive literature on each chemical, and the vast amount of data generated by even a modest experiment. But including multiple disciplines in study design, study conduct, data mining, and data interpretation is proving useful to the NCT teams, and provides an added benefit of a camaraderie that is helpful in combining resources to face a daunting list of differentially expressed genes. Both toxicologists and pathologists have the background and training to contribute to toxicogenomics. The NCT Tox/Path team allows both disciplines to bring their strengths to bear on the issues.

The task of the Tox/Path team is exciting, if at times overwhelming. The potential advantage of adding genomic technology to toxicology evaluations is vast. Shorter studies, fewer animals, and less expense are among the obvious advantages. Far greater potential benefits include the ability to recognize precursor lesions or biomarkers of effect that may be applicable to humans exposed in the workplace or the environment. Furthermore, understanding the pathways and mechanisms of toxicity may lead to better therapeutic interventions and treatment of diseases. **–Michael L. Cunningham, Richard Irwin, and Gary Boorman**

Digital Vision

It's all in the details. Tox/Path researchers are designing toxicogenomics studies to control for a diverse array of variables such as animal strain, circadian cycle, time of tissue sampling, animal housing, and others, all of which may affect differential gene expression.