Innovations

Virtual toxicology
Xenometrix Inc.

Stress is on the minds of pharmaceutical companies these days. The stress they are worried about is not, however, that suffered by their chemists, working long hours to find that elusive next drug. Rather it is the stress responses of single cells, which Xenometrix Inc. (Boulder, Colorado) is using in a reductionist approach to toxicology.

As pharmaceutical chemists amass huge collections of chemicals, they cannot hope to test them all in animals. Using rows upon rows of animals would not be acceptable, either ethically or financially.

To fish out the few active compounds, companies have resorted to assays that test the responses of cultured cells or purified proteins. But even when a compound appears to be active by this measure, it may still be unacceptably toxic. Xenometrix hopes to uncover these toxicities, by examining which of the candidate drugs alters the expression of genes that are induced by stress.

From Ames to Ames II

The first person to substitute single cells for animals in toxicology was Bruce Ames, of the University of California at Berkeley. In the 1960s, Ames developed a strain of *Salmonella typhimurium* to test for the mutagenicity (and so carcinogenicity) of compounds. The strain carried a mutation in one of its histidine synthesis genes that could be effectively repaired by any one of several missense mutations. Ames exposed these bacteria to various chemicals, and found that the number of bacteria that were now able to make histidine was proportional to the ability of the chemicals to induce carcinogenic mutations in animal models.

The Ames II assay, developed by Pauline Gee in Ames' laboratory, is now part of the Xenometrix product line. Gee constructed six *Salmonella* strains, each of which can regain the ability to synthesize histidine only by one of six possible missense mutations. Two other strains, developed as part of the original Ames test, detect a one or two base pair frameshift. Mutagenic compounds vary in the types of mutations that they introduce, so the pattern of histidine synthesis in the test strains acts as a fingerprint for each compound.

Stress alerts

Detecting the mutagenic potential of compounds is a routine procedure in drug companies, but there is more to toxicity than mutagenesis. Chemicals can damage cells and organisms in many ways, and cells have, in turn, developed mechanisms to detect and counteract that damage. This is where the Xenometrix leap of faith comes in. "We're looking at [the induction of] genes — the first response that cells have — and we think that is indicative of the metabolic response further on," says Gee, who is now the Vice President for Research and Development at Xenometrix.

Each Xenometrix assay uses a bank of inducible promoters, some of which are synthetic promoters with the active elements from more complex promoters. Each promoter and its associated reporter gene is inserted into an individual bacterial or human cell line. The read-out is the activity or presence of reporter gene products.

A simplified list of the toxic effects detected by two of the Xenometrix assays is given in Table 1. The real results are, however, more complicated. Any given chemical may cause several types of damage, and genes typically respond to a complicated combination of signals, some of which have not been characterized. "The surprises are probably less than 10%," says Gee, "but it's those that you notice, and you spend 90% of your time on them." One way of making sense of certain responses is to look at the kinetics of induction, in an attempt to determine which responses are primary and which follow the induction of other genes.

But does the system work?

Just as the Ames assay had to struggle for recognition as a measure of genotoxicity, stress gene induction must be validated as a good indicator of other toxicities. Most toxicologists are enthusiastic about the general approach, but have reservations about applying the methods. "I don't think we know enough about what the read-out means," says Richard Morimoto of Northwestern University. The threshold of damage that cells respond to is poorly characterized, and so the significance of the signal is hard to interpret. "These monitors may be a little too sensitive," says Morimoto.

Jeffrey Theiss, the Director of Molecular Toxicology at Parke-Davis, believes that more work is needed. "Is the signal reflective of a particular toxicity, or of adaptive

Competition

Xenometrix is not the only practitioner of molecular toxicology, but it does appear to be the most ambitious. Pharmaceutical companies use individual gene read-outs, but Theiss says that these are devised and used "on a case by case basis that is not very efficient".

Other companies have mapped out specific applications. Azur Environmental (Carlsbad, California) monitors environmental contamination using bioluminescent bacteria. The luminescence of the bacteria varies with their overall respiratory activity. In Vitro International (Irvine, California) uses protein matrices to mimic delicate eye tissue. And although Advanced Tissue Sciences (La Jolla, California) is primarily interested in developing artificial skin for grafting (the skin is grown on nylon scaffolds seeded with discarded foreskins), they also use the skin to test cosmetics for irritant activity.

Table 1

The Xenometrix stress assays.

Damage or cellular insult detected	Examples of promoters or promoter elements used (incomplete list)
The bacterial stress assay detects:	
Oxidative damage	Peroxidase
Heat shock	DnaK (chaperone, hsp70 homolog); clpB (ATP-dependent protease)
DNA damage	Gyrase and topoisomerase (regulate supercoiling, and their promoters are regulated by supercoiling); DNA repair enzymes (ada [methyl transferase], nfo [endonuclease IV])
Heavy metals	Mercury reductase
Loss of cell membrane integrity	MicF (promoter responds to osmotic conditions, as the gene product regulates the production of a membrane channel)
The human stress assay detects:	
Oxidative damage	GST Ya and HMTIIA (glutathione *S* transferase subunit and human metallothionein$_{IIA}$; both promoters have antioxidant response elements); NFκB response element
Heat shock	Hsp70 and grp78 (chaperonins)
DNA damage	GADD153 and GADD45 (growth arrest and DNA damage proteins); p53 response element
Heavy metals	HMTIIA (human metallothionein$_{IIA}$)
Biotransformation	GST Ya and CYP1A1 (glutathione *S* transferase subunit and cytochrome P450; both promoters have a xenobiotic response element that binds the dioxin/aryl hydrocarbon receptor)
Mitogenesis	Fos (through a serum response element); collagenase (through a TPA response element, activated by protein kinase C); NFκB response element
Inflammation	NFκB response element
Cell cycle arrest	p53 response element

changes that protect the cell from toxicity down the road?" he asks. "We don't really know that yet."

Gee agrees with these caveats, but feels that Xenometrix is responding adequately by building a database — a survey of the responses to drugs whose toxicities are known. "We have to decide at what point [the drug has] overwhelmed the cell," she explains, "and we are working to decide what that cutoff might be."

To help interpret the mountain of data, Xenometrix is developing pattern recognition software. Based on the assay results of compounds with known toxicities, the computer designs rules for predicting likely toxicities of novel compounds. This eliminates the bias that could come from specific scientific hypotheses.

Bacteria or human

Xenometrix produces the stress gene assays in both human cultured cells and bacteria. The bacterial assays have several advantages over the human assays, including promoters that are sensitive to osmotic stress, an oxidative stress response that is better understood, and simplicity. To mimic some of the metabolic reactions that happen in humans, a liver homogenate is added to these assays, and a cell-wall mutation is used to increase permeability to drugs.

The human assays use more complicated culture conditions, but they have the obvious advantage of being closer to the clinical situation, an advantage they also have over various animal assays.

Applications

Gee estimates that, by next year, ~60% of Xenometrix's business will come from pharmaceutical and biotechnology companies, with the remainder split between cosmetic, chemical and environmental companies. For pharmaceutical companies, explains Theiss, the assays can be used in one of two settings. "If you have a drug pretty far along in development and identify a toxicity," he says, "with these systems you can understand why a toxicity is there." Once the mechanism of toxicity is identified, researchers can try to rationally modify the drug to remove the toxicity while retaining the therapeutic properties.

The second application comes earlier in drug development, when the number of lead compounds is being whittled down. The stakes here are higher, and the drug companies are correspondingly more cautious about implementing the new assays. "If we develop a high level of confidence, then we could test compounds early on," says Theiss. But a false positive in a toxicity assay could result in a valuable lead being discarded.

Theoretically, these assays could be used to replace either animal or human testing, but neither Xenometrix nor its customers feel that this is imminent. The assays can, however, reduce the number of compounds that need to be tested in animals, by first eliminating those that are obviously toxic. And by using more stress-related genes (a gene-discovery effort is now beginning) and more powerful interpretive software, the detection of stress seems set to become a central feature in drug discovery.

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