Improving Therapy

With the post-genome era comes the challenge of assigning biologic functions to gene sequences and relating those functions to human health and disease. Animal models of human disease have been used to elucidate pathways of normal function, identify abnormalities leading to disease, and develop diagnostics and therapeutics. Better models of human disease are likely to result in more efficient drug discovery and screening and to reduce the likelihood that ineffective or toxic therapies will make it to the clinic.

Mutant Mice

Mice continue to be useful models because they share many genes with humans. At the Centre for Modeling Human Disease (CMHD), Toronto, Canada, they are using ethylnitrosourea (ENU) to mutagenize mice to generate models of specific human diseases, including diabetes, cardiovascular disorders, and abnormal embryonic development. Injection of male mice with ENU causes point mutations in spermatogonial stem cells. High-throughput screening (HTS) is used to identify dominant mutations in the first generation progeny. After confirmation that the trait of interest is heritable, chromosome localization, fine mapping, and cloning follow. Third generation mice are examined for recessive mutations. Physiologic assays and morphologic studies mimicking those used in human patients (e.g., complete blood counts, blood pressure measurements, and electrocardiograms) are used to screen for phenotypes.

“The other side of the coin,” Nisha Kassam, a project manager with the North American Conditional Mouse Mutagenesis Project at CMHD, notes, “is using in vitro gene trapping to create mutant, using poly(A) trap vectors to mutagenize murine embryonic stem (ES) cells. Current goals are to generate gene trap insertions followed by sequence tagging to develop a library of mutagenized ES cell clones freely available to the scientific community.”

S. Lee Adamson, Professor, Department of Obstetrics and Gynecology, University of Toronto, has an appointment at Mt. Sinai Hospital and is also Director of the CMHD Physiology/ENU Core facility. Her laboratory uses the mouse as a model for human pregnancy disorders, including pre-eclampsia and uterine growth restriction. They are taking existing animals with abnormalities predicted to affect adaptations to pregnancy and using monitoring procedures similar to those used in humans, including tail cuff devices to measure blood pressure, the ultrasound biomicroscope for imaging pregnant mice, and Doppler ultrasound to measure uterine artery blood flow waveforms.

The causes of human intrauterine growth restriction are multifactorial, including a genetic component, which they are hoping to identify; although there may be several acting in consort, in the mouse model, they will be able to look at these independently. In small human babies, insulin-like growth factor binding protein 1 (IGFBP-1) prevents IGF from stimulating normal fetal growth. IGFBP-1 levels are also elevated in animal models of fetal growth restriction. It appears that the fetus overexpresses an inhibitor that reduces its growth in response to nutritional supply and other factors. Although growth restriction abnormalities are seen on the fetal side of the placenta, they are also seen on the material side. “The mother’s reaction may be important in leading to new insights into the interaction between mother and fetus and how the fetus controls growth,” Adamson believes.

Adamson’s group at CMHD investigates abnormal cardiac function. “What’s been exciting for me in the mutagenesis program is the development of three lines of mice with abnormal aortic valve development.” The genes in these lines have been mapped to different chromosomes, so should be distinct. “In the literature, there was little about genes in cardiac development, and none known to affect aortic development, even though aortic valve defects are common in people,” she notes. Two of the mouse lines seem to reproduce the pathology of aortic stenosis seen in humans. “This is really new and exciting,” Adamson says. The next step will be to better characterize the genes in mice by determining their normal expression patterns and pathways. Following that, the search for the human genes can begin. “We’re trying to understand what goes wrong in development that leads to aortic stenosis. Because severity increases with age and the mechanism is not understood, we’d like to identify it early and see what’s going wrong,” Adamson says. “Areas of the valve grow as an indi-
vidual matures from infancy to adulthood, suggesting that growth factor pathways are involved.”

High-Throughput Fish
Randall Peterson, Assistant Professor at Harvard Medical School and Massachusetts General Hospital, Boston, MA, uses zebrafish to model human disease states. One might expect that the best animal models of human disease would be those closest to humans (i.e., primates). However, ethical issues aside, working with nonhuman primates is expensive and unwieldy. Peterson looks at the use of zebrafish as a disruptive technology. Disruptive technologies are new technologies that can displace existing dominant technologies, despite the fact that they are very different and frequently perform less well at first. He likens the zebrafish model to cameras in cell phones, which are increasingly smaller and cheaper compared with bigger and better high-end digital cameras with greater resolution. “Zebrafish are like that,” he says. “They won’t totally supplant the mouse, rat, or pig, but will have new applications as animal models.”

One example is the adaptability of zebrafish to chemical HTS. “You can’t do 10,000 chemical screens in a mouse,” he observes. “Fish are small, inexpensive, and easy to phenotype.” Peterson’s group screens thousands of compounds in the fish, looking for those that affect development or suppress disease phenotypes. Genetic screens are also feasible in this model (e.g., to identify genetic modifiers of disease). They are looking at cardiovascular disease, leukemia, anemia, and neurodegenerative disease, among others. Another advantage of zebrafish is that they are transparent. “You can look at living and growing fish, see the heart beat and the blood flow. In the leukemia model you don’t even have to draw blood, you can see a mass of blast cells accumulate in the embryo. We’re really excited about high-throughput imaging using automated scopes. A really cool technique is to use automated screening to find fish in multiwell plates and capture videos of beating hearts. It’s hard to imagine doing this with any other animal model,” Peterson concludes.

Previous models have lacked critical features of the human disease, have been difficult to establish and maintain, or don’t reliably mimic the response to therapy seen in the clinic. In this new mouse model, the expression in a Vκ construct of the human oncogene c-myc, which is disregulated in myeloma, is activated in postgerminal B cells by somatic hypermutation. The mice spontaneously develop monoclonal gammopathies, a hallmark of the human disease, and plasma cells expand at 20 weeks of age, again reflecting the older age of patients at diagnosis with myeloma. As in the human disease, the model mice slowly develop bone disease. “Our model is different because it reproduces the phenotype, the biology of the disease,” Sebag says.

“Preclinical models to date were very deficient, so there has been no good way to predict which drug will work,” Sebag observes. “If cell lines respond to a drug, this usually indicates that it will work in the clinic, but this doesn’t apply to multiple myeloma. Many of the cell lines don’t resemble the human disease.” In addition, because myeloma cells are dependent on interaction with the bone marrow microenvironment, cell lines in culture don’t offer this component. “We were brave enough to test our model against a range of drugs that were useful in the clinic and those that were not,” Sebag says. So far, drugs that are active in human myeloma are active in the mouse model, and those that are inactive in humans are inactive in the mice. “No other model has withstood this kind of analysis. Absolutely, we see the model to be used in drug discovery,” he says. The model could also be used to determine the pathways involved in the disease process, as well as to study the optimal sequencing of drugs used in treatment to overcome the inevitable drug resistance.

Future Directions
Kassam observes that “what’s hot now is a huge international push...
to mutate every gene in the mouse genome.” U.S., Canadian, European, and Chinese groups will each contribute to a Human Genome Project-like effort. “We expect a complete library of mouse knockout stem cells within 3 to 5 years,” she concludes.

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