



EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

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Stem Cell Models for Environmental Health

Introduction

On June 3–4, 2010, the National Research Council's Standing Committee on the Use of Emerging Science for Environmental Health Decisions held a public workshop to further understanding of the utility of stem cell models in evaluating the environmental stressors that affect human health. Toxicologists and environmental health regulators learned about state-of-the-art stem cell models, including human cells that are derived from embryos, induced pluripotent stem cells, and adult lineage-restricted stem cells, which could be used to inform environmental health decisions. Stem cell biologists learned more about the critical questions challenging environmental health scientists.

Stem cells have received much attention because of their potential therapeutic applications, but in the short term their expected value as research tools may be even greater. This is because stem cells have the special property of being able to differentiate to produce a wide variety of cell types—a property that enables them to be used to model aspects of human biology that have been largely inaccessible to study by other means.

Stem Cell Basics

After a human egg and a sperm merge at fertilization, the cells of the resulting two-cell embryo are thought for the first 1 to 2 days to be totipotent, that is, capable of forming every cell type of the placenta and the body. During the next 4 to 5 days, the embryo becomes a blastocyst—a hollow ball of cells with an outer layer that will become the placenta and an inner cell mass that is comprised of cells that are pluripotent, that is, capable of forming every cell type of the body except placenta cells. Human embryonic stem cells, which are collected from the inner cell mass

and continuously self-renew in culture, are also thought to be pluripotent.

Jane Lebkowski (Geron Corporation) explained how pluripotent embryonic stem cells can be manipulated to produce more than 200 specific cell types corresponding to all the major lineages (types of cells) and to more immature cells that can be used for studying developmental processes. The main reason that stem cells can be manipulated to produce so many cell types is that they retain the plasticity to express the DNA sequences required to produce different types of cells, said M. William Lensch (Harvard University). Other cells also contain the full DNA complement that is required to produce all types of cells, but they lack the plasticity that stem cells have. Changing the conditions in which stem cells are cultured, adding components to or subtracting components from the medium, can alter their fate.

Another special property of stem cells is that they are immortal. This is in contrast with somatic cells that make up almost all the body and have a limited life span. Lebkowski pointed out that the

immortality of stem cells allows them to be propagated indefinitely, and it enables companies like Geron to produce very large, well-characterized cell banks for many types of applications. For example, Geron is already producing heart-muscle cells (cardiomyocytes) derived from embryonic stem cells in lot sizes of 100 billion.

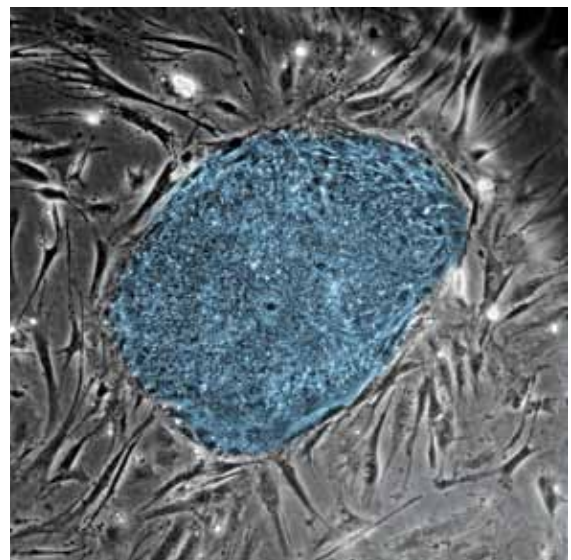
Lebkowski went on to explain that toxicity testing using stem cell models is already under way. Research by ChanTest, a company that performs screening for drug development, shows that cardiomyocytes produced from embryonic stem cells can be used to detect potentially toxic compounds. Cardiomyocytes in culture can produce a coordinated contraction similar to a heartbeat. Hence, it is possible to test effects of chemicals directly by measuring their effects on contractility with methods similar to those used to measure heart function. In fact, she said, the company's testing showed that cardiomyocytes could predict problems beyond those detected with the classic rabbit Purkinje-cell test used to assess a compound's arrhythmic risk. A cardiomyocyte is just one type of cell. Worldwide, many different groups are interested in using embryonic stem cells to produce other cell types for toxicity testing, including neural, intestinal, pancreatic islet, endothelial, hematopoietic, retinal, bone, and cartilage cells. In contrast with many of the screening approaches used currently, some scientists envision that those panels of cells will be much better barometers of potential toxic effects in humans.

In reviewing stem cell basics, Michael Roberts (University of Missouri) summarized two nonscientific concerns that have been expressed. Some people are opposed to the use of embryonic stem cells because creating them requires at least the partial destruction of spare embryos that were produced as a result of in vitro fertilization (IVF) treatments. However, he noted, they would otherwise be discarded. Another concern involves the derivation of human stem cells via cloning techniques similar to the ones that produced Dolly the sheep. The idea of using cloning technologies to

create stem cells is controversial because of the potential, although unlikely, of cloning the person who donated the cells. It should be emphasized that the technology has not been successful in creating embryonic stem cells except in the mouse and would, in any case, cross a second ethical boundary, the destruction of many human eggs.

Roberts next described induced pluripotent stem cells (iPS cells). iPS cells are not derived from embryos or via cloning technologies, so their use sidesteps the ethical issues discussed above. Instead, they are created from cells that are easily obtainable from adults or children. The method of producing iPS cells, realized in the last few years, constitutes a substantial scientific breakthrough. Cells that are given specific "factors" can be reprogrammed to behave more like embryonic stem cells. iPS cells can be created in a matter of weeks and, of interest to researchers exploring stem-cell therapy, theoretically can be put back into the host without eliciting an immune reaction.

Although the use of iPS cells avoids many ethical issues, it raises technical concerns. There are reports that iPS cells have a limited lifespan in culture, although Roberts said that this may depend on the age of the cells from which they were developed. Other reports suggest that iPS cells have a limited potential to differentiate and that cell lines generated from different kinds of



Human embryonic stem cells
Credit: Clay Glennon, University of Wisconsin-Madison

somatic cells behave and differentiate differently, possibly because they carry an “epigenetic memory” of their origins. In this regard, different lineages have different potential. Lensch pointed out that the reprogramming process for producing iPS cells creates a mixed cell population. Some cells are almost completely reprogrammed, and others are not. There is also evidence that the reprogramming process does not completely reset the pluripotent state. Nonetheless, these cells have many interesting applications. For example, researchers have used them to model complex genetic diseases, such as using them to identify genes that make people who have Down syndrome more or less susceptible to some tumors, as described by Lensch.

Using Stem Cells to Understand the Role of Environmental Chemicals in Disease

Autism

Ricardo Dolmetsch (Stanford University) is using human stem cells to investigate autism-spectrum disorders that many believe have both genetic and environmental components. Dolmetsch told conference attendees that the incidence of autism has been rising and that, although some circumstantial evidence points to environmental agents, there is not much strong scientific evidence. Identifying links by using conventional animal models is problematic because scientists know that some important classes of neurons exist only in primates. For example, the von Economo neurons believed to control social interactions are found in primates and in elephants but not in rodents.

Dolmetsch’s group is studying autism by harvesting cells from patients’ skin for the production of iPS cells, which are then converted into a wide variety of functional neurons and glial cells. The group has succeeded in recapitulating key aspects of neuronal development in the laboratory and in identifying genes associated with the calcium-signaling deficits found in patients who

have a rare genetically mediated form of autism called Timothy syndrome.

Although the Stanford researchers have not yet used iPS cells to study environmental triggers of autism, Dolmetsch believes “that at least in principle it is doable.” Relatively homogeneous populations of neuronal precursors, which can be made in large quantities, are good candidates for this kind of screening. Dolmetsch thinks it likely that researchers can use these cells to produce a system that is amenable to high-throughput screening. In summarizing, Dolmetsch cautioned that the goal is complicated by the fact that researchers are still trying to identify genetic links with autism. In addition, researchers lack key information, for example, how much of any given chemical gets into the brain of a developing child and for how long.

Breast cancer

Zena Werb (University of California, San Francisco) researches the role of stem cells in breast cancer. She is using many experimental systems, including mouse models and human tissue grown in mice. Werb told workshop attendees that there are two kinds of pluripotent stem cells: (1) the actively proliferating population (more numerous) and (2) the quiescent type (much rarer). Both help to maintain the body. The actively proliferating cells tend to be involved in routine, day-to-day tasks such as maintaining the intestinal tract, skin, and blood cell populations. The quiescent cells are called on under more acute circumstances, in which case they can proliferate rapidly.

Stem cells are relatively common in some adult tissues and can play an important role in regeneration. For example, in mouse mammary epithelium, stem cells make up 2–4% of the population; similar numbers are found in the mouse intestinal tract. Breast tissue can undergo multiple rounds of massive regeneration in the presence of the demands of pregnancy, lactation, and restoration to a nonpregnant state. Quiescent stem cells are also involved in generation of secretory tissue.

Stem Cell Technical Concerns

James Trosko (Michigan State University) was asked to consider the stem cell concepts presented at the workshop and share his thoughts about other issues that toxicologists and stem cell biologists might bear in mind as they develop stem cell models for toxicologic research. He pointed out a few complications that he sees when stem cell models are used to assess chemical toxicity:

- Scientists use iPS cells to model the body's 200-plus cell types. But the induction methods entail artificial "cocktails" that block or enhance various differentiation pathways. Therefore, the processes may not accurately mimic the analogous events that occur in people.
- Stem cells are evolutionarily designed to be less sensitive to toxics than their differentiated daughters, Trosko said. Indeed, stem cells appear to express genes that are associated with pumping out various types of toxic chemicals (drug-transporter genes) and thereby protect the stem cells. Helmut Zarbl (Robert Wood Johnson Medical School) pointed out that stem cells may use repair enzymes different from those used by developing cells.
- A fundamental problem with testing individual cells is that these models ignore the key roles that intercellular communication plays in health or disease. "Without cell-cell communication and homeostasis, normal development and function cannot occur," he stressed.
- Most of the body's stem cells are found in low oxygen environments. Maintaining them in a high-oxygen environment in vitro may change how they function by mechanisms that include oxidative stress and the resulting damage.
- Stem cells can divide both symmetrically (and produce other stem cells) and asymmetrically (and produce cells that have an established fate). Scientists are just beginning to understand the mechanisms that control these switches, which may be important for understanding carcinogenesis. This is something Trosko thought should be taken into consideration; indeed, Max Wicha (University of Michigan) noted that research is beginning to show that many oncogenes that cause cancer trigger imbalances between symmetric and asymmetric cell division.

Participants also discussed how stem cells vary according to their genetic backgrounds, which might affect their toxicity responses. This might warrant studying stem cell lines from different genetic backgrounds. However, Lensch pointed out that the genetic diversity of human embryonic stem cell lines may be limited because less diverse populations tend to invest in assisted reproductive technologies. Nevertheless, iPS cell approaches may help to expand the genetic diversity of tissue culture models that are used for testing purposes.

A major issue with many in vitro systems is their failure to mimic the metabolic circuitry of the organism as a whole, said Deborah Hansen (US Food and Drug Administration National Center for Toxicological Research). Many

We have to figure out how we are going to include metabolism in these assays.

—Deborah Hansen, U.S. Food and Drug Administration, National Center for Toxicological Research

compounds must be metabolized for them to be active teratogens or carcinogens, she pointed out. "We have to figure out how we are going to include metabolism in these assays," she said, noting that the use of cocultures is one option. David Jacobson-Kram (US Food and Drug Administration Center for Drug Evaluation and Research) agreed, noting that he was "very concerned that Tox 21 is now testing tens of thousands of chemicals with no provision for metabolic activation." He believes that finding a way to achieve in vitro metabolic activation is important to ensure that mass chemical screening is useful.

Richard McFarland (US Food and Drug Administration Center for Biologics Evaluation and Research) pointed out that scientists will need to validate the utility of stem-cell-based models for assessing chemicals by comparing the data that they yield with the relevant existing bodies of knowledge. He said the Interagency Coordinating Committee on the Validation of Alternative Methods has validation paradigms that may prove helpful although they may need to be revised to encompass stem-cell models.

Werb's laboratory has found that stem cells that produce the epithelial cells lining milk ducts can give rise to "mini-mammary glands" if they are placed in a microenvironment that models the three-dimensional (3-D) niche that they normally occupy. The cells behave quite differently when they are grown in a 3-D space rather than in a flat 2-D configuration. Accordingly, the 3-D setting allows researchers to model more complex interactions. Werb described how introduction of chemicals into these mini-mammary glands shows promise as an assay for evaluating the effects of chemicals on breast development, for example, branching of mammary gland ducts.

Stanley Barone (Environmental Protection Agency) commented that migrating simple 2-D systems to a 3-D architecture that recapitulates more of the niche's structural characteristics will be much more informative because these culture conditions are closer to the real-world systems that we are trying to model. Tom Knudsen (Environmental Protection Agency National Computational Toxicology Center) was excited about Werb's work because the extracellular matrix is a critical mediator

of cell behavior that very few in vitro systems are designed to study.

How Stem Cells May Be Used to Assess Toxicity

Generating typical target cells

In addition to their use to study autism, breast cancer, and other diseases that may have environmental contributions, human pluripotent stem cells can be used to generate the cell types that scientists examine for evidence of toxicity in animal assays. For example, liver, kidney, heart muscle, and nerve cells have been difficult to grow in the laboratory with conventional cell culture techniques.

Roger Pederson (Cambridge University) described how scientists could assess how these cells respond to environmental chemicals by looking for evidence of cell damage and other responses that toxicologists know are important. For example, responses of interest might include the effects of oxidative stress and the induction of xenobiotic metabolizing and detoxification networks, programmed cell death (apoptosis), and cell proliferation.

Addressing Regulatory Challenges

Now is a "critical time to be talking about stem-cell research in an environmental-health context," Tracey Woodruff (University of California, San Francisco) told workshop attendees. There is a strong incentive to accelerate the pace of toxicity testing given the increased burden of disease being documented in both adults and children, Woodruff said. Human stem cell models can speed up the process because they reduce the need to translate results obtained from animal testing to humans, according to Woodruff. In addition, stem cell assays enable scientists to use dose ranges that are measured in humans in a high-throughput mode in which the effects of multiple chemicals can be tested simultaneously at much lower costs.

Both Woodruff and Shafer reminded workshop attendees of the vision outlined in the National Research Council's 2007 report, *Toxicity Testing in the 21st Century: A Vision and a Strategy* [http://www.nap.edu/catalog.php?record_id=11970]. That report discusses how advances in molecular biology and toxicology can improve toxicity testing and points out the advantages of reducing animal testing. The report also recommends that testing for environmental agents cover the broadest possible array of chemicals, end points, and life stages. Other recommendations include focusing on cell lines or cellular components, preferably of human origin. Stem cells can help researchers to meet those goals, Woodruff said.

In addition to their use in assessing potential end points of toxicity in target cells, stem cells may allow researchers to study how cells that have major metabolic functions, such as liver cells, detoxify, activate, or otherwise transform chemicals. Pederson described how information about effects could be gained by using reporter systems similar to the ones that have been successful in the mouse embryonic stem cell field. For example, systems could be devised that report the activity and gene expression of enzymes, such as CYP3A4, that help to metabolize xenobiotics. Such reporter systems could also enable the mechanization that is required for high-throughput screening efforts.

Modeling barriers

Pedersen described another potential use of stem cell models: they can mimic the barriers formed by human skin, intestines, and blood vessels. Thus, model systems can be developed to help scientists to study how toxicants enter the body at these points.

Screening with stem cells

Pharmaceutical companies are already using human stem cells in broad-based screens of new drugs for particular kinds of toxicity, said William Pennie (Pfizer). Stem cells have many features that make them “far superior to other cell-based models we have at our disposal” for predicting drug safety, Pennie told workshop attendees. Conventional cell models tend to do a poor job of representing the biology of the human cells that pharmaceutical companies need to model, and they can also be extremely time-consuming to use.

The use of mouse embryonic stem cells to assess teratogenicity, the ability to cause birth defects, has been validated in multicenter studies by the European Centre for the Validation of Alternative Methods, Pennie said. Pfizer has further improved on that validated model for use in its own teratogenicity screening programs. Stem cells are also being used or developed to assess toxic effects on the liver, nervous system, heart, blood vessels, pancreas, and kidneys. Pennie explained that

these kinds of toxicity are difficult to predict with conventional screening models, and stem cells offer an approach to detecting toxicity sooner, before it is manifested at later stages, such as in preclinical testing in animal models or clinical trials.

Human diversity was a recurring theme in the workshop. Pfizer has applied to the National Institutes of Health for funding to create a panel of pluripotent stem cells that represent the diversity of human genotypes. This may help drug companies to identify whether there is a genetic basis of some of the rare, idiosyncratic kinds of toxicity that now are discovered only in clinical trials with large populations—or even later when a drug is on the market.

Shafer described how the Environmental Protection Agency (EPA) is developing ways to use human neuroprogenitor cells to screen chemicals for potential developmental neurotoxicity. These cells can be produced by differentiating embryonic stem cells or iPS cells. Or they can be isolated from fetal or adult brain or spinal cord.

Because there are so many specific targets whereby neurodevelopment can be impaired, Shafer said that he and his colleagues had focused on key processes that could be disrupted by exposure to environmental chemicals, including proliferation, differentiation, neurite outgrowth, formation of synapses (synaptogenesis), migration, myelination, and apoptosis. As the group works toward developing assays to detect chemicals that impact these processes, they are focusing on high-throughput methods that use commercially available stem cells, which will enable other investigators to adopt their models easily.

Delving into a specific example of stem cell use, Shafer described how his group validated a proliferation assay that was developed as part of the ToxCast neurotoxicity battery by testing a small group of chemicals already known to have antiproliferative effects. More recently, the assay was used to screen 309 biologically active chemicals that are being evaluated by the EPA National Computational Toxicology Center’s ToxCast program, which is aimed at using such tools as

high-throughput screening to predict the potential toxicity of untested chemicals. The results showed that about 125 chemicals had a significant impact on neural proliferation. Knudsen pointed out that other researchers in the ToxCast effort are also using stem cells and that they “are seeing . . . some very systematic, very clear correlations between what is happening to the stem cells and what we are measuring in some of the other ToxCast assays.”

Different views were expressed on the doses being evaluated. Shafer’s group has been testing a range that they believe is physiologically relevant—I nanomolar to 100 micromolar. Shafer said that when hundreds and thousands of chemicals are being screened, the concentration range cannot be adjusted for each individual chemical. Some a priori decisions about dose will need to be made even though some chemicals will be missed. However, Barone said that it is imperative that investigators who are developing assays set doses by taking into account concentrations that are measured in biomonitoring (measuring body burden of chemical compounds) and what is actually in the environment. He also recommended using such tools as physiologically based pharmacokinetic modeling and reverse dosimetry to predict potential real-world exposures.

Shafer’s work brought to light other potential issues regarding high-throughput assays that use stem cells. It takes several weeks to differentiate the cells into neural progenitors and later to more specific types of neurons. That is similar to the time required to differentiate other cell types, said Susan Fisher (University of California, San Francisco). Researchers must also make decisions about when and for how long cells should be exposed.

Looking Forward

In a panel discussion that he moderated at the end of the workshop’s first day, Committee Chair William Farland (Colorado State University) summarized the talks and comments by saying that “people are pretty enthusiastic about using stem cells for toxicity testing” and “there are

We are just at the cusp of defining significant and relevant gene–environment interactions and are just beginning to define epigenetic effects of environmental agents. The use of stem cells will certainly contribute new insights and approaches to these areas for further defining, for example, windows of susceptibility, as well as differential population and age-dependent sensitivities.

—Thomas Gasiewicz, University of Rochester

lots of areas where we can see promise with these systems.” He said that the workshop had documented the utility of the systems in three categories, and Thomas Gasiewicz (University of Rochester) added a fourth to this summary:

- Investigating how chemicals may affect the viability and functions of stem cells
- Observing the effects of chemical exposures on stem-cell differentiation as a model of developmental exposures.
- Producing common toxicant targets, such as human liver and kidney cells, for studying the specific effects of chemical exposures.
- Screening for gene–environment interactions

Workshop attendees who made predictions regarding the utility of stem-cell models for toxicity testing included Barone, who believes that they are already proving useful for risk assessment in a qualitative way. Knudsen said that stem-cell systems are now ready for attempts to predict the human pathways that might be the most sensitive to chemicals. He also pointed out that researchers have gained important mechanistic insights by studying zebrafish development, and he recommended that investigators who are studying the effects of chemicals on development collaborate with this community.

Lensch predicted that testing in human stem-cell systems could help to reduce the “false negatives

[that] everybody wants to avoid”—chemicals that did not affect mice or rats but ultimately prove to be acutely toxic in humans.

Finally, Fisher pointed out that scientists know very little about the key issue of how exposure to environmental chemicals affects human development. She believes that systems that assess how chemicals affect the development, self-renewal,

and differentiation of human embryonic stem cells are going to be extremely powerful models. “With the advent of human stem-cell systems, we now have the ability to understand these effects, and we should embrace this possibility rather than run away from it,” she concluded.

*Prepared by Kellyn Betts, with editing
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Related News and Publications

Betts KS. 2010. Using Stem Cells to Study Developmental Neurotoxicology. *Environmental Health Perspectives*. Oct;118(1): a433-37.

About the Committee

At the request of the National Institute of Environmental Health Sciences, the National Academies formed the Standing Committee on the Use of Emerging Science for Environmental Health Decisions to facilitate communication among government agencies, industry, environmental groups, and the academic community about scientific advances that may be used in the identification, quantification, and control of environmental impacts on human health.

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