

# ICE

A newsletter about faculty and activities at the Institute for Cell Engineering at The Johns Hopkins University School of Medicine

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## ICE's New One-Stop Shop

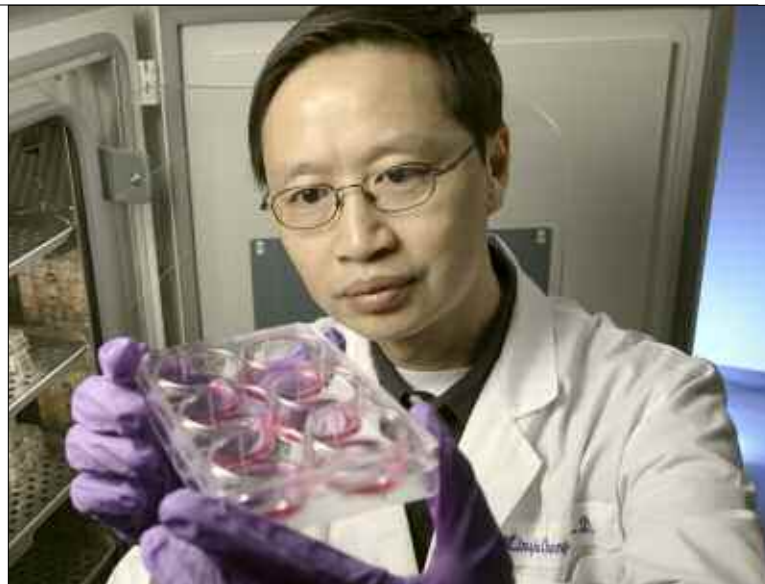
New Stem Cell Resource Center and Oversight Committee Pave the Way for Discovery

Imagine trying to build or remodel a house without the convenience of a nearby Lowe's or Home Depot," says gynecology and obstetrics professor Linzhao Cheng. "You have to drive all across town to find the parts you need, and if one thing is unavailable, you may not be able to start your project." At many academic institutions, prospective stem cell researchers face a similar unpleasant task; without a core facility that supplies materials, methods and support, they essentially have to learn how to build a house by themselves.

To save its researchers the time and hassle of searching for everything they need, Hopkins launched the Stem Cell Resource Center (SCRC), with Cheng as director, to house shared materials and equipment, and an institutional oversight committee—ESCRO (embryonic stem cell research oversight)—to help researchers navigate through the regulatory morass surrounding funding and other issues. Both funded by a private donation, the SCRC and ESCRO have centralized all stem cell lines on campus and approve all research protocols—all in one place.

"I personally would rather use a facility that takes care of a lot of core functions so I don't have to grow and test cells to make sure they're OK to use," says Chi V. Dang, vice dean for research and director of ICE. "We've created a toolbox that enables everyone to plug-and-play, rather than asking each researcher to reinvent the wheel."

As part of the SCRC, ICE has created a proteomics core that allows scientists to look at thousands



Linzhao Cheng in ICE's new resource center.

of proteins at once, as well as a small-animal imaging core so individual researchers do not have to buy their own expensive microscopes. Dang points out that investment in these core facilities is a win-win for Hopkins: the University saves millions of dollars while at the same time having the most cutting-edge technology in the field. "Pooling our resources allows us to buy—and share—the best equipment out there," says Dang.

With the SCRC up and running, one of its many goals is to generate new human embryonic stem cell lines because older lines have been shown to have acquired genetic mutations and other negative changes over time. However, creating new cell lines presents ethical considerations.

"With current technology, scientists must destroy a human embryo to create new embryonic stem cell lines," says Jeremy Sugarman, professor of bioethics and

medicine, a co-chair of the ESCRO Committee along with molecular biology and genetics director Carol Greider. Established under guidelines put forth by the National Academy of Sciences, the ESCRO Committee was created to help make human embryonic stem cell research at Hopkins consistent with ethical standards.

By reviewing all human embryonic stem cell research on campus, the ESCRO Committee aims to ensure that Hopkins' efforts to speed discovery from bench to bedside are handled with care. The ESCRO Committee is also preparing for what will hopefully be a promising future. "There will be new ethical issues that come up when and if the science is of sufficient quality to begin using embryonic stem cell-based interventions in patients," says Sugarman, "and we have to start considering such issues in advance." ■

—By Chris Troxell

## Maryland State Funds at Work

Coaxing a frightened cat out of a tree might require a combination of comforting words and warm milk. Coaxing embryonic stem cells to become a desired cell type is not as easy. Exploiting stem cells' pluripotency—their ability to become nearly any cell when triggered—could lead to limitless therapeutic uses. But although this capacity has been observed for decades, scientists still do not understand how stem cells regulate their pluripotency or how they pass it on to daughter cells after they divide.

"Until we can determine how stem cells control their own fates, it is impossible for us to direct them," says Candace Kerr, a research associate in the laboratory of John Gearhart. This year, Kerr was one of 24 investigators to receive funding from the Maryland Stem Cell Research Commission, and she plans to use that funding to identify the genes that control pluripotency.

Kerr and her collaborators compare the genes that are turned on and off in two separate groups of stem cells: pluripotent cells and a group of cells triggered to become a specific cell type, like nerve cells. "This research will pave the way for identifying the genes exclusively turned on in pluripotent cells," Kerr says. While the work is still in early stages, Kerr's team is taking a closer look at three promising candidate genes.

"I'd like to learn if we could reverse the process," she says. "Perhaps we can revert adult cells and make them behave like stem cells." Instead of injecting foreign cells, physicians could use the patient's own cells to replenish damaged tissue. ■

BY KATHLEEN DAUMER

# MagnetoCaps: Iron Fortification Saves the Day

New cell tracking method solves invisibility problem.

To gauge how well stem cell-based therapies may work in people, it's important for scientists to be able to track where transplanted stem cells go and what they do. There's only one small problem: Once inside the body, transplanted cells are basically invisible. However, a new advancement in cell imaging developed by Hopkins radiologists and ICE researchers may make hidden stem cells a problem of the past.

The recently developed cell-tracking method involves the use of tiny superparamagnetic iron oxide particles that when mixed in with cells provide a natural contrast that can be visualized by magnetic resonance imaging (MRI). "Before, we had to actually remove and test tissue samples to track down stem cells," says magnetoparticle designer and radiology professor Jeff Bulte. "MRI, on the other hand, is noninvasive and allows for serial imaging of cells with a resolution unmatched by any other clinical imaging technique."

And while there have been other research efforts to make cell transplants tractable, Bulte notes that they use heavy metals like gadolinium or radioactive isotopes, both of which can be quite toxic to the body. Iron, in comparison, is a mineral we probably don't get enough of. "It's inherently much safer to use," he says. "Our cells are already used to metabolizing it, recycling it and storing it."

Safe? Check. Easy? Check. Rounding out the trifecta is that magnetoparticle tagging can be applied to a wide range of therapies because the technique doesn't rely on any cell-specific markers. The first application—now in clinical testing—involves tracking dendritic cells, specialized cells employed in cancer therapy to stimulate our immune system to kill cancer cells. However, Bulte and his collaborators have been hard at work conducting imaging studies for potential applications ranging from multiple sclerosis to stroke to diabetes.

The diabetes studies, which Bulte has done along with radiologist Aravind Arepally, have taken magnetoparticles to a whole new level. Transplanting insulin-producing beta-cells into a diabetes patient is a potentially promising treatment, but it's hindered by the fact that the host immune system will attack the transplants and cause them to fail.

Bulte and Arepally have now provided a shelter; by combining their magnetoparticles with an organic polymer found in seaweed, they developed porous



Jeff Bulte, tracking down invisible cells.

microcapsules called MagnetoCaps. The enclosed beta cells can release their insulin into the blood while staying safe from the marauding immune system—and they can still be easily imaged with MRI. "We're really excited because we can track our capsules to make sure they don't move and their protective housing stays intact," says Arepally.

Adds Bulte, "We think that MagnetoCaps will make tissue-type matching and immunosuppressive drug treatment much less of a factor when it comes to cell-based therapies for type 1 diabetes." ■

## A New Stem Cell Superhero

Scientists often use cells, including stem cells, from animals like the mouse to carry out experiments. While there are many similarities between mouse and human biology, human cells hold the obvious advantage because they can lead to more direct clinical applications.

However, one significant disadvantage to using human stem cells is their long doubling time—it takes 36 hours for human stem cells grown in the lab to double in number, compared to 12 hours for mouse. Cells grown in the lab also tend to end up in a resting phase and stop dividing. As a result, it's difficult for scientists to grow enough material to use for experiments.

Linzhao Cheng, professor of gynecology and obstetrics and member of ICE, has recently discovered that growing human stem cells with a protein called Wnt3a increases cell numbers. According to Cheng, Wnt3a probably stimulates cells to leave the resting phase and start dividing again.

To test Wnt3a's ability to prolong human embryonic stem cell growth, Cheng's team grew cells with or without Wnt3a. After nine days, the researchers counted the cells and found more than seven times the number of cells when Wnt3a was present. After 14 days, Wnt3a cells outnumbered the non-Wnt3a cells by 16-fold.

"We really want to use human cells in our research, but it is difficult to get enough of them," Cheng explains. "We call Wnt3a 'the amplifier' because when it's around, we get more cells in less time, which means we can get results much faster."

Other researchers have already begun taking advantage of Cheng's finding. By increasing the speed of experiments, his new technique enhances the advancement of stem cell science. ■

BY KATHLEEN DAUMER

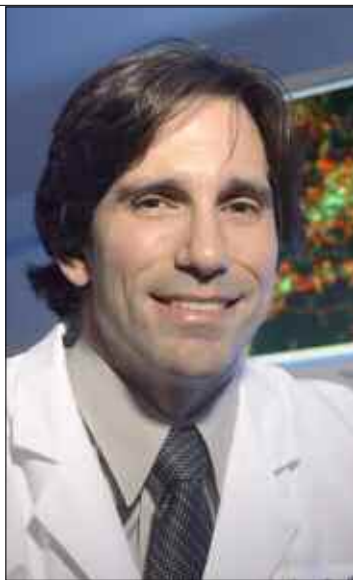
# Putting a Notch in the Stem Cell Belt

Discovering the molecular switches involved in stem cell fate decisions.

To paraphrase a classic Orwellian line: All stem cells are multipotent, some are just more multipotent than others. Sifting through this “equality” has been a major focus of neurology associate professor Nicholas Gaiano, as he is investigating precisely how a developing brain progresses from a handful of undifferentiated neural stem cells to fully functional adult forebrain with distinct regions.

“Stem cells in our brain don’t instantly convert into a neuron or glial cell,” he explains. “They mature in a stepwise fashion, gradually shedding their stem cell potency.” In an embryonic brain, he says, true neural stem cells that can become all manner of brain tissue are intermingled with so-called progenitor cells. While they still have many stem cell properties, progenitor cells have taken that first commitment step and only can mature into neurons.

Gaiano recently found that the stem cell-progenitor cell switch may be a protein called



Nicholas Gaiano follows the stepwise conversion of stem cells to neurons.

CBF1. CBF1 turns some genes on and others off in response to the activation in a cell of the Notch signaling pathway. The Notch signaling pathway is involved in several aspects of development and consists of an antenna-like receptor protein on the

cell’s surface and a relay system of enzymes within the cell that activate each other in sequence to translate a surface signal into changes in gene activity within the cell’s nucleus. Notch and CBF1 are both active in neural stem cells.

Gaiano and his team created genetically engineered mouse embryos that glow green when CBF1 is turned on and observed that during brain development, some of the glowing stem cells began flickering out, indicating that the CBF1 protein was no longer active in them. A closer look revealed that the “dark” cells were no longer true neural stem cells and instead had the characteristics of progenitor cells.

To confirm that CBF1 is the critical switch to cell commitment, Gaiano chemically knocked out the protein in cultured neural stem cells, which caused the cells to rapidly convert to progenitor cells. “Interestingly, if we turned on the normally inactive CBF1 protein in progeni-

tor cells, we couldn’t get them to shift back into stem cells,” says Gaiano. “So whatever happens biochemically once CBF1 is turned off seems to create a one-way street.”

Uncovering the critical first step of stem cell commitment is great, but Gaiano notes that this again is only the first step. “If we really want to manipulate neural stem cells for therapeutic uses, to help rebuild a damaged nervous system for, example, it’s vital to know what triggers the differences between all these classes of stem cells.”

Another exciting development is that gene regulators like CBF1 may not be restricted to neural stem cells. Another group of researchers using Gaiano’s glowing CBF1 mouse found that this protein plays the same role in blood stem cells, leading Gaiano to suspect that his team’s discovery might be a general “switch” distinguishing stem cells from progenitors in many different tissues. ■

—By Nick Zagorski

## Conversation

### Place Your Bets

Stephen Desiderio explains how the immune system is similar to Atlantic City.

**Q: How is the immune system able to sense such a wide variety of pathogens?**

**Desiderio:** In order to enforce the specificity of the immune system and ensure that antibodies don’t respond and react to the wrong things, cells that make antibodies only do so from one copy—either your mother’s or your father’s—of the antibody genes. Moreover, to avoid the potential danger of autoimmune disease, each cell only makes one antibody.

To build the diversity, each antibody protein is built from a different combination of discreet DNA segments from two different genes, the heavy chain and the light chain genes. The heavy chain gene consists of three parts—100 different V parts, 30 different D parts, and about six different J parts—that literally are cut and mixed chromosomes that are pasted back together to make millions of single V-D-J combinations. It’s exactly like a slot machine with three registers. There are billions of possible

antibody combinations, and 10 million cells in your body go through this every day of your life.

**Q: What is your latest finding about how a cell makes these V-D-J combinations?**

We discovered how the lever of the slot machine works. When an antibody-making cell starts this gene recombination process, it marks the chromosome with chemical changes. These changes are recognized by the molecular scissors—the RAG proteins—which home in and bind to the chromosome where it will be cut. The binding then activates the scissors to cut the chromosome.

**Q: Why do the RAG proteins need to be activated by the chemical marks on chromosomes? Isn’t binding enough?**

This too is new to our discovery. Traditionally, these chromosome marks have been thought of as simple landing pads for proteins to come in



and do their things. But we’ve shown that the marks can have a more active role, which is something no one really has considered, and in this case, the marks act as a key to turn on the RAGs.

**Q: What keeps the RAGs, once they’re bound and activated, from recutting a chromosome that already has been cut?**

Just like in slots, the registers spin only once. If you hit three in a row, the winner cashes out. Once the cell has recombined the antibody genes once, the chemical marks on the chromosomes are removed and RAG can’t bind and cut anymore. It’s game over for that cell. ■

## Overseeing Human Embryonic Stem Cell Research

Jeremy Sugarman describes how Hopkins is overseeing embryonic stem cell research on campus.

**Q: What are the issues with federal funding for human embryonic stem cell research?**

A: President Bush's first presidential address concerned human embryonic stem cells. The president attempted to strike a moral compromise by permitting federal funding for research with human embryonic stem cell lines that were created prior to the date of his presidential address, but not to provide such funding for the creation of new embryonic stem cell lines, which would require destroying embryos. So currently, federal funds are made available consistent with the president's remarks.

Scientists argue whether those existing lines are sufficient to do the kinds of work they want to do for a variety of reasons. One of the hopes of stem cell research is that eventually scientists will develop ways of introducing embryonic stem cells or stem cell products into people to treat a variety of diseases. But the stem cells that were created before the address involved the use of a mouse-feeder



layer, likely making them unusable for treating humans.

**Q: What are the ethical issues with human stem cell research?**

A: Some of those issues are related to the moral status of the embryo because using established methods, an embryo must be destroyed to create new human embryonic stem cell lines. However, even if you believe it is appropriate to use embryos to create human embryonic stem cell lines, other questions still are relevant. For example, should scientists use discarded embryos from in vitro

fertilization, or use embryos or gametes that were donated specifically to create embryos just for that purpose?

**Q: Why can't stem cell scientists self-regulate?**

A: It's clear the human embryonic stem cell research raises ethical issues. Not all of these issues relate to the embryo. For example, there are issues related to commercialization and patenting, informed consent, justice and research integrity. Given the complexities of these issues, the understandable excitement over stem cell science, and the financial and moral stakes at hand, it seems prudent to provide some type of oversight, much like in other types of research.

**Q: Why the ESCRO committee?**

A: There is now a confusing moral and legal landscape related to human embryonic stem cell research. To navigate through this landscape, the National Academies of Sciences believed that human embryonic stem cell sci-

entists and their research should have some type of oversight. The National Academies issued guidelines which suggest that institutions that conduct stem cell research establish committees (called Embryonic Stem Cell Research Oversight Committees) to provide local oversight. The leadership at Hopkins established a committee of people in charge of all sorts of research activities across the University, and called it the Human Stem Cell Committee. The committee suggested that an ESCRO committee also be established.

**Q: What current actions is the ESCRO committee taking?**

A: We're reviewing the protocols submitted by scientists at Hopkins to do human embryonic stem cell research. This involves deliberating about the ethical issues associated with each of their protocols as they come through. We're also educating researchers across Hopkins about the ethical issues inherent to human embryonic stem cell research. ■

# ICE

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