The action behind the words: embryonic stem cell research marches on

Talk of policy has dominated talk of science for those interested in embryonic stem cell science. But research is continuing, and the advances are making clear why embryonic stem cells are such an important scientific and medical resource.

Fraud in a South Korean laboratory shoots down a high-flying scientist and nicks the country's pride. A nasty Congressional battle over loosening federal restrictions on embryonic stem cell (ESC) research ends with President George W. Bush's first veto. In California, a protracted lawsuit stalls the state's voter-approved program for funding stem cell work, prompting Governor Arnold Schwarzenegger to offer an emergency loan.

Stem cells have hit the front page again and again this year—for the wrong reasons, researchers say. The ethical, political, and financial commotion has overshadowed the field's scientific progress, which researchers say is accelerating. "The literature is burgeoning—it's sometimes hard to keep up," says Ian Duncan (University of Wisconsin, Madison, WI). Although the United States' regulatory environment and limited involvement in ESC research slows progress the world over, "I'm very pleased with the pace we're going at," says Alan Trounson (Monash University, Melbourne, Australia). He heads the Monash Immunology and Stem Cell Laboratory, where 120 scientists are prodding stem cells to specialize into kidney, liver, blood, and other cell types.

As reviewed recently by Trounson (2006), it was Thomson et al. (1998) who first reported the successful isolation of human ESCs from blastocysts. Derivation without the need for feeder cells was more recent (Klimanskaya et al., 2005), and refinement of culture conditions has continued (Hoffman and Carpenter, 2005). There are now many markers for these human ESCs, and they can be transfected and differentiated into ectodermal lineages (e.g., oligodendrocytes for possible myelination; dopaminergic neurons potentially for Parkinsonism; and motor neurons for possible spinal cord repair), mesodermal lineages (cardiomyocytes and hematopoietic derivatives), and, with more difficulty, endodermal lineages (pancreatic islet–like cells and hepatocyte-like cells). Several disease-specific lines of human ESCs have been developed, thus generating models for diseases such as Fanconi anemia-A and cystic fibrosis.

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In other labs, researchers are shaking off the effects of the South Korean fiasco and restarting work on somatic cell nuclear transfer (SCNT), or therapeutic cloning. The technique, which entails fusing an unfertilized egg with a nucleus from an adult cell, is promising because it would generate cells that are genetically identical to the patient's own and thus might escape immune attack. In August, researchers from Kyoto University in Japan reported identifying four genes that can regress an adult mouse fibroblast to a stem cell–like state (Takahashi and Yamanaka, 2006), and a group at Advanced Cell Technology (Worcester, MA) derived ESCs from single blastomeres of 8–10-cell stage embryos (Fig. 1; Klimanskaya et al., 2006). According to Arnold Kriegstein, who directs the Institute for Regeneration Medicine at the University of California, San Francisco, the Japanese results could offer a solution to "the ethical dilemma that plagues this kind of work": how to obtain embryos for research. Although no treatments that rely on ESCs have made it to the clinic, next spring Geron Corporation of Menlo Park, CA, expects to begin phase I trials of a therapy for spinal cord injuries.

To assess the state of the field, we check in with five bench scientists who are pushing embryonic cells to be all that they can be. The projects they are tackling include a survey of what gives an ESC its identity, new attempts at deriving ESCs from SCNT, perfecting the transformation of ESCs into either oligodendrocytes that make myelin or pancreatic cells that make insulin, and creating an ESC-based model for Alzheimer's disease.

Profile of potential

An ESC has the capacity to transform into myriad cell types, but it is unclear what gene activities confer that ability. One possible factor that intrigues researchers such as Jeanne Loring (Burnham Institute for Medical Research, La Jolla, CA) is the distribution of methyl groups on the cell's DNA. Methyl groups don't just flip genes off; their effect on gene expression is complicated, Loring says. But methylation patterns do seem to shape a cell's developmental decisions. After fertilization, for example, eggs shed their methyl groups as they prepare to divide and differentiate. Although other researchers have measured methylation on individual ESC genes, nobody has tracked the tags across the genome.

Loring and colleagues used microarrays to check for methyl attachments at more than 1,500 sites scattered across 371 genes. The researchers compared the marks in 14 ESC lines to those in adult stem cells and cancer cells. Previous work on gene expression suggests that more genes are

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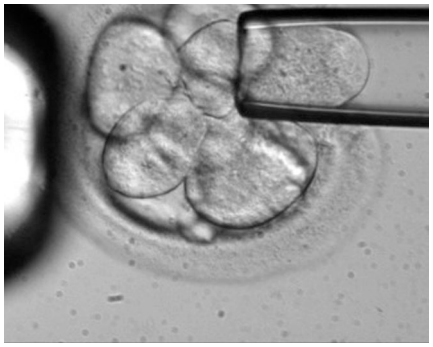

ADVANCED CELL TECHNOLOGY

Figure 1. **Research on embryonic stem cells marches on.** One of the new methods for generating ESCs involves using a single blastomere.

active in ESCs than in more specialized cells, and Loring expected a similarly "wide-open" arrangement when it came to methylation. However, 35% of the sites bore methyl groups in ESCs, 3% more than in adult stem cells (Bibikova et al., 2006).

Although ESCs weren't as free and easy as the researchers expected, the cells did show a distinct methylation signature that separated them from adult stem cells, cancer cells, and differentiated cells. That pattern might underlie the cell's versatility, Loring says, and might help researchers track down the genes that bestow this ability. Loring and her colleagues are developing larger arrays to assess more genes. She would like to test a cell produced through SCNT to see whether its methylation signature matches that of ESCs.

Mergers and acquisitions

Once is definitely not enough for Alison Murdoch (Newcastle Fertility Centre at Life and University of Newcastle in the UK). Last year, her group used SCNT to create a human blastocyst, a feat matched by only one other researcher, discredited South Korean scientist Woo Suk Hwang. The blastocyst perished before yielding any stem cells, and Murdoch and colleagues see it as only a start. They hope to refine the SCNT technique until minting a cell line that matches a patient's tissues is "reliable and routine," she says. The procedure is illegal in some countries that permit other forms of ESC work (Fig. 2), and no other lab in the UK has government sanction for this type of research, Murdoch says. But she'll soon have company. Scientists at Harvard, the University of California, San Francisco, and at least four other institutions plan to pursue SCNT.

Murdoch's position at the head of the pack is ironic because, when her team published its results in June of last year (Stojkovic, et al., 2005), it looked like an also-ran. The month before, Hwang and colleagues had announced fantastic success with SCNT, claiming not only to have created human blastocysts, but to have turned them into 11 ESC lines. Murdoch and company, meanwhile, reported comparatively modest results using 36 unfertilized eggs and donor nuclei from ESCs. Nuclei from ESCs are presumably easier to genetically reprogram to an uncommitted state. To unite eggs and nuclei, the researchers goaded them with a combination of chemical and electrical stimulation. Only three of the fused cells launched into division, and only one went further. Compared with Hwang's abundance of blastocysts and ESCs, the results "seemed like a tiny, tiny advance," Murdoch says. By the time Hwang's scam came to light in late 2005—investigators determined that his group had produced a few human blastocysts but no ESC lines—Murdoch's team had diverted to other questions. "We thought the technology [for SCNT] was there," she says. "It set us back a year at least."

Now that the lost year is over, the group is trying to pin down the problems that impede SCNT. Murdoch won't reveal what obstacles they've identified, but one limitation has loomed from the start: a shortage of eggs. SCNT works best on eggs freshly removed from a woman's body. But Murdoch and colleagues found that, even if they convinced in vitro fertilization patients to donate spare eggs, they could only garner a grand total from all the patients of about 10 eggs per month (Choudhary, et al., 2006). The Harvard team plans to solicit egg donations from young, healthy women. However, the UK body that regulates ESC research has rejected on ethical grounds Murdoch's application to tap the same source. She plans to apply again. With good eggs scarce, researchers like her who have worked with in vitro fertilization have an advantage, says Murdoch. They are adept at coaxing star performances from a single, recalcitrant egg.

Good conduct

Eggs aren't the only cells vexing researchers. Human ESCs follow a different timetable than do the rodent stem cells that scientists are more accustomed to working with, says Duncan. Like the animals themselves, rodent stem cells are frenetic, dividing and differentiating swiftly. By comparison, human ESCs are leisurely—and fragile. Moreover, human ESCs shrug off the growth factors that galvanize their mouse counterparts. Because researchers know so little about rearing human ESCs, the obstacles holding up progress in the field are more technical than intellectual, he says.

For seven years, Duncan and colleagues have been trying to prod ESCs to mature into oligodendrocytes. In the central nervous system, these cells make myelin, the fatty material that insulates nerves. An infusion of oligodendrocytes might help ease the symptoms of multiple sclerosis—in which myelin deteriorates under immune system assault—and several inherited diseases in which the material breaks down or doesn't form.

Duncan and colleagues started by growing mouse ESCs into oligodendrocytes and transplanting them into the spinal cords of rats that lacked myelin. The implants settled in and laid down new insulation (Brüstle et al., 1999). Next,

Country	Status
Ireland; Poland; Lithuania; Austria	Forbid research
Germany; Italy	Permit work on imported cell lines
France; Brazil; Australia; Canada; Japan; Netherlands	Permit derivation of stem cells only from donated embryos; forbid SCNT
Belgium; China; India; Israel; Singapore; South Korea; Sweden; United Kingdom	Permit SCNT
United States	No national regulations

Figure 2. **A world of regulation.** Current regulatory status for embryonic stem cells by country.

Duncan teamed up with University of Wisconsin colleague Su-Chun Zhang and other researchers to try a similar experiment with human cells (Zhang et al., 2001). They nudged human ESCs into differentiating into nervous system precursor cells. In the lab dish, these cells would mature into several types of cells, including neurons, astrocytes, and a few oligodendrocytes. But when the researchers inserted the precursors into immunodeficient mice, no oligodendrocytes developed, and no new myelin formed. Hans Keirstead of the University of California, Irvine, and colleagues (Nistor et al., 2005) have achieved these feats, Duncan notes. To improve their cells' performance, Duncan and colleagues are focusing on a molecular decision maker, the transcription factor Olig2. They plan to follow its expression during differentiation to understand how it helps a cell choose a particular path.

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To β or not to β

The options for patients with type I diabetes are sour: continued insulin doses or an infusion of pancreatic islets that contain hormone-making β cells. Although islet transplants can help control blood sugar levels, donors are scarce and the benefits often wane after a few months or years. Henrik Semb's lab (Lund University, Sweden) is one of several around the world hunting for a recipe that will drive ESCs to morph into β cells, thereby providing an alternative source for transplants. Nobody has gotten an ESC to go all the way, but with a helping paw from mice, Semb and colleagues have cultivated human cells that manufacture insulin.

Even under normal culture conditions, human ESCs began to differentiate and produce a transcription factor, Pdx1, that characterizes β cells, Semb's group found. Pdx1's presence suggests that the cells had started along the right road. However, they wouldn't go further and crank out insulin. To provide a boost, Semb and colleagues packed the cells into a section of mouse pancreas and tucked them under a rodent's kidney. The change of surroundings did the trick. Not only did the cells harbor three transcription factors—including Pdx1—that mark β cells, but they made the raw form of insulin (Brolén et al., 2005). "We are fairly confident that the cells are really close to a β cell," Semb says. This technique couldn't produce transplant-ready β cells because of the danger of contamination with mouse proteins, which could trigger an attack by the recipient's immune system. To overcome that problem, the researchers are working to decipher the signals between the endoderm, which gives rise to β cells, and the surrounding mesenchyme in the developing pancreas.

Stem cell stand-ins

Mouse models capture some aspects of Alzheimer's disease (AD), amassing the β-amyloid plaques that riddle the brains of patients with the fatal illness. But the animals share one big drawback, says Larry Goldstein (University of California, San Diego, CA): "They don't really get AD." Why the rodents don't lose their memories isn't certain—their physiology might differ in a key way from ours, or they might not live long enough to develop symptoms, he says. Searching for a more human alternative, Goldstein and colleagues turned to models that don't even have brains—ESCs. The cells are an improvement over existing neural cultures because they live longer and are easier to grow, and therefore are easier to genetically modify, says Goldstein.

For the last 18 months, Goldstein's group has been coaxing the versatile cells into specializing into neurons. The researchers are still learning how to keep human stem cells happy—Goldstein describes them as "high-maintenance"—and nudge them down the right path. "We've had to develop a lot of the methodology ourselves," he says.

Goldstein plans to deploy the neurons to test an alternative hypothesis for AD's cause. The leading explanation blames β-amyloid buildup for starting the disease. But Goldstein wants to determine whether AD begins when the cell's transportation system goes haywire. In the axon of a neuron, a protein called kinesin-I trucks vesicles, organelles, and other molecular cargo away from the cell body, running on microtubules that function like railroad tracks. Last year, Goldstein's team reported that they found train wrecks of vesicles and organelles in brain neurons from an AD mouse model and from patients in the early stages of the illness (Stokin et al., 2005). More pile-ups formed in mice that fashioned 50% less kinesin than normal. Furthermore, the brains of kinesin-poor animals accrued more β-amyloid plaques, suggesting that the transportation tie-up somehow spurs the protein's accumulation. To evaluate that explanation, the researchers are genetically altering ESCs to cut kinesin levels and to carry mutations associated with inherited varieties of AD.

Lab-reared neurons won't supplant mouse models, but they'll be essential for probing what instigates AD and for testing possible treatments, Goldstein says. "I don't see how you can generate a molecular description of the pathogenic events if you don't work on human cells," he says.

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