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Innovations

Advanced Cell Technology: Embryonic-Stem-Cell-Based Regenerative Medicine

Stem cells never stray too far from public attention. This was particularly true in May 2005, when researchers and politicians again brought focus to the topic. In the May 20, 2005 issue of *Science*, South Korean researchers announced the cloning of human embryos with greater efficiencies than ever seen before. The next week, on May 24, the U.S. House of Representatives voted 238 to 194 in favor of reinstalling federal funding for human embryonic stem (ES) cell research.

And like stem cells themselves, one U.S. company, Advanced Cell Technology (ACT, Worcester, MA), remains firmly at the center of it all. After a previous life as Avian Farms, Inc., an animal-genetics research company in Maine, ACT's current focus on human ES cell research began in earnest under the guidance of its founders, including Michael D. West, Ph.D. West, founder of Geron Corporation, joined the ACT effort in 1998 and brought with him 10 years of human ES cell research experience. "I joined the company to advance the research of making personalized human embryonic stem cells by nuclear transfer," says West. His intention was to bring ACT into the human medical arena. Today, ACT applies human ES cell research in the field of regenerative medicine.

Focus on Nuclear Transfer

"ACT is a valued part of the embryonic stem cell field," according to Leonard I. Zon, M.D., Chairman, Executive Board, Harvard Stem Cell Institute and President, International Society for Stem Cell Research. "At various points in the stem cell discussion, ACT has been very important to the argument of moving embryonic stem cell research forward, particularly regarding what the clinical aspects will be."

In 2001, ACT became the first research group to announce it had cloned a human embryo. ACT drew worldwide attention—and skepticism—over its results. "When we published our first paper in the human system in 2001, all we proved was the activation and cell division in the early embryo," recalls West. "People said it wouldn't work, saying we had gone through 100 oocytes and didn't get a single stem cell line." The ACT team recruited women to donate eggs and simultaneously sought cells to be cloned from other individuals. With nuclear transfer, the nucleus from the donated egg is removed, and it is replaced with the nucleus of the cell to be cloned. The new hybrid embryo is activated and then allowed to divide. In the 2001 experiments, none of the new ACT clones grew sufficiently to yield a stem cell line. Only one reached a 6 cell state. For this reason, the results were judged as premature and seen in the research community as more of a failure than a success. "ACT is often out there in doing important preliminary experiments that get published and stimulate the field," says Zon, "but the company still has yet to publish meaty pieces with a tremendous set of data, particularly in the clinical aspect of embryonic stem cell research."

"We envision building an ES cell bank representative of the major histocompatibility genes that would match up to 80%–90% of the US population, akin to the kind of match you try to make when doing a liver transplant," explains Michael D. West, Ph.D., of ACT.

West is not discouraged by the criticism. "We always said it would require fine tuning of the culture media and activation protocols," says West. The South Korean team's May 2005 announcement confirms this. Using a modified nuclear-transfer technology, Woo Suk Hwang's group at Seoul National University announced that from 242 human eggs modified with genetic material taken from skin cells from people with one of a variety of illnesses, 11 new ES cell lines were created. Each new ES line is a genetic match to the person donating the skin cell. "Sure enough, after four years, the South Koreans have improved efficiencies now to about one in 20 oocyte nuclear transfers," says West. Adds ACT CEO William Caldwell, "They have validated what we have believed to be the most elegant technique for developing new therapies: nuclear transfer. They have shown the world the efficiencies that are potentially embodied by this technique, and their announcement will really accelerate things."

ACT's own efforts in animal embryo cloning followed a similar path. In 1998, ACT published a paper showing that therapeutic cloning (with somatic nuclear transfer) worked in the bovine model. "There was real skepticism about this regarding the efficiency of the stem cell lines," recalls West. At first, the group created 1 stem cell from 200 or 300 nuclear transfers. "But then we got it up to 1 in 10 or 20," says West. "Then we collaborated with Teruhiko Wakayama, the man who cloned the first mouse." Again, the efficiencies were one in several hundred but then improved to one in a dozen or so nuclear transfers.

Target: Histocompatibility

Now that the proof of concept regarding human ES cell cloning is firmly established, ACT is looking for ways to further refine the potential in human clinical applications. "As I see it, stem cell applications

will fall into two major highways now that nuclear transfer is relatively easy to do," according to West. The first is to create a perfect genetic match by taking a patient's own cells and reprogramming them back to an ES cell state. The other is building a bank of off-the-shelf ES cells suitable for large groups of patients—similar to the concept of blood banking. ACT is in pursuit of both.

"Probably 85% of my efforts in the last 7 years at ACT have been in building our technology, our patent portfolio, in the area of histocompatibility. When I left Geron, I felt this was the one problem left to solve—and it was solvable." West believes that any ES cell therapy is subject to rejection—similar to organ donation today—owing to histocompatibility problems. "I came to ACT and thought nuclear transfer provided a pathway around this issue."

"For people with acute medical conditions, like acute myocardial infarction, there won't be time to do nuclear transfer," explains West. For these patients, ACT is developing a technology called reduced complexity library (RCL). The plan is to build a bank of pathogen-free ES cells that are homozygous in the HLA region. "We envision building an ES cell bank representative of the major histocompatibility genes that would match up to 80%–90% of the US population, akin to the kind of match you try to make when doing a liver transplant," explains West. "You could have the attitude that you need to create a large bank of ES cell lines to treat all patients to include racial diversity and genetic diversity," comments Zon. "But if you believe that the South Koreans have perfected the nuclear-transfer procedure, then you should be able to make stem cell lines that include the same MHC genes as the patient." This is why West and colleagues will pursue the ES cell bank idea exclusively for use in acute situations. "This idea is for use when you need to take cells out of a freezer and inject them within the hour," says West.

Other potential applications include acute spinal cord injury prior to destruction of the motor neurons and burn treatment. Although development of skin equivalents has been in process for years—à la Organogenesis—ACT envisions using human ES cells for superior burn treatment than is possible today. "We envision making off-the-shelf, embryonic skin equivalents," says West. Embryonic skin has a unique property. In the first two trimesters of human development, skin cells can regenerate completely. During the last trimester and in adult skin, they do not. Skin heals but does so with scarring. "But if you use embryonic skin cells, there is complete regeneration of skin without scarring," says West. "Those cells, we believe, can be easily made from human ES cells and then banked in a homozygous HLA state." A patient would present in the emergency room, and the doctor would match the HLA type and inject matching premade embryonic skin cells. "They could probably be made overexpressing platelet-derived growth factor (PDGF) with the potential to enhance wound repair in the burn setting," theorizes West. ACT recently acquired a Good Manufacturing Practice (GMP) facility in Worcester, MA, where the 30-person company is currently headquartered, to build this bank of cells.

"When it comes to cell therapy of any kind, one of the most limiting factors is protection of the cells after transplantation," says stem cell researcher Kevin Eggan, Ph.D., Assistant Professor of Molecular and Cell Biology at Harvard University and a member of the Harvard Stem Cell Institute. But he points out that it is not clear yet how immunogenic human ES cells are. "Some people claim that human ES cells and derivatives do not express high levels of major histocompatibility complex," he says. "I'm not sure that is true, but if so, it would make them less antigenic than other cell types." He comments that until the antigenic properties of human ES are fully understood, it is not unreasonable at this point in time to believe they would have the same antigenic properties as most other transplanted cells. "If that is the case, then histocompatibility is an important issue and one that has to be resolved," he adds. "If the ACT approach works, it should improve acceptance of the new cells." Eggan and West both believe that if an ES cell bank is developed, immunosuppressive therapy would still be required even if the major histocompatibility loci are matched because minor loci imcompatibilities will remain. Zon is enthusiastic that ACT is pursuing this line of research, even if the outcomes are uncertain at this point. "There are relatively few people working on the immune system and embryonic stem cells, so I think it is an area that really deserves some thought."

"While nuclear transfer is a cornerstone of what we built at ACT, we believe it is going to be possible to do other interesting things," says West. One of those second-generation cloning technologies skips the need for a donated egg cell. "We hope to be able to reprogram a patient's own cells in extracts," he says. If successful, this type of second-generation cloning technique would lend itself to patient-tailored cell therapy in the future, thus bypassing the need for creating or destroying embryos. "We would all love this to be the output," acknowledges Zon. "It sounds great, and I actually believe that by studying all these processes over time we will have better understand how to reprogram cells, but right now we do not have any evidence it really can happen." Zon adds that the proof of concept has not been established. "Nobody has been able to take a somatic cell and reprogram it," he says. "That idea is much, much longer down the road."

Compass Pointing Westward

ACT, along with many other institutions and academic centers based in California, is taking advantage of the state's passage of Proposition 71, an act passed in November 2004 that will provide $3 billion in state funds for human ES cell research. Caldwell believes Proposition 71 will enable extensive collaboration opportunities in California with numerous academic centers. "This will provide opportunities unprecedented in scope for a biotech company," he says. ACT will trade its expertise in ES cell growth and nuclear transfer for information on creating new cells they have yet to work on. "What you'll see going forward is extensive collaborations between academia and biotech companies like ACT," Caldwell says.

During the May 2005 Congressional ES cell debate, House Minority Leader Nancy Pelosi of California said, “We in California will become the regenerative capital of America, indeed probably the world.” With its senior managers already residing in California and a planned corporate expansion into the state, ACT is well situated to remain at the core of ES cell research in the United States.

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