What We Know About Embryonic Stem Cells

by Maureen L. Condic

Back at the beginning of 2002, there was considerable optimism regarding the promise that embryonic stem cells were said to hold for millions of people suffering from fatal or debilitating medical conditions. Stem cells derived from human embryos, it was claimed, provided the best hope for relief of human suffering. Despite the profound ethical concerns regarding the use of human embryos for medical and scientific research, many Americans embraced this promise and the seemingly miraculous hope it offered.

The challenges facing embryonic stem cells were formidable. First, there was the concern that the cells and their derived tissue would be rejected by the patient's immune system, requiring the patient to undergo lifelong immune suppression. The three proposed solutions to this incompatibility problem (generating large banks of stem cell lines, cloning human embryos to provide a source of cells that perfectly match the patient, or genetically engineering stem cells to reduce immune rejection) were either socially, scientifically, or morally problematic (or all three). Second, there was the serious problem that embryonic stem cells form tumors when transplanted to adult tissues, and the tumorogenic capability of these cells is difficult, if not impossible, to control. Finally, there was the disturbing fact that science had thus far provided essentially no convincing evidence that embryonic stem cells could be reliably differentiated into normal adult cell types, as well as the disturbing possibility that overcoming this barrier would prove a difficult scientific endeavor.

Despite these concerns, many continued to regard embryonic stem cells with hope, believing that further research would overcome these difficulties and harness the power of embryonic stem cells for the benefit of mankind. Such optimists asserted that it was simply a matter of investing sufficient time, money, and research.

Since 2002, considerable resources have been devoted to just such research. A recent query of the grant database maintained by the National Institutes of Health (NIH) indicates that more than eighty research projects investigating human embryonic stem cells have been funded over the past five years. A research effort of this size represents millions of dollars in public money invested in the medical promise of embryonic stem cells. Indeed, the NIH reported to Congress in September of last year that anticipated spending on human embryonic stem cell research in 2006 was "just $24,300,000." Since 2002, approximately nine hundred research papers have been published on investigations of human embryonic stem cells, with more than a thousand additional papers investigating the properties of embryonic stem cells derived from animals. Clearly, research on embryonic stem cells has advanced considerably over the past five years, and it is therefore important to revisit the promise in light of current findings.

Stem cell-based therapies propose to treat human medical conditions by replacing cells that have been lost through disease or injury. Unlike an organ transplant, where a damaged or diseased tissue is removed and then replaced with a comparable organ from a donor, stem cell therapies would involve integration of replacement cells into the existing tissues of the patient. The dispersed integration of the transplanted cells throughout the targeted organ (indeed, throughout the entire body of the patient) would make it impossible to remove the stem cell derivatives surgically should

any problems arise. Thus, the problem of immune rejection is of particular concern-if transplanted cells are attacked by the immune system, the entire tissue in which the foreign cells reside becomes the target of a potentially disastrous immune attack.

Over the past five years, the scientific community has focused almost exclusively on somatic-cell nuclear transfer, or cloning, as the best resolution to the problem of immune rejection. During somatic-cell nuclear transfer, the genetic information of an unfertilized human egg would be removed and replaced with the unique genetic information of a patient. This would produce a cloned, one-cell embryo that would mature for several days in the laboratory and then be destroyed to obtain stem cells genetically matched to the patient. Based on the success of animal cloning, human cloning was optimistically predicted to be a simple matter. Once we were able to clone human embryos, those embryos would provide patient-specific stem cell repair kits for anyone requiring cell-replacement therapies.

Human cloning has proved to be more challenging than anticipated. Human eggs, as it turns out, are considerably more fragile than eggs of other mammalian species, and they do not survive the procedures that were successfully used to clone animals. Multiple attempts by several research groups worldwide have been unsuccessful in generating human clones. The few reports of the successful cloning of human embryos were either unverifiable press releases or clear chicanery promoted by a quasi-religious group for its own publicity.

The elusive prize to generate the first human clone appeared to be won in March 2004, when a South Korean group led by Hwang Woo-Suk reported in the prestigious professional journal Science that they had generated a human stem cell line from a cloned human embryo. A year later, in June 2005, this same group sensationally reported that they had successfully generated eleven patient-specific stem cell lines from cloned human embryos and had dramatically improved their success rate to better than one in twenty attempts, bringing cloning into the realm of the possible for routine treatment of human medical conditions. Hwang was hailed as a hero and a pioneer, and his reported success evoked an almost immediate clamor to remove the funding restrictions imposed by the Bush administration on human embryonic stem cell research, lest America fall hopelessly behind South Korea in developing therapies.

By fall 2005, however, the cloning miracle had begun to unravel. Colleagues of Hwang raised serious concerns about his published studies, launching an investigation into possible scientific fraud. By December, it was conclusively shown that all the claimed cloned stem cell lines were fakes. To date, no one has successfully demonstrated that it is indeed possible to clone human embryos, and, based on the failed attempts of Hwang and others, human cloning is not likely to be a simple task, should it prove possible at all.

The scandal surrounding Hwang’s audacious fraud raised multiple concerns about the ethics of embryonic stem cell research. Investigations revealed that Hwang had used thousands of human oocytes for his unsuccessful attempts, not the hundreds as he had originally claimed. The medical risks associated with egg donation (the potential complications include both sterility and death) raise serious questions about the morality of conducting basic research on human cloning. Given that Hwang pressured junior female colleagues into donating eggs for his research, how can the interests of female scientists be protected from such professional exploitation? Given that thousands of human eggs from more than a hundred women were used by Hwang and not even a single viable

cloned human embryo resulted from this research, how can the medical risks to women entailed by this research possibly be justified?

The technical challenges encountered by Hwang are not particularly surprising. Experience from multiple laboratories over the past decade confirms that it is extremely difficult to clone any animal. Cloned embryos are generally quite abnormal, with those that are sufficiently normal to survive to live birth typically representing between 0.1 and 2 percent. The problems do not end with the technical difficulty of somatic-cell nuclear transfer itself. Extensive evidence indicates that even the cloned animals that make it to birth are not untarnished success stories. Following Ian Wilmut's production of Dolly the sheep, the world's first cloned mammal, it was almost immediately evident that Dolly was not normal; she experienced a number of medical problems that resulted in her being euthanized, due to poor health, at the age of six years, about half the lifespan of a healthy sheep. Dolly was the only clone to survive to live birth out of the 277 cloned embryos Wilmut's group generated, yet this success did not prove that cloning can produce a normal sheep. Dolly was merely normal enough to survive to birth.

In the past five years, a number of studies have carefully examined patterns of gene expression in mice and other cloned animals that survived to birth. Not one of these animals is genetically normal, and multiple genes are aberrantly expressed in multiple tissues. Both the severity and the extent of these genetic abnormalities came as a surprise to the cloning field, and yet, in retrospect, they are not surprising at all. The fact that most cloned embryos die at early stages of development is entirely consistent with the conclusion that somatic-cell nuclear transfer does not generate normal embryos, even in the rare cases where clones survive to birth. Thus, the optimistic contention that "therapeutic cloning" would fix the immune problem facing potential embryonic stem cell-based therapies for humans seems thus far entirely unsupported by the scientific evidence.

The dwindling numbers of therapeutic-cloning supporters defend this procedure by asserting that the genetic abnormalities are only a problem if you are attempting to produce a live birth. Thus, in a 2004 New York Times article, George Daley, a stem cell researcher at Children's Hospital in Boston, acknowledged that cloned animals show multiple genetic abnormalities, yet optimistically asserted, "Cloned tissues are not likely to have the same problems." In light of the mounting evidence that cloned animals experience severe genetic disregulation, such tentative reassurance is wearing thin, with even Daley admitting that his optimistic prediction that cloned tissues will prove normal enough for medical purposes has "yet to be proven."

The question of how normal cloned tissue needs to be is not merely a detail that needs to be worked out. It is, in practice, a fundamentally unanswerable question. If cloned human embryos are to be used as a source of stem cells, we will be faced with this simple question for every single patient: How normal is this particular cloned embryo, the one we are going to use to generate stem cells to treat this particular patient? Without allowing that embryo to develop and observing precisely how abnormal it proves to be, it is simply impossible to know whether it is normal enough for medical use. Every patient will be an experiment with no quality control. Perhaps the particular cells will be normal enough to cure this particular patient, but then again perhaps they will be so grotesquely abnormal that they will create a condition worse than the one they were intended to treat.

The limitation in our ability to determine which cloned embryos are of sufficient normalcy to generate medically useful replacement tissue is one that no research can address unless scientists develop some kind of test to determine in advance which cloned embryos are normal enough.

Developing such a test would almost certainly require the horrific scenario of growing human embryos to a sufficient state of maturity that the normalcy of their developing tissues could be empirically determined. This would mean implanting cloned embryos into surrogate wombs and then aborting them at specific times to examine the embryo's development. Based on this information, it might be possible (although difficult) to identify features of very early embryos that predict whether they are capable of generating therapeutically useful tissue. Whether Americans are willing to accept the unknown (yet potentially large) risk of being treated with stem cells of undetermined (and essentially undeterminable) quality or whether we would prefer to accept the kind of experimentation on human embryos and fetuses that would be required to ensure embryonic stem cell safety are questions of profound social and moral importance.

It was unambiguously clear five years ago that embryonic stem cells robustly form tumors (teratomas) when transplanted into adult tissues, and this remains the case today. Teratomas are benign tumors that contain a variety of differentiated cell types (hair, teeth, muscle, etc.). These tumors can often prove fatal because of their rapid growth, but they are not malignant or cancerous tumors, which metastasize into multiple locations within the body. Embryonic stem cell advocates were well aware of the tumor-forming potential of these cells. (Indeed, teratoma formation following injection of embryonic stem cells into adult mice is still today the test of whether a researcher has successfully generated a bona fide embryonic stem cell line.) Embryonic stem cell advocates dismiss the threat of these tumors, however, claiming this would prove a problem only for undifferentiated embryonic stem cells.

These optimistic predictions have not held up to scientific experimentation. The tumor-forming potential of embryonic stem cells has proved a significant problem that does not show signs of being resolved any time soon. More than a dozen papers over the past five years (five papers within the past year alone) have shown tumor formation in animals treated with differentiated embryonic stem cell derivatives. In several of these studies, a shocking 70 to 100 percent of the experimental animals succumbed to fatal tumors. In all cases, tumors were believed to be derived from embryonic stem cells that either failed to differentiate or from cells that somehow de-differentiated once transplanted. Although experimental approaches designed to reduce tumor formation from differentiated embryonic stem cell derivatives are under investigation, it is not clear whether these approaches will ever prove successful, especially if the tumors are due to uncontrolled de-differentiation of the embryonic stem cell-derived tissues back to a more primitive state once they are transplanted to an adult environment.

Even more alarming than formation of benign (albeit, fatal) tumors, several studies over the past five years have raised concerns that the longer embryonic stem cells are maintained in the laboratory (or, presumably, in the tissues of adult human patients), the more likely they are to convert to malignant cancer cells. Embryonic stem cells spontaneously accumulate the genetic abnormalities associated with embryonal carcinoma (a form of testicular cancer). Embryonal carcinomas are believed to be the cancerous equivalent of embryonic stem cells and are a highly metastatic form of cancer. Although the finding that embryonic stem cells spontaneously convert to cancer cells over time remains contested, it is clear that some, if not all, embryonic stem cells undergo this conversion, and the factors controlling the transition are not well understood.

The assertion that embryonic stem cells in the laboratory can be induced to form all the cells comprising the mature human body has been repeated so often that it seems incontrovertibly true. What is missing from this assertion remains the simple fact that there is essentially no scientific evidence supporting it. Experiments have shown that embryonic stem cells are able to participate in

normal embryonic development, an observation that is also true of cancerous embryonal carcinoma cells. When injected into early mouse embryos, both embryonic stem cells and embryonal carcinoma cells randomly contribute to every tissue of the developing body.

Even more dramatically, when embryonic stem cells are injected into mouse embryos under specific experimental circumstances (a procedure known as tetraploid complementation), they can be induced to form all the cells of the postnatal body. These experiments prove that embryonic stem cells (and embryonal carcinoma cells) remain capable of responding appropriately to the developmental signals that regulate tissue formation in the embryo, and from these results we can conclude that if embryonic stem cells were intended to provide cell replacement therapies for embryos, they would represent a very promising therapeutic approach. The problem, of course, is that embryos are not the intended targets of stem cell therapies, and there is little reason to believe that the capabilities of embryonic stem cells in an embryonic environment are relevant to their therapeutic potential for non-embryonic patients.

Five years ago, most scientists working in the field of embryonic stem cell research confidently predicted that we would soon determine the precise recipe of molecular factors required to replicate in the laboratory the mysterious inner life of the embryo. David Anderson, a stem cell researcher at Caltech, boldly asserted in a New York Times opinion piece that once science had figured out the factors required to replicate embryonic development, specific molecules could simply be “thrown into the bubbling cauldron of our petri dishes,” where they would transform embryonic stem cells into an unlimited source of replacement cells for any tissue we chose to produce.

Skepticism regarding this claim was well warranted. While there have been hundreds of papers published over the past five years that stridently claim “cell type X produced from embryonic stem cells,” under closer inspection these successes have all been less miraculous than they appeared. It is relatively easy to generate stem cell derivatives in the laboratory that have at least some of the properties of normal, mature cell types. But the test of whether an embryonic stem cell-derived brain cell, for example, is indeed a normal adult brain cell is to put it into the brain of an adult animal and determine whether it survives and contributes to normal brain function. In addition, if laboratory-generated cells are to be therapeutically useful for the treatment of human disease and injury, they must be shown to have therapeutic value in adult animals: It is not sufficient that embryonic stem cell-derived cells merely survive in adults; they must also be able to repair the underlying disease or injury. It is precisely this kind of test that embryonic stem cell-derived tissues have proved unable to pass.

When cells derived from embryonic stem cells are transplanted into adult animals, their most common fate is to die. Indeed, most such transplanted tissue does not survive beyond a few weeks in an adult environment (the only exception is blood cells, where small numbers of cells survive long term in mature animals). The rapid death of transplanted embryonic stem cell-derived cells stands in striking contrast to the robust survival of bona fide adult cells when transplanted to adult tissue. Typically, even the most promising experiments involving the transplant of embryonic stem cell derivatives have reported modest positive effects that persist for only a few weeks. In the few cases where tiny fractions of the transplanted cells survive for months (rather than weeks), this straggling band of survivors typically provides no therapeutic benefit.

The failure of embryonic stem cell-derived tissues to survive when transplanted to adult tissues strongly suggests that science has not yet determined how to generate normal adult tissue from

embryonic stem cells. Why then do some studies show modest, short-term benefits from transplantation of such tissues? In many cases, the authors of these studies speculate that embryonic stem cell-derived transplants are not providing benefit because of replacement of lost or damaged cells but rather because the transplanted cells are supporting the survival or function of damaged adult tissues by secreting generic survival factors. Thus, the modest and transient benefits reported for embryonic stem cell-derived cell transplants over the past five years do not appear to require stem cells at all and are likely to be replicated by simply identifying the beneficial factors produced by the transplanted cells and supplying these factors directly.

In light of the serious problems associated with embryonic stem cells," I noted in 2002, "there is no compelling scientific argument for the public support of research on human embryos." Serious scientific challenges are, by definition, problems that have stubbornly resisted the best attempts of science to resolve them. Over the past thirty years, hundreds of billions of dollars and countless hours of research by dedicated professionals worldwide have been devoted to solving the problems of immune rejection and tumor formation, yet these issues remain serious scientific and medical challenges. The mysteries of embryonic development have been plumbed for more than a hundred years by some of the most brilliant biologists of history, and yet, despite the clear progress we have made, we are nowhere near the point of having a "recipe book" for cooking up cellular repair kits to treat human disease and injury. Immune rejection, tumor formation, and embryonic development have proved themselves to be profoundly serious scientific challenges, and they are likely to remain so for decades into the future.

The hubris of scientists in the field of embryonic stem cell research who confidently asserted "Give us a few years of unrestricted funding and we will solve these serious scientific problems and deliver miraculous stem cell cures" was evident in 2002, and it is even more evident today. For the past five years, researchers have had completely unrestricted funding to conduct research on animal embryonic stem cells, and yet the serious scientific problems remain. They have had every conceivable tool of modern molecular research available to them for use in animal models, and yet the serious scientific problems remain. Millions of dollars have been consumed, and hundreds of scientific papers published, and yet the problems still remain. The promised miraculous cures have not materialized even for mice, much less for men.

In June 2004, Ron McKay at the National Institutes of Health acknowledged in a Washington Post interview that scientists have not been quick to correct exaggerated claims of the medical potential of embryonic stem cells, yet McKay justified this dishonesty by stating: "To start with, people need a fairy tale. Maybe that's unfair, but they need a story line that's relatively simple to understand." Isn't it time Americans recognize the promise of obtaining medical miracles from embryonic stem cells for the fairy tale it really is?