Notes on Bioethics

SCIENCE

Eggs, Embryos, and Human Cloning

Last year, Professor Woo Suk Hwang and his team working in Korea reported that they had cloned the first human embryo in order to harvest embryonic stem (ES) cells. This past quarter, the same group described their successful efforts to derive ES cell lines from embryos cloned from patients with inherited diseases or spinal injuries ("Patient-Specific Embryonic Stem Cells Derived from Human SCNT Blastocysts," *Science* 308.5729 [June 17, 2005]). The first published example of so-called therapeutic cloning, the ES cell lines are genetically identical to the patients who provided the genetic material for cloning. The group also improved the efficiency of their cloning technique. Last year, they were able to obtain one ES cell line from 242 cloned human embryos. This year, they report a success rate of one ES cell line per about twelve cloned human embryos, a significant increase. Their sum total of cell lines established was also higher: eleven cell lines from nine somatic cell donors. It now appears that cloning human embryos is more efficient than cloning other mammalian species. Why this is so is not clear.

Feminist critics of so-called therapeutic cloning often argue that current cloning techniques exploit women as egg donors. It now appears that this argument will soon become moot. In a recent paper, Antonin Bukovsky and his colleagues at the University of Tennessee show that they are now able to culture human ovarian surface epithelium (OSE) cells scraped from the surface of adult ovaries in the laboratory and to transform them into egg-producing stem cells and mature human eggs ("Oogenesis in Cultures Derived from Adult Human Ovaries," *Reproductive Biology and Endocrinology* 3.17, [May 5, 2005]). The ability to develop human eggs from OSE cells may help women with reduced fertility and premature menopause to conceive through in vitro fertilization. This technological advance may also allow physicians to transplant laboratory-grown egg-producing stem cells into the ovaries of older women to delay menopause. Clearly, this scientific paper raises a host of bioethical issues surrounding the very meaning of human procreation.

Embryonic and Adult Stem Cells

The political debate over the destructive embryo research that is currently associated with human ES cell research continues in the United States at both the federal and the state levels. This past quarter, several scientific reports that affect the debate were published.

We begin with four studies related to ES cells. First, Paul J. Tesar at the University of Oxford reported that he has successfully isolated ES cells from mouse embryos that had not yet developed to the blastocyst stage ("Derivation of Germ-Line-Competent Embryonic Stem Cell Lines from Preblastocyst Mouse Embryos," *Proceedings of the National Academy of Sciences USA* 102.23 [June 7, 2005]). This novel approach yielded ES cell lines from 48 percent of the preblastocyst embryos, a number significantly higher than the 25 percent success rate associated with conventional approaches developed with blastocysts. The preblastocyst-derived stem cells had the morphology and all the properties associated with blastocyst-derived pluripotent ES cells and were able to develop into all the tissues of the mouse when injected into a blastocyst. These results suggest that bona fide ES cells can be derived from embryos at any stage of preimplantation development in the mouse.

Next, the Pedersen laboratory at the University of Cambridge looked at the behavior of six imprinted genes, genes that are selectively turned on or off during normal development, in human ES cell lines ("Epigenetic Status of Human Embryonic Stem Cells," *Nature Genetics* 37.6 [June 2005]). They showed that these genes in human ES cells, unlike those in sheep and mouse ES cells, are relatively stable when maintained in long-term culture. The possibility of genetic instability had been a safety concern for scientists working with human ES cells. However, with this study, the researchers conclude that the epigenetic status of human ES cells—the status of its imprinted genes—"would not be a barrier to their therapeutic use."

Finally, two studies published this past quarter illustrate the therapeutic potential of human ES cells. First, Keirstead et al. ("Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitor Cell Transplants Remyelinate and Restore Locomotion after Spinal Cord Injury," *Journal of Neuroscience* 25.19 [May 11, 2005]) undertook a proof-of-concept experiment to demonstrate that neural progenitor cells derived from human ES cells, when injected into rats whose spines had been crushed, could improve the ability of the animals to walk after the injury. The researchers induced human ES cells to develop into oligodendrocyte progenitor cells (OPCs), neural precursors that can become the oligodendrocytes that form the insulating sheath that protects the axons that conduct nerve signals throughout the body. When these OPCs were injected into rats seven days after the rats had experienced crushing spinal injuries, they appeared to repair the insulation that protects the damaged nerves, and the rats exhibited significant improvement in various measures of motor control, compared to rats not treated with stem cells. Although their reported results were good—the results played a prominent public relations role in the debate over Proposition 71 in California—it is significant that the research team did not include any controls to determine whether their transplanted ES-cell-derived OPCs would develop into teratomas. Teratoma formation remains a major safety concern for any

therapeutic strategy involving ES cells, and several animals that had received the transplanted cells should have been allowed to live longer to see if tumors would have developed.

Finally, in a paper published in the *Journal of Experimental Medicine,* Wang and colleagues compared the properties of hematopoietic (blood-forming) cells derived from human ES cells with those of hematopoietic cells derived from human umbilical cord blood ("Generation of Hematopoietic Repopulating Cells from Human Embryonic Stem Cells Independent of Ectopic *HOXB4* Expression," *J. Exp. Med* 201.10 [May 16, 2005]). Intriguingly, the two types of cells were *not* identical. In contrast to cord-blood-derived human hematopoietic cells that were able to reconstitute the blood system of mice lacking their own blood cells, the ES-cell-derived blood-forming cells were unable to do the same. Instead, the ES-cell-derived blood-forming cells clumped together, producing clots that eventually killed the mice. Molecular analysis showed that these abnormal ES-cell-derived blood-forming cells had a distinct gene expression pattern, which was unlike that of normal blood-forming cells. The authors conclude that their study "underscores the importance of functional and molecular comparison of hESC [human embryonic stem cell]-derived progeny with their somatic counterparts for clinical regenerative therapies." In other words, this study shows that there is still much work to be done before we can say that human ES cells will give rise to cells with a therapeutic value comparable to that of equivalent cells derived from adult sources.

Moving on to adult stem (AS) cells, we highlight several papers that illustrate the diversity of the research being pursued in this field. First, given the hype to promote ES-cell-based therapies for juvenile diabetes, it is striking that Sapir and colleagues now report that they have successfully used genetic engineering and gene therapy to transform adult human liver cells into human beta cells that secrete insulin ("Cell-Replacement Therapy for Diabetes: Generating Functional Insulin-Producing Tissue from Adult Human Liver Cells," *Proceedings of the National Academy of Sciences USA* 102.22 [May 31, 2005]). Furthermore, when transplanted into diabetic mice, these human insulin-secreting cells were able to lower the high blood sugar levels of the mice and to improve their condition for a prolonged period of time. This study suggests that reprogramming adult liver cells could potentially cure diabetes by allowing patients to be the donors of their own insulin-producing tissues. However, before this approach can be used with human patients, it would be important to observe the long-term behavior of these transplanted cells, to make sure that they do not become tumors. Again, tumor formation remains a primary safety concern for all cell-replacement-based therapies.

Next, Sigurjonsson et al. have discovered a strategy to transform human blood stem cells into neurons using the microenvironment of the regenerating spinal cord of the chicken embryo ("Adult Human Hematopoietic Stem Cells Produce Neurons Efficiently in the Regenerating Chicken Embryo Spinal Cord," *Proceedings of the National Academy of Sciences USA* 102.14 [April 5, 2005]). They showed that human blood stem cells are able to become nerve cells when transplanted into areas where the embryonic chick spinal cord is regenerating. These experiments provide

compelling evidence that human AS cells have the capacity to become cells of a completely different cell type, and they open up the possibility that a patient's own bone marrow may one day be used to produce neurons for therapeutic use.

Critics often claim that it is more difficult to culture AS cells than it is to culture ES cells. Scientists at the Massachusetts Institute of Technology have discovered an experimental strategy to multiply AS cells quickly and efficiently ("Ectopic Expression of *Oct-4* Blocks Progenitor-Cell Differentiation and Causes Dysplasia in Epithelial Tissues," *Cell* 121.3 [May 6, 2005]). Konrad Hochedlinger and his colleagues report that the overexpression of a gene called *Oct4* in AS cells forces the cells to continue dividing without producing specialized tissue. Significantly, this mechanism is reversible—when *Oct4* was turned off, the cells stopped dividing, and normal differentiated or mature cells appeared. These results suggest that the transient expression of *Oct4* in AS cells could be used to mass-produce these cells for therapeutic use. For instance, growing large numbers of skin stem cells could benefit burn victims.

Finally, one paper that attracted a lot of media attention this past quarter reported that AS cells, if grown in culture for an extended period of time, could develop into tumors ("Spontaneous Human Adult Stem Cell Transformation," *Cancer Research* 65.8 [April 15, 2005]). Rubio et al. grew human adult mesenchymal stem cells extracted from fat tissue for up to eight months. When transplanted into animals, these old stem cells formed tumors, probably because they had acquired the ability to produce a molecule called telomerase. It is important to note that current protocols involving AS cells do not involve the type of extensive tissue culturing used by this Spanish team. Indeed, it is hard to imagine how such extreme culture conditions would ever become routine, since stem cells for therapeutic use can be harvested in a shorter period of culture time. Nevertheless, these results should be a potent reminder that regenerative medicine in all its forms involves some risk.

A Biological Basis for Homosexuality?

The causes of homosexuality remain obscure. Two papers published this quarter are sure to contribute to the debate over whether same-sex attraction is caused by nature or by nurture.

First, Ebru Demir and Barry Dickson, working at the Institute of Molecular Biotechnology in Austria, have shown that a single gene can determine major aspects of a fruit fly's sexual courtship behavior ("*fruitless* Splicing Specifies Male Courtship Behavior in *Drosophila*," *Cell* 121.5 [June 3, 2005]). They report that female flies with the male version of the gene called *fruitless* behave like males. These mutant females attempt to court other females with a complex and normally male-associated behavior display, involving tapping, singing, wing vibrating, and licking. In contrast, male flies that have been genetically altered to possess the female version of *fruitless* rarely attempted to court female flies. In fact, males with the female form of *fruitless* were also more likely to court other male flies than were males with the male form of the gene. In sum, these elegant experiments show that mating behavior is hardwired in fruit flies, but can also be genetically manipulated. Having said this, however, it is hard to extrapolate from these studies of fly behavior to human behavior. For one,

the courtship behavior of human beings is not only driven by biology, but also influenced by culture. It would not be surprising if sexual orientation is rooted in both biological and psychodynamic causes.

Next, a new study has reported that smelling a male pheromone prompts the same brain activity in homosexual men as it does in heterosexual women ("Brain Response to Putative Pheromones in Homosexual Men," *Proceedings of the National Academy of Sciences USA* 102.20 [May 17, 2005]). Savic and colleagues observed thirty-six healthy men and women and showed that smelling 4,16-androstadien-3-one (AND), a testosterone-derived pheromone detected primarily in male sweat, activated the anterior hypothalamus and medial preoptic area in homosexual men and heterosexual women. Animal studies have shown that these areas of the brain are highly involved in sexual behavior. In contrast, AND did not excite these regions in the brains of heterosexual males, although estra-1,3,5(10),16-tetraen-3-ol (EST), an estrogen-derived pheromone found in female urine, did. The authors conclude that their findings "show that [the human] brain reacts differently to the two putative pheromones compared with common odors, and suggest a link between sexual orientation and hypothalamic neuronal processes." It is important to note that this study did not address the cause-and-effect question. It did not show whether sexual orientation caused the brain to function in a particular way, or whether the brain's particular way of functioning determined sexual orientation.

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