SCIENCE

Human-Animal Chimeras

Human-animal chimeras are popular models for testing the pluripotency of human embryonic stem (ES) cells. In a recent paper, Muotri et al. report that they transplanted human ES cells into the brains of embryonic mice ("Development of Functional Human Embryonic Stem Cell-Derived Neurons in Mouse Brain," *Proceedings of the National Academy of Sciences USA*, December 20, 2005). The ES cells were able to differentiate into functioning human neurons and supporting cells including glia, which were able to integrate into the adult mouse brain. Only a small fraction of the cells in the chimeric brain were human—about 100 to 100,000 of the eighty million cells found in a typical mouse brain. This study demonstrates for the first time that human ES cells can develop into functional human neuronal cells inside a living animal and that they can reach out to make connections with surrounding brain cells. Significantly, in contrast to ES cells transplanted into adult mouse brain, these ES cells did not develop into teratomas or tumors, suggesting that tumor formation is influenced by the age of the host. Furthermore, there was no evidence of any immunological rejection, suggesting that the embryonic mammalian brain is immunotolerant. The authors predict that the human-animal chimera will permit the study of human neural development in a live environment, paving the way for the generation of new models of human neurodegenerative and psychiatric diseases, as well as speeding up the screening process for therapeutic drugs. The age of human-animal chimeras is upon us.

Alternative Sources for Human Pluripotent Stem Cells?

The debate over alternative sources for human pluripotent, i.e., embryonic, stem cells that began with the President's Council on Bioethics' white paper on *Alternative Sources of Pluripotent Stem Cells* continues.[1] Several papers this past quarter describe experiments that test three of the four approaches described in the white paper.

[1] President's Council on Bioethics, *Alternative Sources of Human Pluripotent Stem Cells: A White Paper* (Washington, DC: President's Council on Bioethics, 2005), http://www.bioethics.gov/reports/white_paper/.

First, Chung et al. report that they were able to generate ES cells from single mouse blastomeres ("Embryonic and Extraembryonic Stem Cell Lines Derived from Single Mouse Blastomeres," *Nature*, January 12, 2006). Blastomeres (single cells in the developing embryo) were isolated from eight-cell stage embryos using a protocol similar to that used in preimplantation genetic diagnosis (PGD) of human embryos generated with in vitro fertilization. The five ES cell lines produced from these single blastomeres manifested the properties associated with pluripotent stem cells, although they were generated at a relatively low efficiency rate with this novel protocol. When injected into mouse embryos, the blastomeres were able to differentiate into all of the recipient's tissues, including its gametes. Significantly, the donor embryos from which the blastomeres had been taken developed to term without a reduction in their developmental capacity. Although the authors suggest that the ability to generate human ES cells from PGD blastomeres without killing the donor embryos could circumvent the ethical concerns voiced by many, several questions remain. Most important, were the isolated blastomeres totipotent? In other words, when separated from the embryo, can they themselves become embryos? If so, then the ethical concerns remain.

Next, Meissner and Jaenisch report that they have used altered nuclear transfer (ANT) to generate mouse ES cells from *Cdx2*-deficient blastocysts ("Generation of Nuclear Transfer-Derived Pluripotent ES Cells from Cloned *Cdx2*-deficient Blastocysts," *Nature*, January 12, 2006). The team introduced mouse somatic cell nuclei containing a reversible mutation of the *Cdx2* gene into 526 enucleated mouse oocytes; sixty-one blastocysts were created, and they were able to derive ES cells from some of them. The *Cdx2*-deficient blastocysts were morphologically abnormal, and were unable to implant into the uteri of pseudo-pregnant mice. However, when cultured, they were able to generate ES cell lines with an efficiency comparable to that of blastocysts derived from the nuclear transfer of normal nuclei. Once the *Cdx2* deficiency was reversed in these ES cells, they were able to generate all the cell lineages found in a mouse embryo, demonstrating that they were as pluripotent as ES cells derived from normal blastocysts. This report provides proof-of-principle for ANT. However, as the authors point out, because the *Cdx2*-deficient embryo is not obviously abnormal before the onset of *Cdx2* expression at the pre-blastocyst stage, ANT with *Cdx2*-deficiency may not alleviate the ethical concerns raised by those who oppose destructive embryo research—the *Cdx2*-deficient embryo could simply be an abnormal embryo rather than a non-embryo. It is more probable that it is an abnormal embryo, given a recent report that suggests that *Cdx2* gene expression is limited to, and is only important for, the development of the trophectoderm ("Interaction between Oct3/4 and *Cdx2* Determines Trophectoderm Differentiation," *Cell*, December 2, 2005). This is one more piece of data that suggests that the *Cdx2* gene is not involved in the earliest stages of mammalian embryonic development.

Finally, two teams report that they have used ES cells to reprogram somatic cell nuclei so that they become stem cell-like. Strelchenko et al. fused somatic cells with enucleated human ES cells ("Reprogramming of Human Somatic Cells by Embryonic Stem Cell Cytoplast," *RBMOnline*, January 2006). These fused cells, called "cybrids," had the properties of stem cells, demonstrating that ES cells are able to reprogram the somatic cells so that they acquire "stemness." In another paper, Taranger et al. used

extracts from undifferentiated human carcinoma cells to reprogram the gene expression of human epithelial kidney cells so that they acquired characteristics of pluripotency ("Induction of Dedifferentiation, Genomewide Transcriptional Programming, and Epigenetic Reprogramming by Extracts of Carcinoma and Embryonic Stem Cells," *Molecular Biology of the Cell*, December 2005). None of the cells described in both papers were tested in vivo to see if they truly had become pluripotent. Nevertheless, along with the paper by Cowan and colleagues noted in the last issue of the *Quarterly*, these studies constitute proof that somatic cell reprogramming remains a potential alternative to somatic cell nuclear transfer into human oocytes and the logistical and societal concerns associated with it. (This research does, however, raise serious moral questions involving material cooperation with evil, as the embryonic stem cells used in these experiments were derived from the destruction of human embryos.)

On Cloning

We note an intriguing paper that sheds more light on the behavior of cloned embryos. Smith et al. report data that suggest that faulty nuclear reprogramming may not be responsible for the high failure rate associated with cloning as had been previously believed ("Global Gene Expression Profiles Reveal Significant Nuclear Reprogramming by the Blastocyst Stage after Cloning," *Proceedings of the National Academy of Sciences USA*, December 6, 2005). The team compared the gene expression profiles of three types of cow embryos: artificially inseminated (AI) embryos, in vitro fertilized (IVF) embryos, and cloned (nuclear transfer, or NT) embryos. They discovered that less than 1 percent of the five thousand analyzed genes differed more than two-fold between AI and NT embryos, suggesting that the cloned embryos resembled the AI embryos more closely than they resembled IVF embryos. This report showed that nuclear reprogramming had successfully occurred in the cloned embryos, implying that the high death rate of cloned animals results from still unknown problems later in development.

Finally, on January 10, 2006, the Seoul National University panel that was investigating the work of Professor Woo Suk Hwang in Korea concluded that both of his landmark papers published in the journal *Science* were based on fraudulent data. The first paper had reported that his team had successfully isolated an embryonic stem cell line from a cloned human blastocyst for the first time ("Evidence of a Pluripotent Human Embryonic Stem Cell Line Derived from a Cloned Blastocyst," *Science*, March 12, 2004). The second paper had reported that his team successfully established eleven patient-specific embryonic stem cell lines from cloned embryos ("Patient-Specific Embryonic Stem Cells Derived from Human SCNT blastocysts," *Science*, June 17, 2005). The panel's final report concluded, "The research team of Professor Hwang does not possess patient-specific stem cell lines or any scientific bases for claiming having created one."[2] Both papers, which were noted in these pages in the *Quarterly*, were retracted by the editors of *Science* on January 12, 2006.

[2] Seoul National University Investigation Committee, "Summary of the Final Report on Hwang's Research Allegation" (January 10, 2006), http://www.snu.ac.kr:6060/sc_sne_b/news/1196178_3497.html.

With the retraction of these two papers, it is now clear that after nearly ten years of research, there is still no evidence that human cloning is even possible. After the publication of the second *Science* paper, much was made of the increased efficiency of cloning. In contrast to the hundreds of oocytes needed to obtain animal embryonic stem cell lines, only approximately seventeen oocytes were supposedly needed to obtain a single human embryonic stem cell line. Now, the panel has confirmed that Hwang's team had access to 2,061 oocytes extracted from 129 women, a not insignificant number of human oocytes![3] Clearly, the technical obstacles to human cloning remain. Moreover, the investigation revealed that there were numerous ethical violations in the procurement of these donated human oocytes. It appears that Hwang purchased oocytes—an ethical offense, since it commercializes living human tissue and attracts economically vulnerable donors—and obtained oocytes from junior scientists working in his own laboratory—an ethical violation because it opens up the possibility that he had obtained the oocytes with subtle coercion.[4] In the end, the cloning scandal has revealed that there are still many technical and ethical problems associated with so-called therapeutic cloning.

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[3] Ibid.

[4] Constance Holden, "Korean Cloner Admits Lying about Oocyte Donation," *Science* 310.5753 (December 2, 2005): 1402–1403.