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Ethics Report on Interspecies Somatic Cell Nuclear Transfer Research

This report considers whether research involving the creation of human-animal interspecies somatic cell nuclear transfer (iSCNT) embryos raises new ethical issues, and if so, whether it requires additional or special criteria and oversight distinct from research on human-animal chimeras.

Introduction

This report was undertaken to clarify the ethical issues in human-animal interspecies somatic cell nuclear transfer (iSCNT) research and to determine if there are justifiable arguments for proceeding and, if so, under what constraints. While there is some overlap with a report on the ethical standards for human-animal chimera experiments published previously by the ISSCR Ethics and Public Policy Committee (Hyun et al., 2007a)—hereafter the Chimera Report—we note that the creation of iSCNT embryos (also known as cytoplasmic hybrid embryos or "cybrids") may raise additional ethical issues from those associated with chimeras. As the creation of both iSCNT embryos and chimeras for research purposes involves mixing human and nonhuman animal materials, the ethical foundations appear the same. However, as the ultimate methods are different and result in different entities, then one may reasonably ask whether the moral landscape is different as well. For this reason, in the light of the further scientific development and of recent public education/consultation efforts (HFEA, 2007), we present this follow-up report. We confine this report primarily to any additional and special issues worthy of distinct discussion. We begin with definitions and then proceed to a review of the arguments for and against the research.

The chimeric organism contains cells originating from more than one zygote. The Chimera Report was limited to biological entities formed by transferring multipotent or pluripotent human stem cells and their derivatives into animals in embryonic, fetal, and postnatal stages. In contrast, we refer here to human-animal iSCNT, where embryos are formed by transferring the nucleus of a human cell into an enucleated animal oocyte for the purpose of research, in particular, to produce human embryonic stem cells (hESCs) and to study nuclear reprogramming. In political debates, the term "hybrid" is sometimes used in the word's broadest sense—something heterogeneous in origin or composition—to describe the iSCNT embryo, as the resultant embryo includes genetic material from more than one species in the same cell. The iSCNT embryo contains human nuclear DNA, animal mitochondrial DNA (contained in the oocyte's cytoplasm), and traces of human mitochondrial DNA (transferred with the nucleus). The goal of the nuclear transfer is to reprogram the human somatic DNA to an embryonic state. If the resultant embryo develops to the blastocyst stage, a line of hESCs might then be derived from the inner cell mass; this process would destroy the iSCNT embryo.

Scientific Justification

For the reasons stated in the Chimera Report, a necessary precondition for any such research to be justifiable is that the research:

(a) has scientific merit;
(b) has social or humanitarian importance;
(c) has no reasonable, alternative means of answering the specific research question without the use of the proposed technology;
(d) satisfies animal research and welfare requirements; and
(e) meets standards of human subjects and stem cell research oversight review as appropriate; in particular, it is approved by qualified reviewers who take into consideration the special issues associated with the creation of research embryos and chimeric animals.

At the outset, it is therefore important to consider whether iSCNT research is scientifically justified.

A core argument in support of the research can be made in the classic consequentialist sense that medical research strives to produce beneficial outcomes and in the deontological premise that we have a duty to try to relieve human suffering with medical advances. New avenues of human stem cell research have the potential to enhance the understanding of serious diseases and ultimately the development of new ways to treat them.

Through iSCNT, scientists may be able to create and study new hESC lines, including those that carry mutations for human diseases, and to compare iSCNT lines to stem cell lines derived through other methods, such as direct reprogramming and human-human SCNT (hSCNT). The practical and ethical considerations in obtaining unfertilized human eggs have been central to recent interest in exploiting iSCNT as a complementary approach to hSCNT to create patient- or disease-specific ESC lines and in other avenues of research. Thus, in addition to providing new hESCs for research, iSCNT research may enable scientists to observe the development of early embryonic cells. This may assist understanding of some fundamental questions of stem cell biology, such as stem cell migration, development, and characterization. Furthermore, iSCNT research may also be useful in understanding the function of mitochondria in various diseases and conditions. Finally, knowledge from iSCNT research could be applied to efforts to protect endangered species or to recover animals that have become extinct (Beyhan et al., 2007).

While it is hoped that iSCNT embryos could become a source for deriving new hESC lines, there is scientific debate over whether iSCNT embryos will actually produce normal human stem cells. The biggest challenge facing iSCNT research is that the original experiment in which hESCs were derived using rabbit oocytes (Chen et al., 2003) has not been repeated. To date, there are no reports of human stem cell lines derived using any type of animal eggs. The difficulties are manifest. Genetic divergence and interspecies differences lead to transcription failures and

genome inactivation, and the presence of animal and human mitochondria leads to metabolic disruptions. A recent report demonstrates that bovine and rabbit oocytes did not fully reprogram the donor (human) genome (Chung et al., 2009). As a result of such difficulties, iSCNT embryos do not seem to develop properly and have not yet survived long enough for stem cell cultivation, calling into question the potential use of animal eggs to generate human patient-specific stem cells. However, most scientists agree that the only way to settle the debate about whether these embryos will produce viable and useful stem cells is to continue conducting the research, perhaps by using different animal eggs and timing nuclear transfer so that genome activations are synchronized (Fulka et al., 2008; St John and Lovell-Badge, 2007; Academy of Medical Sciences, 2007). Hence, it is possible that iSCNT may inform researchers' understandings of SCNT technology and processes, assisting in later research using SCNT embryos formed from donated human eggs.

Complementary Steps Forward

Given this array of potential options and the difficulties encountered so far by research on iSCNT embryos, it is important to situate this line of inquiry into the broader context of nuclear reprogramming research and the exploitation of reprogramming techniques to obtain patient- or disease-tailored pluripotent stem cell lines. Gene-based reprogramming of adult human skin fibroblasts into pluripotent stem cells, referred to as induced pluripotent stem cells (iPSCs), that share many characteristics with hESCs (Takahashi et al., 2007; Yu et al., 2007; Park et al., 2008) has clearly changed the practice of this research field and has placed the regenerative perspectives of human pluripotent stem cells on an altogether more solid footing. By far the most important feature of iPSC technology is the fact that it is remarkably simple, enabling many laboratories worldwide to develop it further. This is in sharp contrast with the field of hSCNT and also iSCNT, in which the dedicated set of specialized skills and resources needed prevented many laboratories from entering the arena and hence contributing to the success of the field.

Yet, despite these considerations, the very differences between iPSC and SCNT (including iSCNT) technologies indicate that, at least for the foreseeable future, the advent of the former does not render the latter obsolete or redundant, but rather complementary. Starting from the needs of basic research and proceeding to the clinical applications, the reasons for continuing iSCNT research are the following.

First, in terms of basic biology, the epigenetic reprogramming entailed in iPSC versus iSCNT experiments is completely different (Gurdon and Melton, 2008). In the former, the forced expression of variable combinations of transcription factors changes one differentiated cell type (such as adult skin fibroblasts) into a pluripotent cell type (iPSCs) that shares many characteristics with ESCs derived from the inner cell mass of a blastocyst stage embryo. The process takes weeks and, in "leaping" from a differentiated cell type to another defined, albeit pluripotent, cell type, does not recapitulate the initial phases of embryonic development. In SCNT, the oocyte reprograms the somatic cell nucleus within hours and coaxes it into a reenactment of the developmental stages that follow fertilization. Hence, if one is interested in understanding the basic biology of oocyte-driven reprogramming on human somatic cell nuclei, it is clear that iPSCs cannot substitute for SCNT. And given the problems associated with the procurement of human eggs, iSCNT, if successful, would constitute an alternative way to investigate this issue. Of course, one could ask how much in iSCNT the admittedly artificial combination of reagents could illuminate the genuine potential of human oocyte-mediated reprogramming. This is a fundamental concern, but one that can only be addressed by performing a thorough comparison between pluripotent cell lines derived by hSCNT versus iSCNT. It is also possible that recent developments yield a less limited supply of in vitro-generated human oocytes, such as functional oocytes produced from hESC or iPSC lines, that would bypass the difficulties associated with animal eggs. Furthermore, if human zygotes could be used for reprogramming somatic nuclei, in analogy to what was recently demonstrated in mice (Egli et al., 2007), the number of supernumerary IVF embryos worldwide could constitute an important resource.

Second, by recapitulating the early steps of human embryogenesis, iSCNT opens an opportunity for the investigation of its physiological mechanisms and pathological aberrations. This is an area of significant medical need that, again, is not simply amenable to research given the scarcity of human eggs. Also in this case the objection as to the relevance of iSCNT to the physiology of human postzygotic development is warranted, but it is again an objection that can only be solved through additional research. Finally, also concerning this aspect, it is possible that the in vitro derivation of gametes from ESCs or iPSCs, and the ability to combine them for large-scale genetic screens of early in vitro development, would outpace the potential of iSCNT. When and if these developments materialize, it will then be useful to reappraise the scientific rationale for iSCNT.

Third, moving to the potential medical applications, we still need to learn much more about iPSCs before we can confidently abandon all other research pathways aimed at obtaining pluripotent cells. The reason is that we still need to determine, through the contribution of a large enough number of laboratories working on a wide enough array of cell types and disease targets, the extent to which iPSCs are able to generate in vitro, in a predictable and robust manner, differentiated cell types that are then integrated into living tissues. The preliminary evidence gives ample reason to hope this will be the case. Yet what we call iPSCs represent the stochastic result of an epigenetic adaptation, selected in culture on the basis of a variety of assays. Of these, the main one is the ability to generate chimeric mice and contribute to their germline. Even in this stringent assay, the cells are actually asked to operate in the physiologic context of normal cells from the host embryo, and we simply do not know how much iPSCs are "aided" through additional reprogramming cues in vivo, and how much this aid contributes to the final outcome that we assay for. This potential second layer of reprogramming would clearly be very different in the human setting, since in many applications human iPSCs would be expected to rely primarily on in vitro cues without the help of surrounding developing tissue. Could it be that the way in which the epigenome is reset in iPSCs makes them better suited, in vitro, for differentiating into certain cell types than others? Could it be that, for the way in which reprogramming is achieved,

they may prove to be less robust than ESCs—even iSCNT-derived ESCs—for certain applications? We simply do not know. It bears mentioning that, by using tetraploid complementation in the mouse, iPSCs have been used to generate midgestation mice (Wernig et al., 2007); however, no group has yet shown that iPSCs can generate live-born pups, in contrast to work with ESCs. It is surely a simple exercise of caution not to abandon at this stage other avenues of research, even when, as in the case of iSCNT, they appear from the outset more difficult and less likely to succeed (Hyun et al., 2007b). The trajectory of science has surprised us before.

Thus, we believe that iSCNT research remains a valuable option to be considered and evaluated in any research trajectory that strives for the eventual clinical translation of human stem cells and the development of stem cell-based therapies. That is, if one accepts that the clinical translation of human stem cells and their direct derivatives is of social or humanitarian importance—in fact of compelling importance in the ethical justifications of stem cell research in some traditions—then one has a very strong presumptive reason to allow iSCNT embryos to be created for the advancement of basic research where proposals meet criteria (c), (d), and (e) above.

Ethical Factors

Although hESCs could theoretically be obtained by the process of hSCNT, the process requires the use of donated human eggs, which are in short supply. Some commentators have expressed ethical concerns about the possibility of undue inducement and exploitation of women that arise from questions about compensation for egg donation (Check, 2006). Insofar as this ethical controversy remains unsettled, the successful use of animal eggs could provide the benefit of reducing the need for human egg donors in research. If scientists are able to transfer human genetic material into enucleated animal eggs to produce human stem cells, many more stem cells will be available for research. This ethical point favors iSCNT research.

One commentator has expressed the worry that successes in iSCNT research will lead to the widespread exploitation of egg donors pressured by researchers to provide human oocytes to fuel a subsequent increased interest in hSCNT studies (Baylis, 2008). We, along with several other bioethicists, do not believe this slippery-slope argument actually supports the conclusion that iSCNT research is unethical in its own right (Nelson, 2008; Savulescu and Skene, 2008). While we agree that potential exploitation of oocyte donors is a serious issue, we maintain that the best course would be to deal with the threat of exploitation squarely by scrutinizing and eliminating recruitment practices that unduly induce women to participate in research, rather than through an unproven and unnecessarily stringent strategy of banning all iSCNT research.

Most other ethical concerns about iSCNT embryos are no different than the ethical concerns addressed in the Chimera Report. Specifically, we adhere to the answers expressed in that report concerning the mixing of species as unnatural, a violation of taboos, and a violation of human dignity. One additional concern with iSCNT embryos is that they generate cells with a new genetic composition that includes also human genetic material. Insofar as this fact raises animal welfare or human dignity arguments, we have dealt with those arguments in the Chimera Report. What is different about iSCNT embryos is that they contain not a mixture of genetically different cells but a mixture of genetic material within the same cell. An iSCNT embryo is composed almost entirely of human DNA, but the embryos (and stem cells derived from them) contain a very small amount of mitochondrial DNA from the animal egg. If argued that the level of human DNA makes iSCNT embryos, in effect, fully human, we believe the answers lie in the arguments in support of hSCNT and hESC research itself. Moral status issues have already been debated in the context of research on IVF-generated human embryos and also those created by hSCNT for research. Those embryos contain entirely human genetic material, and yet it has been recognized that they can be ethically created and used in research under strict ethical and regulatory controls (ISSCR, 2006). Notably, in accordance with the ISSCR Guidelines for the Conduct of Human Embryonic Stem Cell Research (ISSCR, 2006), resultant embryos would not be cultivated for more than 14 days and not transferred to a uterus for further development. Thus, we do not believe that the creation and destruction of iSCNT embryos for research raise special moral status concerns that exceed the concerns already placed on human embryo use for stem cell science.

Conclusions

(1) The creation of human-animal iSCNT embryos for research is ethically justifiable by the reasons set out in our Chimera Report (conditions [a]–[e] above).
(2) As with chimera research, the creation of iSCNT embryos for research should not be prohibited on the grounds that it crosses the human/animal species boundary per se, nor because it may be thought to violate human dignity. Critiques of such arguments included in our chimera report have equal force with regard to the creation of iSCNT embryos.
(3) Special additional reasons justify the creation of iSCNT embryos for research.
(4) The creation of iSCNT embryos for research is consistent with our previous recommendations regarding chimeras, and as various regulatory, oversight, and funding bodies consider whether iSCNT should be permitted or included in their portfolio, we encourage them to allow this research to move forward.

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