Production of Pluripotent Stem Cells by Oocyte-Assisted Reprogramming

Joint Statement with Signatories

As described in the President's Council on Bioethics' May 2005 White Paper,[1] altered nuclear transfer (ANT) is a broad conceptual proposal for producing pluripotent stem cells without creating and destroying embryos. In the description set forth below, we outline a research program for a form of ANT that should allow us to produce pluripotent stem cells without creating or destroying human embryos, and without producing an entity that undergoes or mimics embryonic development. The method of alteration here proposed ("oocyte-assisted reprogramming," or OAR) would *immediately* produce a cell with positive characteristics and a type of organization that from the beginning would be clearly and unambiguously distinct from, and incompatible with, those of an embryo. Incapable of being or becoming an embryo, the cell produced would itself be a pluripotent cell that could be cultured to establish a pluripotent stem cell line. Significantly, this cell would not be totipotent, as a zygote is.

Our proposal is for initial research using only nonhuman animal cells. If, but only if, such research establishes beyond a reasonable doubt that oocyte-assisted reprogramming can reliably be used to produce pluripotent stem cells without creating embryos, would we support research on human cells.

With few exceptions, all human cells contain a complete human genome; i.e., the complete DNA sequence characteristic of the human species. Specifically, one-celled human embryos, pluripotent human embryonic stem (or ES) cells, multipotent

NOTE: In this presentation of the statement, we have denoted the names of genes with italicization and the names of proteins and transcription factors without italicization.—*Ed.*

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

human adult stem cells, and differentiated (specialized) adult human cells such as neurons all contain a complete human genome. Thus, possession of a human genome is a *necessary* but not *sufficient* condition for defining a human embryo with its inherent dignity. Rather, the nature of each cell depends on its epigenetic state, i.e., which subset of the approximately thirty thousand human genes is switched on or off and, if on, at what level. For example, the gene for albumin, a liver-specific protein, is found both in human embryos and in adult human liver cells called hepatocytes. However, neither the messenger RNA (mRNA) for albumin nor the protein itself is found in single-celled embryos, because in them the gene is silenced.

This fundamental observation has given rise to the concepts of cell fate plasticity and epigenetic "reprogramming." If successful, reprogramming converts a cell from one kind to another by changing its epigenetic state. The ability to clone animals, such as Dolly the sheep, by transfer of a specialized adult nucleus to an enucleated oocyte, demonstrates the power of epigenetic reprogramming: the oocyte cytoplasm is sufficient to reprogram the somatic nucleus to a totipotent state. Human cloning has been proposed as a means of generating human embryos whose pluripotent stem cells would be used in scientific and medical research. Here, through a form of altered nuclear transfer, we propose to utilize the power of epigenetic reprogramming in combination with controlled alterations in gene expression to *directly* produce pluripotent cells using adult somatic nuclei, without generating and subsequently destroying embryos.

How do pluripotent stem cells differ from totipotent single-celled embryos? Several key transcription factors essential for establishing and maintaining the pluripotent behavior of ES cells have been identified. Importantly, some of these are specifically expressed only in pluripotent cells, such as embryonic stem cells or the cells found in the inner cell mass (ICM) of the week-old embryo or blastocyst. They are not expressed in oocytes or single-celled embryos. Expression of these factors therefore positively defines and distinguishes mere pluripotent cells from embryos. These factors instruct a cell to have the identity of a pluripotent cell. Currently, the best studied example is the homeodomain transcription factor called Nanog.[2] Nanog

[2]Kaoru Mitsui et al., "The Homeoprotein Nanog Is Required for Maintenance of Pluripotency in Mouse Epiblast and ES Cells," *Cell* 113.5 (May 30, 2003): 631–642. [*Abstract:* Embryonic stem (ES) cells derived from the inner cell mass (ICM) of blastocysts grow infinitely while maintaining pluripotency. Leukemia inhibitory factor (LIF) can maintain self-renewal of mouse ES cells through activation of Stat3. However, LIF/Stat3 is dispensable for maintenance of ICM and human ES cells, suggesting that the pathway is not fundamental for pluripotency. In search of a critical factor(s) that underlies pluripotency in both ICM and ES cells, we performed in silico differential display and identified several genes specifically expressed in mouse ES cells and preimplantation embryos. We found that one of them, encoding the homeoprotein Nanog, was capable of maintaining ES cell self-renewal independently of LIF/Stat3. *Nanog*-deficient ICM failed to generate epiblast and only produced parietal endoderm-like cells. *Nanog*-deficient ES cells lost pluripotency and differentiated into extraembryonic endoderm lineage. These data demonstrate that Nanog is a critical factor underlying pluripotency in both ICM and ES cells.]

is *not* present in oocytes or single-celled embryos, but first becomes expressed weakly in the morula and then highly in the ICM.[3] Deletion of the *Nanog* gene does *not* prevent early cleavage stages of embryogenesis, including formation of the ICM, but does prevent the formation of an epiblast.[4] ES cells in which *Nanog* is blocked lose their pluripotency—which clearly shows that Nanog is a positive factor instructing cells to be pluripotent, i.e., to behave like ES cells. Furthermore, ES cells which constitutively express Nanog can no longer be differentiated, i.e., they are forced to remain in their undifferentiated state.[5]

We propose a procedure that combines epigenetic reprogramming of a somatic nucleus with forced expression of transcription factors characteristic of embryonic stem cells, to produce a pluripotent stem cell. As a result of this procedure, Nanog and/or other, similar factors,[6] would be expressed at high levels in somatic cells *prior* to nuclear transfer, to bias the somatic nucleus towards a pluripotent stem cell state. Such altered nuclei would then be epigenetically reprogrammed by transplantation into enucleated oocytes. Alternatively or concomitantly, the mRNA for these same factors could be introduced into the oocyte prior to nuclear transfer. This procedure could ensure that the epigenetic state of the resulting single cell would immediately be different from that of an embryo and like that of a pluripotent stem cell: the somatic-cell nucleus would be formed into a pluripotent stem-cell nucleus and *never* pass through an embryonic stage. Therefore, unlike some other proposed methods of

[3]Ibid. See also Shin-ya Hatano et al., "Pluripotential Competence of Cells Associated with *Nanog* Activity," *Mechanisms of Development* 122.1 (January 2005): 67–79. [*Abstract: Nanog* is a novel pluripotential cell-specific gene that plays a crucial role in maintaining the undifferentiated state of early postimplantation embryos and embryonic stem (ES) cells. We have explored the expression pattern and function of *Nanog* and a *Nanog*-homologue, *Nanog-ps1*. *Nanog-ps1* was mapped on Chromosome 7 and shown to be a pseudogene. Immunocytochemical analysis in vivo showed that the Nanog protein was absent in unfertilized oocytes, and was detected in cells of morula-stage embryos, the inner cell mass of blastocysts and the epiblast of E6.5 and E7.5 embryos, but not in primordial germ cells of early postimplantation embryos. In monkey and human ES cells, Nanog expression was restricted to undifferentiated cells. Furthermore, reactivation of the somatic cell-derived *Nanog* was tightly linked with nuclear reprogramming induced by cell hybridization with ES cells and by nuclear transplantation into enucleated oocytes. Notably, mouse *Nanog* (+/-) ES cells, which produced approximately half the amount of Nanog produced by wild-type ES cells, readily differentiated to multi-lineage cells in culture medium including LIF [leukemia inhibitory factor]. The labile undifferentiated state was fully rescued by constitutive expression of exogenous *Nanog*. Thus, the activity of *Nanog* is tightly correlated with an undifferentiated state of cells even in nuclear reprogrammed somatic cells. *Nanog* may function as a key regulator for sustaining pluripotency in a dose-dependent manner.]

[4]Mitsui et al., "The Homeoprotein Nanog."

[5]Ibid.

[6]Nanog is only one example of a growing list of candidate factors, numbering probably at least ten. Oct3/4 is another well-studied example, and is noteworthy because it is also expressed at high levels in pluripotent adult stem cells.

ANT, this method would achieve its objective, not by a gene deletion that precludes embryonic organization in the cell produced, but rather by a positive transformation that generates, ab initio, a cell with the distinctive molecular characteristics and developmental behavior of a pluripotent cell, not a totipotent embryo. This should allow us to produce a pluripotent stem cell line with controlled genetic characteristics.

Endorsers

Institutional affiliations are provided for purposes of identification only and do not necessarily represent the views of organizations with which endorsers are affiliated. Endorsers who are not themselves specialists in biomedical science do not put themselves forward as experts in that field. Their endorsement of the proposal pertains to the ethics of ANT-OAR, assuming its technical feasibility.

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