Stem Cell Technology and Other Innovative Therapies
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NUCLEAR CLONING, EMBRYONIC STEM CELLS AND THE PROMISE FOR TRANSPLANTATION THERAPY

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Summary

An emerging consensus is that somatic cell nuclear transfer (SCNT) for the purpose of creating a child (also called 'reproductive cloning') is not acceptable for both moral and scientific reasons. In contrast, SCNT with the goal of generating an embryonic stem cell line ('therapeutic cloning') remains a controversial issue. Although therapeutic cloning holds the promise of yielding new ways of treating a number of degenerative diseases, it is not acceptable to many because the derivation of an embryonic stem cell line from the cloned embryo (an essential step in this process) necessarily involves the loss of an embryo and hence the destruction of potential human life.

In this article, I will develop two main arguments that are based on the available scientific evidence. 1) In contrast to an embryo derived by *in vitro* fertilization (IVF), a cloned embryo has little if any potential to ever develop into a normal human being. This is because, by circumventing the normal processes of gametogenesis and fertilization, nuclear cloning prevents the proper reprogramming of the clone's genome, which is a prerequisite for development of an embryo to a normal individual. It is unlikely that these biological barriers to normal development can be solved in the foreseeable future. Therefore, from a biologist's point of view, the cloned human embryo, used for the derivation of an embryonic stem cell and the subsequent therapy of a needy patient, has *little if any potential* to create a normal human life. 2) Embryonic stem cells developed from a cloned embryo are functionally indistinguishable from those that have been generated from embryos derived by in vitro fertilization (IVF). Both types of embryonic stem cells have an *identical potential* to serve as a source for therapeutically useful cells.

It is crucial that the ongoing debate on the possible therapeutic application of SNCT is based on biological facts. The goal of this article is to provide such a basis and to contribute to a more rational discussion that is founded on scientific evidence rather than on misconceptions or misrepresentations of the available scientific data.

It is important to distinguish between 'reproductive cloning' and 'nuclear transplantation therapy' (also referred to as 'SCNT' or 'therapeutic cloning'). In reproductive cloning a cloned embryo is generated by transfer of a somatic nucleus into an enucleated egg with the goal to create a cloned individual. In contrast, the purpose of nuclear transplantation therapy is to generate an embryonic stem cell line (referred to as 'ntES cells') that is 'tailored' to the needs of a patient who served as the nuclear donor. The ntES cells could be used as a source of functional cells that would be suitable for treating an underlying disease by transplantation.

There is now experience from cloning of seven different mammalian species that is relevant for three main questions of public interest: 1) Would cloned human embryo be 'normal'? 2) Could the problems currently seen with cloning be solved in the foreseeable future? 3) Would ES cells derived from a cloned human embryo be 'normal' and useful for cell therapy? The arguments summarized in this article are based upon a review prepared for the President's Bioethics Committee. Here I will only summarize the key arguments and the reader is referred for details and literature to (Jaenisch, 2003)

Most Cloned Animals Die or Are Born With Abnormalities

The majority of cloned mammals derived by nuclear transfer (NT) die during gestation, and those that survive to birth frequently display 'Large Offspring Syndrome', a neonatal phenotype characterized by respiratory and metabolic abnormalities and enlarged and dysfunctional placentas. In order for a donor nucleus to support development into a clone, it must be reprogrammed to a state compatible with embryonic development. The transferred nucleus must properly activate genes important for early embryonic development and also suppress differentiation-associated genes that had been transcribed in the original donor cell. Inadequate 'reprogramming' of the donor nucleus is most likely the principal reason for developmental failure of clones. Since few clones survive to birth, the question remains whether survivors are fully normal or merely the least affected animals carrying through to adulthood despite harboring subtle abnormalities that originate in faulty reprogramming but that are not severe enough to interfere with survival to birth or beyond.

Adult Cloned Animals: How Normal Are They?

The observation that apparently healthy adult cloned animals have been produced in seven mammalian species (albeit at low efficiency) is being used by some as a justification for attempting to clone humans. In fact, even those that survive to adulthood, such as Dolly, may succumb relatively early in adulthood because of numerous health problems. The available evidence indicates that most clones die soon after implantation. But even those that survive to birth an beyond are not 'normal'. A direct comparison of gene expression profiles of over 10,000 genes (of the 30,000 or so in the mammalian genome) showed that approximately 4% of the expressed genes in their placentas differed dramatically in expression levels from those in controls, and that the majority of abnormally expressed genes were common to both types of clones. When imprinted genes, a class of genes that express only one allele (either from maternal or paternal origin), were analyzed, between 30 and 50% were not correctly activated. These observations represent strong molecular evidence that cloned animals, even those that survive to birth, suffer from serious gene expression abnormalities.

Nevertheless, despite these widespread gene expression abnormalities, a small fraction of clones developed to seemingly normal adults. However, when cloned mice were aged, serious problems, not apparent at younger ages, became manifest leading to serious pathological alterations in multiple organs and premature death. Thus, severe abnormalities in cloned animals may often become manifest only when the animals age.

It is a key question in the public debate whether it is ever possible to produce a normal individual by nuclear cloning, even if only with low efficiency. The available evidence suggests that it may be difficult if not impossible to produce normal clones for the following reasons: 1) All analyzed clones at birth showed dysregulation of hundreds of genes. The development of clones to birth and beyond despite widespread epigenetic abnormalities suggests that mammalian development can tolerate dysregulation of many genes. 2) Some clones survive to adulthood by compensating for gene dysregulation. Though this 'compensation' assures *survival*, it may not prevent maladies to become manifest at later ages. Therefore, most if not all clones are expected to have at least subtle abnormalities that may not be so severe as to result in an obvious phenotype at birth but will cause serious problems later as seen in aged mice.

Is it Possible to Overcome the Problems Inherent in Reproductive Cloning?

It is often argued that the 'technical' problems in producing normal cloned mammals will be solved by scientific progress that will be made in the foreseeable future. The following considerations argue that this may not be so.

A principal biological barrier that prevents clones from being normal is the 'epigenetic' difference between the chromosomes inherited from mother and from father, i.e. the difference between the 'maternal' and the 'paternal' genome of an individual. Such methylation of specific DNA sequences is known to be responsible for shutting down the expression of nearby genes. Parent-specific methylation marks are responsible for the expression of imprinted genes and cause only one copy of an imprinted gene, derived either from sperm or egg, to be active while the other allele is inactive. For cloning to be made safe, the two parental genomes of a somatic donor cell would need to be physically separated and separately treated in an 'oocyte-appropriate' and a 'sperm-appropriate' way, respectively. At present, it seems that this is the only rational approach to guarantee the creation of the epigenetic differences that are normally established during gametogenesis. Such an approach is beyond our present abilities. These considerations imply that *serious biological barriers* exist that interfere with faithful reprogramming after nuclear transfer. It is a safe conclusion that these biological barriers represent a major stumbling block to efforts aimed at making nuclear cloning a safe reproductive procedure for the foreseeable future.

Therapeutic Applications of SCNT

In spite of the biological and ethical barriers associated with reproductive cloning, nuclear transfer technology has significant therapeutic potential that is within our grasp. There is an enormous distinction between the goals and the end product of these two technologies. The purpose of reproductive cloning is to generate a cloned embryo that is then implanted in the uterus of a female to give rise to a cloned individual. In contrast, the purpose of nuclear transplantation therapy is to generate an embryonic stem cell line that is derived from a patient (referred to as 'ntES cells') and can be used subsequently for tissue replacement.

In a 'proof of principle' experiment, nuclear cloning in combination with gene and cell therapy has been used to treat a mouse genetic disorder that has a human counterpart. To do so, the well-characterized *Rag2*

mutant mouse was used as 'patient'. This mutation causes *severe combined immune deficiency (SCID),* because the enzyme that catalyzes immune receptor rearrangements in lymphocytes is non-functional. Consequently, these mice are devoid of mature B and T cells, a disease resembling human *Omenn syndrome*. The successful treatment of the mutant mice indicates that, unlike the situation with reproductive cloning, no *biological* barriers exist that in principle prevent the use of SCNT to treat human diseases. The *technical* issues in using SCNT and human stem cells for therapeutic purposes need, however, to be solved, but there are no indications at present that these represent formidable problems that will resist relatively rapid solution.

SCNT for Cell Therapy: Destruction of Potential Human Life?

A key concern raised against the application of the nuclear transplantation technology for tissue therapy in humans is the argument that the procedure involves the destruction of potential human life. From a biological point of view, life begins with fertilization when the two gametes are combined to generate a new embryo that has a unique combination of genes and has a high potential to develop into a normal baby when implanted into the womb. A critical question for the public debate on SCNT is this one: is the cloned embryo equivalent to the fertilized embryo?

In cloning, the genetic contribution is derived from one individual and not from two. Obviously, the cloned embryo is the product of laboratory-assisted technology, not the product of a natural event. From a biological point of view, nuclear cloning does not constitute the creation of new life, rather the propagation of existing life because no meiosis, genetic exchange and conception are involved. Perhaps more important is, however, the overwhelming evidence obtained from the cloning of seven different mammalian species. As summarized above, the small fraction of cloned animals that survive beyond birth, even if they appear 'normal' upon superficial inspection, are likely not so. The important conclusion is that a cloned human embryo would have little if any potential to develop into a normal human being. With other words, the cloned human embryo lacks essential attributes that characterize the beginning of *normal* human life.

Taking into account the potency of fertilized and cloned embryos, the following scenarios regarding their possible fates can be envisaged. Fertilized embryos that are 'left over' from IVF have three potential fates: disposal, generation of normal embryonic stem cells or generation of a nor-

mal baby when implanted into the womb. Similarly, the cloned embryo has three potential fates: it can be destroyed or could be used to generate a normal ntES cell line that has the same potential for therapy as an ES cell derived from a fertilized embryo. In contrast to the fertilized embryo, the cloned embryo has little if any potential to ever generate a normal baby. An embryonic stem cell line derived by nuclear transfer may, however, help sustain existing life when used as a source for cell therapy that is 'tailored' to the need of the patient who served as its nuclear donor.

If SCNT were accepted as a valid therapeutic option, a major concern of its implementation as medical procedure would be the problem of how to obtain sufficient numbers of human eggs that could be used as recipients. Commercial interests may pressure women into an unwanted role as egg donors. The recent demonstration that embryonic stem cells can be coaxed into a differentiation pathway that yields oocyte-like cells may offer a solution to this dilemma. If indeed functional oocytes could be generated from a generic human ES cell line, sufficient eggs could be generated in culture and serve as recipients for nuclear transfer without the need of a human egg donor. It seems that technical issues, not fundamental biological barriers, need to be overcome so that transplantation therapy can be carried out without the use of human oocytes.

REFERNCE

Jaenisch, R., 'The biology of nuclear cloning: embryonic stem cells and their potential for transplantation therapy', Appendix N in *Monitoring Stem Cell Research: A Report of the The President's Council on Bioethics*, pp. 385-434, Washington, DC Government Printing Office, 2004.