ARGUMENTS FOR HUMAN THERAPEUTIC CLONING

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INTRODUCTION

For over 30 years, many Western governments have regulated scientific research involving human subjects. According to Knoppers (1) implementation of regulation followed a long and checkered history of research abuse. The regulations evolved largely in response to ethical violations. The Nuremberg Codex exemplifies the progression. It was adopted in 1947. At the conclusion of Nazi Doctors Trial.

Spectacular technical and conceptual advances in modern biology and molecular medicine have solved many problems in a short time. Genetic diagnostics extended well beyond simple inheritance testing, and is now moving into all areas of pathology. Gene therapy, although in a phase of consolidation after an exuberant youth, holds real promise. Understanding of the molecular basis of tissue differentiation, perhaps with the use of nuclear transfer techniques, may allow creation of histocompatible tissue for transplantation purposes (2).

Scientific work will have profound long-term consequences for medicine, leading to the elucidation of the underlying molecular mechanisms of disease and thereby facilitating the design of rational diagnostics and therapeutics targeted at those mechanisms.

All molecular medicine must operate within a social and ethical context.

Prominence of ethical controversy (i.e. presymptomatic genetic testing, or human therapeutically cloning) will very likely diminish with time, as the products of molecular medicine range further away from establishing pure diagnostic and into therapy.

One of the major issues of today’s modern medicine is therapeutically cloning. The main practical purpose of cloning is to generate genetically modified animals to serve as bioreactors. The cloning of mammals is fascinating biological problem, although it is difficult to perform and attempts are rarely successful. The reproductive cloning of humans is likely to cause more individual concern than real social effects, as it is unlikely to become a widespread method of reproduction even if possible and safe.

HUMAN THERAPEUTIC CLONING

Human therapeutic cloning is potentially limitless source of cells for tissue engineering and transplantation medicine. What is human therapeutic cloning? It involves the transfer of a patient’s somatic cell nuclei into enucleated oocytes, development of embryo to the early stage - morula or blastocyste, and isolation of stem cells that can differentiate into immunologically matched tissues. For example, cardiomyocytes could be used to treat patients with heart disease, pancreatic islet cells, for patients with diabetes, or hepatocytes, in a tissue-engineered liver. The main purpose of embryonic stem cell cloning techniques would be to create tissue that would not be subject to graft rejection. This procedure has a great potential, in producing specialized, replacement cells to treat a variety of diseases and conditions including parkinsonism, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, rheumatoid arthritis (2).

The cloning of mammals from adult cells has been achieved in several species in the past few years. The first mammal to be successfully cloned from a differentiated animal cell was the sheep (3), despite the fact that there had been previous cloning successes using embryonic cells. The sheep "Dolly" was cloned from an adult somatic cell by the somatic cell nuclear transfer method.

Authors who cloned the sheep proved that the differentiation of adult cells (in this case derived from the mammary epithelium) does not involve the irreversible modification of genetic material that is required for the development of the animal to term. Despite this success, somatic cell nuclear transfer cloning is still insufficient, because we still do not know the main factors that distinguish successfully developed clones from clones that do not develop normally (4).

Low cloning efficiency (1% of nuclear transfer embryos develop to adulthood) is not really an impediment for agricultural use of cloning because breeding from a single cloned genetically modified individual should be sufficient (5).

Because we do not know precise mechanisms that are involved in the abortions, neonatal deaths and postnatal disease associated with cloning, the human cloning is still dangerous and ethically unacceptable. In a future, we have to give much more emphasis to the development of the nuclear-transfer technology itself and to the genetic and epigenetic mechanisms that are involved in clone failure. Although human reproductive cloning is unacceptable today, production of cells from cloned embryos could offer many potential benefits. Therapeutic cloning according to Davor Solter may also not be affected by low cloning efficiency because this technique does not require a nuclear transfer embryo to develop to adulthood but only to the blastocyst stage, which has a higher success rate (close to 50% on average) (5).
What is present legal status of cloning? Human cloning for any purpose – reproductive or therapeutic – is illegal in Japan. In the United Kingdom, a government-appointed panel recently recommended that scientists should be permitted to create cloned embryos by nuclear transfer for research purposes only, and that these embryos cannot be maintained for longer than 14 days. There are many other countries without any laws whatsoever regarding human cloning, where cloners could move and set up laboratory (6).

**EMBRYONIC AND ADULT STEM CELLS**

Stem cells are clonogenic self-renewing progenitor cells that can generate one or more specialized cell types. Stem cells can be divided (in vertebrates) in two groups: embryonic and organ or tissue specific stem cells. Embryonic stem cells are pluripotent stem cells derived from the inner cell mass of the blastocyst, capable of generating all differentiated cell types in the body. Embryonic cells generate second group - organ/tissue specific stem cells. Such multipotent stem cells generate the cell types comprising a particular tissue in embryos or even some cases in adults. More research is needed to solve some current problems and questions: how to reprogram the nucleus of the adult cells without the need for an enucleated egg, how to put the cells together to create or recreate functional structures, how to modify the genome of the patient’s cells before the nuclear transfer procedure etc. It is our hope that by understanding how the cytoplasmic component directs development, we may eventually be able to reprogram the nucleus of adult cells without the need for an enucleated egg. Furthermore, it may be possible to modify the genome of the patient’s cells (through targeted gene alterations or engineered chromosomes) before the nuclear transfer procedure, so that after “reprogrammation”, the clones develop only into groups of specialized cells and tissues, rather than into a whole organism (7).

There is a still the task of putting the cells together to create or recreate functional structures. For relatively simple tissues, such as skin and blood vessels substitutes, this may involve seeding cells onto masses or sheets of polymeric scaffold. Creating vital organs will be much greater challenge, and will require assembling different cell types and materials with great combinatorial and architectural complexity.

The creation of embryos for the purpose of research has been ethically and politically contentious. The term human embryo is defined as any organism, not protected as a human subject … that is derived by fertilization, parthenogenesis, cloning, or any other means from one more human diploid cells. American National Institute of Health (NIH) has concluded that pluripotent stem cells are not themselves “organisms” under the definition. NIH may fund research on such stem cells; It raises the question of where the embryos are obtained and the possibility of complicity in embryo destruction (8).

According to Winston (9) many of ethical objections regarding embryonic stem cells could be resolved by more research. In time destruction of large number of embryos might be avoidable. One possibility is to derive embryonic stem cells from embryonic blastomeres before blastocyst formation; blastomeres can be removed from the embryo without risking damage; it may be possible to collect cells mass at slightly later development stage. Cells could be banked and preserved in prolonged culture. A possible solution is to study heterokaryons produced by fusion of an embryonic cell (rather than egg) with somatic-cell nucleus; such hybrids could have potential for targeting Duchenne muscular dystrophy.

Although the destruction of a human embryo is lamentable there is a considerable moral difference between creating and destroying embryos solely to obtain stem cells and destroying unwanted human embryos that will never be used for reproductive purposes, to achieve benefit for those with serious disease and disorders (8).

The question of the definition and status of the human embryo is emerging as one of the most problematic issues for scientists. J.F. Mattei feels that, on the one hand, we cannot deprive ourselves of the therapeutic potential of the embryo solely on the basis of protecting it; on the other hand, Mattei wonders whether these few cells at the bottom of a test tube truly merit the name embryo. Does the embryo results from fertilization? Yes, when fertilization took place in utero. But when the fertilized egg is at bottom of a test tube, its spontaneous process is not to develop into a living being. Therefore, Mattei thinks that is not possible to combine, in the same concept and the same name, the in vitro embryo and the in vivo embryo. All this reinforced by the progress in therapeutic cloning (9).

The cloning of human beings has been officially unlawful in Europe since the Additional Protocol of the Council of Europe Convention on Human Rights and Biomedicine came into force in March 2001. On the other hand, the Council of Europe decided in favor of therapeutic cloning. Some experts think that is the first stage leading to reproductive cloning (6,8,10).

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Indeed, there are strong arguments for embryonic stem cells research: our legal approach to abortion, our readiness to remove ectopic pregnancies, human preimplanta-
tion have only a limited potential to become humans. Most are lost before menstrual period. Finally, there is general public approval of in vitro fertilization; only around 10% of transferred IVF embryos produce a baby.

The promise of stem cell research for millions of patients may afford an outcome in which the ethical debate can be resolved. We can ask ourselves where be morality in letting millions of people continuing to suffer from chronic life-threatening disease. Human pre-embryos should be treated with respect. But, as Lanza pointed does a blastocyst warrant the same rights and reverence as that accorded a living soul - a parent, a child or a partner - who might die because we failed to move the moral line (11).

It seems increasingly likely that somatic cell nuclear will be developed and tested in humans, not in an attempt to create a child, but in effort to prevent and treat a long list of diseases.

**ADULT STEM CELLS**

Adult stem cells, the multipotent cells, exist in many adult organs and could serve as potential tool in future therapy. They can be isolated and in some cases expanded ex vivo. And even, they can be transplanted back to adult animals where they can differentiated and function approximately like in the normal organ.

Organ-specific stem cells can overcome their intrinsic restrictions upon exposure to a novel environment perhaps via genomic reprogramming. A dult stem cells from one tissue/organ can be induced to differentiate into cells of other organs (bone marrow-to-brain, bone marrow-to-liver, skin-to-brain, brain-to-heart).

There are some problems with adult stem cells, which could be possibly resolved by future research. Here are some of those problems: it is difficult to expand them and impossible to grow in large numbers, they don't have the same plasticity or broad range of potential as embryonic stem cells, we don't know the impact the aging process would have the same gene defect (this problem is also applied to embryonic stem cells) (2,5,7).

There are some other options. Recent observations on cell cultures from amniotic fluid and on amniotic epithelial cells provide evidence that they may represent new sources for the isolation of cells with the potency to differentiate into different cell type. A wide variety of investigations have provided evidence that cells of all three germ layers (ectoderm, mesoderm and endoderm) depending on the gestational age, fetal pathology, etc. can be detected in human amniotic fluid. Amniotic fluid can serve as a source of cells for fetal tissue engineering (12).

**CONCLUSION**

What are expectations? Cloning has the potential to contribute to improvements in veterinary and human medicine, with the prospect that non-reproductive human cloning strategies might provide future therapies for severe, incurable disease. Any stem cell can turn into any tissue given the appropriate conditions. More research has to be done before we understand whether there are restrictions on this process, whether it involves reprogramming that can lead to other unpredictable cellular behaviors and finally whether it even occurs at sufficient high frequency to be clinically useful. Until then there are no ethical and moral reasons to forbid stem cell therapeutic cloning. Before we start seriously with human therapeutic cloning, we have to learn more about the basic molecular mechanisms that are involved in nuclear reprogramming.
REFERENCES

(1) Knoppers B.M.: From medical ethics to "genetics". The Lancet Perspectives, 356 Suppl. 10; S38, 2000.


