Somatic cell nuclear 'reprogramming' in livestock species is now routine in many laboratories. Here, Robert Lanza, Jose Cibelli and Michael West discuss how these techniques may soon be used to clone genetically matched cells and tissues for transplantation into patients suffering from a wide range of disorders that result from tissue loss or dysfunction.

Human therapeutic cloning

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Many technologies have been developed and refined in the past few years that set the stage for human therapeutic cloning as a potentially limitless source of cells for tissue engineering and transplantation medicine. These technologies include the identification and isolation of pluripotent stem cells that are capable of generating all of the cell types in the body[1–3], genetic and cell engineering techniques enabling the design of custom tissues and organs[4–6], and advances in somatic cell nuclear transfer to clone ungulates such as sheep[7,8], cattle[9,10] and now goats[11]. It is likely that the confluence of these technologies will lead to means for developing tissue therapies that will overcome the present difficulties related to immune compatibility and graft rejection, and thus the requirements for use of immunosuppressive drugs and/or immunomodulatory protocols.

Since the cloning of Dolly in 1996 (ref. 7), the successful application of nuclear transfer to a range of mammalian species, especially livestock, has brought the possibility of cloning a human being significantly closer to reality. Although the procedure is conceptually simple, many parameters still need to be optimized. For example, protocols for the *in vitro* maturation of human oocytes are unreliable and still need to be developed. The parameters for cell fusion and activation also have yet to be determined. In the long term, however, the greatest obstacle to human therapeutic cloning may well be access to an adequate source of oocytes. Although the use of 'surrogate' oocytes from animal sources would insure an adequate supply both for research purposes and, eventually, for large-scale clinical implementation, questions continue to surround the scientific feasibility of this approach, as well as the appropriateness of mixing DNA across species.

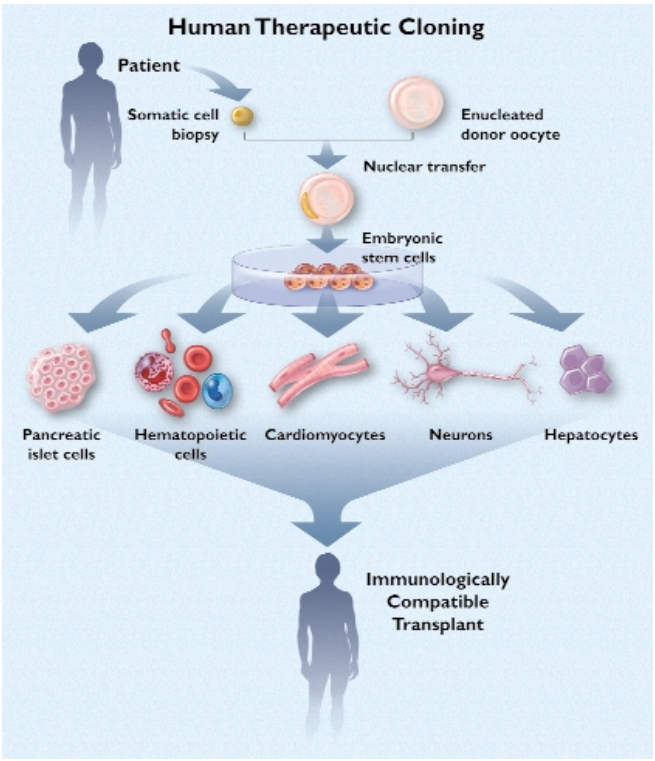

Fig. 1 Procedure for human therapeutic cloning. A cell from the patient is fused with an enucleated donor oocyte using the nuclear transfer techniques pioneered in murine and livestock species. Embryonic stem cells are isolated from the resulting clone, and then differentiated *in vitro* into genetically matched cells and tissues for transplantation. 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.

Cross-species transfer

Several years ago, we transferred nuclei from human somatic cells (18 lymphocytes and 34 oral mucosal epithelial cells) into enucleated bovine oocytes to form a preimplantation embryo ('pre-embryo') that, in theory, could have been used to create cells for transplantation. Of the 56 nuclear transfer units produced, 6 (26%) grew to the 4- to 16-cell stage, whereas only 1 (3%) reached the 16- to 400-cell stage. This last clone was plated onto a fibroblast feeder layer, and began to propagate as a colony with a embryonic stem (ES) cell-like morphology[12]. *In vitro*-derived cells such as these—each genetically identical to the individual from which the nuclei came—might one day help patients with disorders such as cancer, Parkinson disease and diabetes. After being 'coaxed' with growth factors to develop into cells and tissue with specific vital differentiated functions, they could, in turn, be incorporated into scaffolds to build more complex 'neo-organs', such as pancreases, livers and kidneys.

For all its therapeutic potential, however, the use of this methodology raises some profound new issues and anxieties that will need to be more fully considered in our individual and public lives. This need became especially apparent last November, when President Clinton requested that the National Bioethics Advisory Commission consider the implications of the above work. "[The] creation of an embryonic stem cell that is part human and part cow raises the most serious of ethical, medical and legal concerns," wrote the President."I am deeply troubled by this news of experiments involving the mingling of human and nonhuman species." We and of course most other thoughtful scientists share the President's concerns about the mixing of DNA across species. However, the research described above resulted in cells that contained only a small percentage of nonhuman (bovine) DNA. Perhaps more importantly, however, this mitochondrial DNA does not code for species-specific traits such as eye color, intelligence or other distinctive features, and can be eliminated from the bovine oocytes before nuclear transfer. Thus, we do not believe that the resultant 'pre-embryo', the cells or organism itself, could be considered a

chimera (that is, part-human, part-cow), as some have feared.

Questions have also been raised as to the ability of 'surrogate' oocytes to support the development of 'pre-embryos' produced by interspecies nuclear transfer. It is obvious that mitochondrial DNA alone does not contain enough genetic information to code for all mitochondrial components, and that the nuclear and mitochondrial genetic systems must interact in the formation of the protein systems in the mitochondria. Recent results indicate that these essential nuclear–mitochondrial interactions can occur, in primates, not only between different species but also between different genera, up to approximately 8–18 million years after species radiation[13]. Nevertheless, the mitochondrial genome of vertebrates is extremely specialized, and there are likely to be incompatibilities between distantly related species. This may explain the failure of most human–bovine nuclear transfer units to grow and divide beyond the 4- to 16-cell stage, as well as why it may eventually be necessary to enrich the 'surrogate' eggs with human mitochondria.

Irrespective of the species-specific nature of these nuclear–-mitochondrial genome interactions, a paper published recently by Neal First's group confirms the ability of bovine oocyte cytoplasm to support mitotic cell cycles under the direction of differentiated somatic cell nuclei of several mammalian species[14]. Nuclear transfer units produced using cells from cows, sheep, pigs, monkeys and rats underwent transition to interphase accompanied by nuclear swelling and further progression through the cell cycle. As in our own studies, some units progressed further and developed to advanced stages, as evidenced by successive cell division and formation of a blastocoele cavity at the time appropriate for the species of the donor nuclei. It is unclear whether such 'pre-embryos' have the potential to develop into viable offspring, as so far, no pregnancies have been carried to term after transfer of these units into surrogate animals.

In this work, no 'pre-embryos' containing human DNA were placed in the uterus (human or otherwise), and that we believe it is unacceptable (and unsafe) to use this or any other cloning technique to produce a human child. It is our hope that by understanding how the cytoplasmic components direct development, we may eventually be able to reprogram the nucleus of adult cells without the need for an enucleated egg. As well as being rich in maternal mRNA and protein, the cytoplasm of the unfertilized oocyte contains all the components required to direct the earliest phases of embryonic development up to the activation of the zygotic genome. In most species, the main activation of the embryonic genome occurs around the 4- to 16-cell stage. After successive cleavage divisions, a fluid-filled cavity appears within this ball of cells and enlarges until the embryo resembles a hollow sphere, the blastocyst. A knob of cells to one side of the central cavity, the 'inner cell mass' (from which ES cells are derived), will give rise to the adult organism.

The ethical quandary

To accomplish human therapeutic cloning, it will be necessary to isolate and culture ES cells from these preimplantation stage embryos. Although the embryo does not have human form or sentience at this early stage, some believe human 'personhood', and thus claims of moral status and dignity, begins at conception, or—as in cloning and nuclear transfer—at the genetic beginning. To subvert the development of a potential human being would be considered morally objectionable. To others, the primitive streak is the first trace of the embryo. This does not occur until the end of the second week after fertilization, when implantation is underway. Before this stage, twinning and recombination are possible, and developmental individuality or 'singleness' has not been established. The potential therapeutic benefits of the procedure, it is argued, far outweigh the harm. Where is the morality in letting millions of people continue to suffer from chronic and life-threatening disease? Yes, a human 'pre-embryo' should be treated with respect. But 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?

Avoiding the ethical and legal quandary surrounding the use of 'pre-embryos' might be accomplished with advances in this technology. It may eventually 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. For example, it may be possible to direct the developmental capabilities to develop into only one or two embryonic germ layers. The use of growth factors and specific regulatory promoter systems could further ensure that the cells differentiate into specific types of tissue; for example, into cardiomyocytes that could be used for heart repair, chondrocytes for osteoarthritis and rheumatoid arthritis, pancreatic islets for patients with diabetes, hematopoietic lineages for leukemia or breast cancer patients, or dopaminergic neurons to treat Parkinson disease.

Differentiation of stem cells

Transplanted ES cells spontaneously differentiate into any of a variety of ectodermal, endodermal and mesodermal cell types—sometimes into a disorganized mass of neurons, cartilage and muscle; sometimes into teratomas containing an eye, hair or even teeth[15]. Progress has been slow, but some success has already been achieved in directing the development of stem cells into specific lineages. For example, bone morphogenic protein 4 has been shown to induce ES and teratocarcinoma cells to produce mesenchymal cells. Another molecule, the vitamin A derivative retinoic acid, has been shown to turn ES cells into neurons. Guiding cells down the endoderm lineage has proven far more difficult, although Douglas Melton has achieved some success even here—he has managed to 'persuade' ES-derived endoderm cells to become pancreatic cell precursors (only a few steps away from the β cells needed to treat diabetes) by exposing them to pancreatic bud tissue[15].

Tissue engineering

Many of these differentiated cell types could be useful in medicine as individual or small groups of cells. However, to realize the full potential of therapeutic cloning, it will be important to understand how to reconstitute more complex tissues and organs *in vitro*. Although cloning would eliminate the most essential problem of immune compatibility, there is still the task of putting the cells together to create or recreate functional structures. For relatively simple tissues, such as skin and blood vessel substitutes, this may involve seeding cells onto masses or sheets of polymeric scaffold. Creating vital organs, such as the kidney, the liver or even the heart, however, will be a much greater challenge, and will require assembling different cell types and materials with great combinatorial and architectural complexity.

Some of these hurdles will certainly challenge our scientific ingenuity. But it should be remembered that when Dolly was

cloned a few years ago, the event brought the scientific community a powerful new technology that many thought was impossible. While scholars, the public and the Congress have been debating the complex moral, ethical and legal issues that could emerge, the technology continued to move forward in a careful and deliberate fashion. We now find the stage set for therapeutic cloning as a principal means for treating human disease. It seems increasingly likely that as we approach the threshold of the new century, somatic cell nuclear transfer will be developed and tested in humans, not in an attempt to create a child, but in an effort to prevent and treat a long list of diseases that have claimed the health and lives of millions.

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