SOMATIC CELL NUCLEAR TRANSFER (CLONING) - SCIENCE

Introduction

1. Nuclear transfer involves transferring the nucleus from a diploid cell (containing 30-40,000 genes and a full set of paired chromosomes) to an unfertilised oocyte from which its chromosomes have been removed. The technique involves several steps: synchronization of the donor nucleus into G0 phase of its cell cycle; transfer into an "enucleated" oocyte; fusion of the 2 cells; activation of the "hybrid" cell; and growth of the cell into an embryo. The nucleus itself can be placed into the peri-vitelline space of the oocyte or the intact cell can be injected directly into the oocyte. In the former case, the oocyte and donor cell are normally fused and the 'reconstructed embryo' activated by a short electrical pulse. In the sheep (as with Dolly), the embryos are then cultured for 5-6 days and those that appear to be developing normally (usually about 10%) are implanted into foster mothers.

2. Nuclear transfer is not a new technique. It was first used in 1952 to study early development in frogs and in the 1980's the technique was used to clone cattle and sheep using cells taken directly from early embryos. In 1995, Ian Wilmut, Keith Campbell and colleagues created live lambs - Megan and Morag - from embryo derived cells that had been cultured in the laboratory for several weeks. This was the first time live animals had been derived from cultured cells and their success opened up the possibility of introducing much more precise genetic modifications into farm animals.

3. In 1996, Roslin Institute and PPL Therapeutics created Dolly, the first animal cloned from a differentiated somatic cell taken from an adult animal (Wilmut et al, 1997). In August 1998, Wakayama et al published a report of the cloning of over 50 mice by nuclear transfer. Since then, the cloning of cattle, sheep, mice, goats and pigs have been reported, but not for rabbits, rats, monkeys, cats or dogs.

4. There are differences in early development between species that might influence success rate. In sheep and humans, the embryo divides to between the 8- and 16- cell stage before nuclear genes ("genomic activation") take control of development, but in mice this genomic activation occurs at the 2 cell stage. In 1998, a Korean group claimed that they had cloned a human embryo by nuclear transfer but their experiment was terminated at the 4-cell stage and so they had no evidence of successful reprogramming; there was no publication.

5. Currently, success rates remain very low in all species, with published data showing that on average only about 1% of 'reconstructed embryos' leading to live births. Many cloned

offspring die late in pregnancy or soon after birth, often through respiratory or cardiovascular problems. Abnormal development of the placenta is also common and this is probably the major cause of fetal loss earlier in pregnancy. Many of the cloned cattle and sheep that are born are much larger than normal ("large fetus syndrome"). The high incidence of abnormalities is not surprising. Normal development of an embryo is dependent on the methylation state of the DNA contributed by the sperm and egg, and on the appropriate reconfiguration of the chromatin structure after fertilization. Somatic cells have very different chromatin structure to sperm and 'reprogramming' of the transferred nuclei must occur within a few hours of activation of reconstructed embryos. Incomplete or inappropriate reprogramming will lead to dysregulation of gene expression and failure of the embryo or fetus to develop normally or to non-fatal developmental abnormalities in those that survive.

6. A major effort is now being made to identify systematic ways of improving reprogramming, through: (1) known mechanisms involved in early development, and in particular on the 'imprinting' of genes; (2) technological advances in genomics to screen the expression patterns of genes to identify differences between the development of 'reconstructed embryos' and those produced by in vivo or in vitro fertilization.

Applications of SCNT

i. Cloning in Farm Animal production

7. Nuclear transfer can in principle be used to create an infinite number of clones of the very best farm animals. In practice, cloning would be limited to cattle and pigs because it is only in these species that the benefits might justify the costs. Cloned elite cows have already been sold at auction for over $40,000 each in the US but these prices reflect their novelty value rather than their economic worth. To be effective, cloning would have to be integrated systematically into breeding programmes and care would be needed to preserve genetic diversity. It would also remains to be shown that clones do consistently deliver the expected commercial performance and are healthy and that the technology can be applied without compromising animal welfare.

ii. Production of Human Proteins for Therapy

8. Human proteins are in great demand for the treatment of a variety of diseases. Whereas some can be purified from blood, this is expensive and runs the risk of contamination by HIV or Hepatitis C. Proteins can be produced in human cell culture but costs are very high and output small. Much larger quantities can be produced in bacteria or

yeast but the proteins produced can be difficult to purify and they lack the appropriate post-translational modifications that are needed for efficacy in vivo.

9. By contrast, human proteins that have appropriate post-translational modifications can be produced in the milk of transgenic sheep, goats and cattle. Output can be as high as 40 g per litre of milk and costs are relatively low. PPL Therapeutics has produced alpha-1-antitrypsin through such an approach, and this protein is due to enter phase 3 clinical trials for treatment of cystic fibrosis and emphysema in 2001. Nuclear transfer allows human genes to be inserted at specific points in the genome, improving the reliability of their expression and allows genes to be deleted or substitutes as well as added.

iii. Xenotransplantation

10. The chronic shortage of organs means that only a fraction of patients who could benefit actually receive transplants. Genetically modified pigs are being develop as an alternative source of organs by a number of companies, though so far the modifications have been limited to adding genes. Nuclear transfer will allow genes to be deleted from pigs and much attention is being directed to eliminating the alpha-galactosyl transferase gene. This codes for an enzyme that creates carbohydrate groups which are attached to pig tissues and which would be largely responsible for the immediate rejection of an organ from a normal pig by a human patient.

iv. Cell Based Therapies

11. Cell transplants are being developed for a wide variety of common diseases, including Parkinson's Diseases, heart attack, stroke and diabetes. Transplanted cells are as likely to be rejected as organs but this problem could be avoided if the type of cells needed could be derived from the patients themselves. The cloning of adult animals from a variety of cell types shows that the egg and early embryo have the capability of 'reprogramming' even fully differentiated cells. Understanding more about the mechanisms involved may allow us to find alternative approaches to 'reprogramming' a patient's own cells without creating (and destroying) human embryos.

12. With such cells, the potential in clinical use will include the following:

a) Replacement tissues & organs;

b) Prevention of immunological tissue rejection;

c) Enhancement of immunological survellance; and

d) Gene therapy

13. The implications of such clinical applications include the ability to treat and overcome aging, disease, cancers, myocardial infarctions, renal failure, liver failure, and genetic disorders.

14. These cells will form the basis of new therapies in the battle against death and disease – cell-based therapies will be the next major approach in medicine. The simplest approach is to seed satellite cell clusters of healthy donor progenitor cells in a diseased or dysfunctioning organ, and this may be all that is necessary. The next level is to produce primordial or rudimentary organs with primordial cells which can replace the diseased organ in part or in whole. The final step is to develop the organ completely ex-vivo, probably in conjunction with xenotransplantation, before transplant.

Limitations of nuclear transfer

15. SCNT has many limitations currently, especially its low success rates, but this is due to the infancy of the technique. As basic understanding of this fundamental manipulation improves, success rates will improve.

16. Other requirements for cloning are an appropriate supply of oocytes and surrogate mothers to carry the cloned embryos to term. Use of animal oocytes is an alternative, but this approach poses many questions, both scientific and ethical. In fact, the fusion / introduction of human nuclei into animal oocytes is not permitted in many guidelines related to SCNT.

17. Cloning of endangered species will be possible by using eggs and surrogates from more common breeds of the same species. It may be possible to clone using a closely related species but the chance of successfully carrying a pregnancy to term would be increasingly unlikely if eggs and surrogate mothers are from more distantly related species. Proposals to 'save' the Panda by cloning, for example, would seem to have little or no chance of success because it has no close relatives to supply eggs or carry the cloned embryos.

18. Plans to clone extinct species have attracted a lot of publicity. An Australian project aims to resurrect the 'Tasmanian tiger' by cloning from a specimen that had been preserved in a bottle of alcohol for 153 years. Another research group plans to clone a mammoth from

20,000 year old tissue found in the Siberian permafrost. Unfortunately, the DNA in such samples is likely to be fragmented and the chances of reconstructing a complete genome is highly unlikely. Moreover, nuclear transfer requires an intact nucleus, with functioning chromosomes.

Reproductive and Therapeutic Cloning

19. There are 2 forms of SCNT: reproductive and therapeutic. The former results from replacement of the cloned embryo into a surrogate mother, to allow pregnancy and a live-birth. This approach is important in animal technology and farming, as well as in the pursuit to clone endangered animals. Reproductive cloning of a human is not permitted by many governments and agencies.

20. Therapeutic cloning is the production of cloned cells to produce tissues and/or organs, mainly to improve healthcare treatments. This approach is that taken by many research groups and companies.

21. Because SCNT requires the production of an embryo, the cells produced are completely toti-potent, ie able to produce a complete individual, and that is the basis of reproductive cloning. As the embryo develops further, it is possible to collect only the inner cell mass cells of the embryo (the part of the embryo that forms the fetus and hence all the possible tissues in the body, except the placenta and placental membranes which come from the trophectoderm) and hence embryonic stem cells.

Strategies to produce Stem Cells

22. All cells contain the genetic material and instructions in its DNA to form all the proteins and enzymes in the life of the animal or person from whom it comes. There is now a major research effort in unraveling the time sequence and relational positioning to understand developmental processes. With this understanding and knowledge it will be possible to produce progenitor cells that can develop into specific tissues that are needed.

23. It is now appreciated that adults have stem cells in certain tissues to enable repair and re-population, and that these stem cells can de-differentiate to re-populate tissues of different types. Hence one strategy is to de-differentiate adult stem cells, from tissues that have them in abundance, eg adipose and bone marrow. Because the age of the individual may have a

bearing on the telomerase length of the stem cell, it is logical to move to stem cells which can be collected at birth. Umbilical cord stem cells are found in the umbilical cord and the placenta that are usually discarded following the birth of the child. Many institutions are now realizing the potential benefits to collect such cells, which can be stored for the child's own use in the future, or matched for donation if necessary. These cells, obtained from a fully formed individual, though at different ages, are multipotent, in that they can form several types of cells.

24. Another strategy is to go even earlier into a developing embryo or fetus to obtain stem cells that are pluripotent. This has been discussed by Ariff Bongso in his submission.

25. The last strategy is to produce a cell that is completely totipotent, and that can only come from an embryo that is able to produce a complete individual, ie with the cells that can produce the placenta and membranes in addition to the fetus. This is different from embryonic stem cells that can only produce the embryo, and not the placenta. This is achieved through somatic cell nuclear transfer to re-program its nucleus to "go-back" completely to its very first division ("cloning"). The added advantage of this approach is that the genetic material is that of the donor, and hence, there is no ethical repulsion, cf a donated cell / organ, or immunological rejection.

26. The best strategy, with the least controversy, is to re-instruct an adult differentiated somatic cell to form a progenitor cell of a specified tissue type without the need to form an embryo.

Embryonic Stem Cells

27. The source of embryonic stem cells can be classified into 3 main groups: Wild-type ES cells; Genetically-altered ES cells; and ES cells from SCNT.

28. To limit ES cells to a few cell-lines can have major potential repercussions. These ES cells are genetically identical to the donor. Widespread use of these cells would be similar to producing a large number of chimeras with a link to only a few donors; as there is no one without any form of recessive genes, it would be tantamount to allowing widespread propagation of a gene mutation.

29. Another potential problem is the propensity of ES cells to form teratomas; in fact it is this property that characterizes an ES cell. Hence introduction of ES cells which are not properly differentiated into a particularly cell line may result in formation of a tumour (Solter, 1999).

SOMATIC CELL NUCLEAR TRANSFER (CLONING) - ETHICS

30. Many ethical and moral concerns have arisen over the potential applications of the cloning technology. The technology is still in its infancy and in the meantime, society as a whole has time to contemplate which uses of the technology might be acceptable and which would not. It is also impossible to predict all potential applications of a new technology. Most will be beneficial but all technology can be misused in one way or another. The solution is not to regulate the technology itself but how it is applied.

31. There is also concern that scientists are "playing at God". However, mankind has always been altering nature. Animals were first domesticated about 5000 years ago and selective breeding since has produced modern strains of livestock, plants and pets which are very different from their original progenitors. In medicine, our current life expectancy of well over 70 years is a result of direct intervention in nature, from improved prenatal care, vaccination and use of antibiotics. The human condition is still far from perfect and there is no particular reason now to call a general halt to what most people view as progress.

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As this submission is to be part of the deliberations of the Bio-Ethics Advisory Committee on Human Stem Cell Research Sub-Committee, it will be relatively concise.
This submission is based on a review paper in preparation by Ng et al (2001).