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Cloning/Nuclear Transfer : Novel Biotechnology for the Year 2000

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Introduction

Nuclear transfer is the process involving the introduction of genetic material (in form of an individual cell nucleus removed from either an embryonic, a fetal or an adult cell) into the cytoplasm of an unfertilised egg or embryo, whose own genetic material (nucleus) has been removed.¹

The nuclear genes of clones produced by this technique would be identical, although the mitochondrial DNA of such clones would be different. However, unlike the embryonic splitting technique, nuclear replacement has the potential to create a clone of an adult organism, as well as the potential to produce many more clones.

The nuclear replacement technique to clone animals is relatively new. The first evidence that it was possible to clone vertebrate animals using nuclear replacement was in 1952, using frogs.² The more recent developments of nuclear replacement technology have brought with them the potential to contribute towards the genetic improvement of livestock. Dolly is the first example of an adult vertebrate cloned from another adult by any technique. She was cloned using a nuclear transfer technique, where the nucleus from a cell, which had two chromosome sets, was fused with an unfertilized egg from which the nucleus had been removed. This experiment was the first time that a fully developed animal had been born following transfer of a somatic cell nucleus from an adult animal.³ There are wide ranges of potential benefits using this latest biotechnology which will subsequently be discussed in this article.

Potential benefits of nuclear transfer technology

1. GENE TARGETING IN FARM ANIMALS

At present the only way of producing genetically modified farm animal is by a procedure called "pronuclear injection". This involves the physical injection of 200-300 copies of the gene of interest into recently fertilised eggs and their subsequent implantation into surrogate mothers. Only 2-3% of the animals born are transgenic (that is they carry the added gene from one generation to the next) and only a proportion of these express the added gene at a high level. It is also only possible to add genes. The ability (via nuclear transfer) to derive live animals from cultured cells provides an alternative way of producing transgenic farm animals. Moreover, the ability to manipulate many millions of cells at once opens up the possibility of much more specific genetic modifications, including the deletion or substitution of specific genes or the introduction of the single letter changes in the whole genetic code that are typical of many human genetic diseases.⁴

2. APPLICATIONS IN MEDICINE

Human therapeutic proteins

Transgenic sheep, goats and cattle are already being used as 'bioreactors' to produce human proteins in milk. PPL Therapeutics, one of the leaders in this field and collaborators in production of Dolly, recently announced

that alpha-1-antitrypsin produced from a transgenic flock is now being used to treat cystic fibrosis patients in phase 2 clinical trials. Nuclear transfer will provide a more reliable way of producing transgenic animals, reducing the number of animals needed to establish each transgenic line.⁵

Xenotransplantation

Over the past 20 years transplantation of hearts and kidneys has become almost routine. Nevertheless there is a shortage of suitable organs for transplant (about 5000 each year in the UK) and many patients die as a result. Transgenic pigs are being developed as sources of organs to meet the shortfall. At present these pigs contain added human proteins that coat the pig tissues and are intended to prevent immediate rejection of the transplanted heart or kidney.

In the future, nuclear transfer should improve the chances of success because it will allow pigs to be produced in which the pig proteins responsible for rejection are removed and replaced by their human counterparts.^{4,6}

Nutriceuticals

Cow's milk is ideal for calves but not for premature infants. Gene targeting using nuclear transfer will allow milk to be produced in which one or more of the normal cow's proteins have been replaced by human proteins, thereby improving its nutritional quality for these special consumers.^{5,6}

Animal models of disease

Mice in which specific mutations have been deliberately introduced have often provided very useful models for studying human genetic diseases such as cystic fibrosis and obesity. In some cases, differences between mice and humans means that the effects of the introduced mutation are not the same as in the human genetic disease. Nuclear transfer will extend the range of species in which gene targeting will be possible and thereby proved better models to test treatments for human diseases.⁷

Cell therapy

Intact cells are already used to treat patients suffering from a number of diseases, including leukemia and Parkinson's diseases. In most cases these cells have

to be obtained from close relatives to avoid problems of immune rejection.

The fact that Dolly was cloned from a cell taken from an adult ewe shows that even specialised (or differentiated) cells can be 'reprogrammed' into all the cell types that make up an intact animal. When we know more about this process, there is prospect of using the patient's own cells in such therapies. Cells would be removed from the patient, converted into the desired cell type in the laboratory and then reintroduced into the patient for treatment.^{6,8}

3. NEW TOOLS FOR RESEARCH

Aging & cancer

Each of us starts as a single cell, but by the time we are born most of the cells in our bodies have divided and multiplied at least 20-30 times. Small mistakes in the replication of the DNA occur at each of cell division and these so-called somatic mutations are believed to contribute to the aging process and to the increased incidence of cancer as we get older.

One of the more interesting questions about Dolly concerns her age. As far as her DNA goes, is she two years old or older? And will she age prematurely? These questions cannot be answered with just one animal : we will need to be able to carry out nuclear transfer in a shorter lived species such as the mouse and produce sufficient large numbers of offspring to generate statistically meaningful results. The more recent study by Wakayama and his colleagues in Hawaii showed that full-term development of mice can be achieved from enucleated oocytes injected with cumulus cell nuclei⁷. The careful follow-up of these cloned mice will hopefully uncover the mystery about the age of the cloned animals.

Alternatives to ES cells

Embryo stem (ES) cells are used for gene targeting in mice. ES cells have been recovered from very early embryos and grow well in culture. They can be genetically modified in the laboratory and then introduced into early mouse embryos which are then implanted in surrogate mothers. A proportion of the cells in the resulting pups are derived from the manipulated cell and some of their offspring carry the desired modification.

Embryo stem cells have only been recovered from two specific strains of mice (and not from any livestock species). Nuclear transfer would allow gene targeting in other strains of mice and in other laboratory species such as rabbits and rats.^{4,6,7}

Cloning

An ability to produce large numbers of genetically identical animals would have important benefits in experimental design. Genetic variation between individuals tends to confound the often subtle responses to test diets or drugs. The advantages of genetic uniformity have been amply demonstrated in studies with inbred lines of mice. Nuclear transfer from cultured cells could provide alternative approach in species where repeated inbreeding is impractical or prohibitively expensive.⁹⁻¹¹

Conclusion

The birth of "Dolly the sheep", the first cloned animal, aroused interest and controversy all over the world, especially focusing on the possibility of human reproductive cloning, namely the production of genetically identical human beings. This issue leads to the call for "banning" on the study using nuclear transfer technology in humans in some countries. However, there are wide ranges of scientific and therapeutic applications of nuclear transfer technology, which do not involve the creation of genetically identical individuals. It is fortunate that most societies have now come to understand the potential benefits of the so-called "therapeutic cloning". Further studies using this newly established technology will certainly provide us more useful information regarding reproductive biology, genetics and cancer medicine.

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