statement

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statement 02/00

February 2000

Registered Charity No 207043

THE ROYAL SOCIETY

Therapeutic cloning

A submission by the Royal Society to the Chief Medical Officer's Expert Group

Summary

- This submission focuses on stem cells because of their application to a wide range of therapeutic interventions. Stem cells are defined here as cells that retain the capacity to renew themselves and produce more specialized progeny.
- Patients suffering from damage to their organs through extensive burns, complex fractures or degenerative diseases, such as hepatitis, diabetes or leukaemias, are likely to benefit from stem cell therapy. Damaged organs or tissues would be colonized with sufficient normal cells to restore their physiology or accelerate repair, or organs replaced by providing stem cells with an appropriate scaffold for their reconstruction.
- After the early stages of embryo development, stem cells are more difficult to obtain in significant numbers and typically are capable of forming only one or a limited number of different types of specialized cells.
- The therapeutic use of stem cells raises two major problems: tumour formation from incompletely or inappropriately differentiated stem cell transplants, or rejection. Most of the scientific issues that need to be addressed to exploit stem cells effectively for therapeutic purposes relate to fundamental problems in the fields of cell and developmental biology.
- We strongly recommend that a working party should be set up to investigate the feasibility of establishing frozen banks of various categories of stem cell that have been both tissue-typed and screened comprehensively for pathogenic viruses.

Background

On 29 January 1998, the Human Genetics Advisory Committee (HGAC) and the Human Fertilisation and Embryology Authority (HFEA) issued a consultation paper entitled *Cloning issues in reproduction, science and medicine*. The results of the consultation were published in December 1998, and the Government responded by asking the Chief Medical Officer (CMO) to establish an expert group to look in more detail at the issue of therapeutic cloning.

Following confirmation of its membership, the CMO invited comments and contributions that would be useful to the expert group. This submission, which has been endorsed by the Council of the Royal Society, has been prepared by a working group, chaired by Professor Richard Gardner (Dept of Zoology, University of Oxford), and comprising Professor Christopher Graham (Dept of Zoology, University of Oxford), Sir John Gurdon (Wellcome CRC Institute, Cambridge), Sir Aaron Klug (member of the MRC Laboratory of Molecular Biology, Cambridge, and President, Royal Society), Dr

Anne McLaren (Wellcome CRC Institute, Cambridge, and member of the Human Fertilisation and Embryology Authority), Dr Robert Moor (Babraham Institute, Cambridge), Professor Azim Surani (Wellcome CRC Institute, Cambridge) and Professor Cheryll Tickle (Dept of Developmental Biology, University of Dundee), with support from Mr Bob Ward (Secretariat, Royal Society).

This submission should be considered in conjunction with the Royal Society statement *Whither cloning?* (published in January 1998), and the response by the Royal Society to the HGAC/HFEA consultation document (published in May 1998).

Although the terms of reference for the CMOs expert group are to examine the potential benefits, risks and alternatives to therapeutic cloning research, the Royal Society working group has focused on stem cells because of their wider applications to a range of therapeutic interventions. Consequently, this document has addressed the question: What are the current research areas on stem cell studies and which are the most important?

Terminology

The January 1998 HGAC/HFEA consultation document defined therapeutic cloning as:

> *Medical and scientific applications of cloning technology which do not result in the production of genetically identical fetuses or babies. These techniques may be undertaken to advance fundamental research and therefore not all such applications will lead to immediate therapeutic utility.*

The December 1998 HGAC/HFEA report noted that the consultation document had drawn a distinction between therapeutic cloning and reproductive cloning (defined as the reproduction of an entire animal from a single cell by asexual reproduction). Therapeutic cloning is, however, an ambiguous term as it has been taken by some to include reproductive cloning as a therapy for infertile couples.

The January 1998 HGAC/HFEA consultation document defined a stem cell as an undifferentiated cell which is a precursor to a number of differentiated cell types. This is too restrictive for present purposes as certain adult tissues, such as skin, contain cells that continue to divide but produce only one or a few types of differentiated or specialized cell.

Cells are generally regarded as stem cells if they retain the capacity to renew themselves as well as to produce more specialized progeny. During the course of embryonic development, stem cells with the potential to form a wide variety of specialized types of progeny are succeeded by those with a more restricted potential. Furthermore, as organs and tissues grow and begin to function, stem cells constitute a progressively decreasing proportion of the total. Tissues like the skin, intestine and blood which undergo continual turnover of cells, retain a population of dividing stem cells throughout life and are able to replace the losses continuously. Other tissues have quiescent stem cells that resume division only following damage (eg muscle) or, as may be the case in the central nervous system, do not retain any stem cells into adulthood.

Reduction of new cell production in adults means that, in practice, stem cells are obtained more easily from early embryos. These are called embryonic stem (ES) cells, and they retain the ability to form most, if not all, of the specialized cell types of the adult. Later, particularly after birth, stem cells are harder to obtain in significant numbers and, typically, are capable of forming only one or a limited number of different types of specialized cells. A general classification of stem cells might be as follows:

- *Totipotent stem (TS) cells*, that can differentiate into all types of cells in the fetus or adult, including eggs or sperm. They are exemplified by ES cells derived from the blastocyst. So far, such cells have only been obtained from certain strains of mice.
- *Pluripotent stem (PS) cells*, that have been derived either from blastocyst-stage embryos or from fetal primordial germ cells in a variety of mammals, including man. Those originating from blastocysts in mammals other than the mouse can differentiate into many types of cells, but have not yet been shown to be capable of yielding eggs or sperm. Those of primordial germ cell origin, which are termed embryonic germ (EG) cells, can also produce a wide variety of cell types. However, even in the mouse, relatively little work has been done on EG cells, when compared with ES cells, and this needs to be addressed before contemplating use of human EG cells therapeutically.
- *Multipotent stem (MS) cells* (eg neural, haemopoietic), that can differentiate into a smaller range of cell types and arise later during fetal stages of development.
- *Stem cells with more restricted potency*, that are able to differentiate into only one or a few types of specialized cells.

Introduction

Studies originally undertaken in amphibia, and more recently in mammals, have shown that nuclei from specialized cells may support normal development following transplantation into unfertilized eggs, that have had their nuclei removed. Hence cells can retain intact the full complement of nuclear genes during the course of specialization so that the loss of stem cell properties is not irreversible in genetic terms. Both these and other experiments, in which different types of cells are fused together, suggest that the differentiated state of cells is maintained by the continuous active regulation of expression of distinct constellations of genes. Understanding the basis of this regulatory process is of considerable potential significance for stem cell therapy, as it offers the prospect of being able to alter or reverse differentiation and then to send cells down the desired alternative pathway. This research falls under the general heading of developmental biology.

Stem cell therapy in humans

The scope for therapy is considerable. Organs damaged by trauma or disease do not always need replacing, and repair often would be possible if a suitable source of cells was available. Stem cells are a potential source. Patients suffering from certain degenerative diseases of the brain, liver (hepatitis), pancreas (diabetes), blood (leukaemias), joints (rheumatoid arthritis), heart and kidneys are likely to benefit from stem cell therapy. Other diseases which might be alleviated thus include muscular dystrophy and cystic fibrosis.

Treatment of extensive burns and complex fractures are among other conditions that could benefit from this approach. The aim would be to colonize host organs or tissue with sufficient normal cells to restore their physiology or accelerate the repair of damage, or to assemble replacement organs by providing stem cells with an appropriate scaffold for tissue reconstruction. Where pathology was due to lack of secreted gene products such as hormones or growth factors, introduction of stem cells that had been genetically-modified to correct the deficit is a

possibility, or the cells may produce such factors as one of their normal properties. Use of already defined types of MS cells and those of more restricted potency offers the closest prospect of application and, indeed, use of bone marrow grafts as a source of haemopoietic stem cells has already been practised for some time. At present, however, the lineage of origin is only known for a fraction of the estimated 200 or more different types of specialized cells of which the human body is composed.

Rejection

Use of stem cells for the above purposes raises two major problems. One is tumour formation from incompletely or inappropriately differentiated stem cell transplants, and the other is their rejection. Rejection is obviously not a problem if the cells used for therapy come from the patient or are made to carry, via nuclear replacement in eggs, the patient's complement of nuclear genes. Where use of cells from the patient was not possible, potential problems of graft rejection might require tissue matching and/or immunosuppression. Stem cells could be genetically manipulated to reduce immune recognition in any human host.

Some of the published work on hearts donated by genetically manipulated pigs is relevant. While the most recent studies of xenotransplantation do not look promising, transplanting entire organs from pigs to humans represents, of course, a much greater immunological barrier than that of transplanting isolated cells from one human to another. The obvious alternative would be to establish banks of different types of stem cells that, like blood, represent the range of tissue types in the population and have been extensively characterized for chromosomal defects and viral infection. However, mesenchymal stem cells, ES cells derived from the mesoderm which differentiate into muscle and connective tissue, are noteworthy in this context. The transplantation of mesenchymal stem cells, which can accelerate repair of damaged bone for example, has proved effective even between mouse strains that show very rapid rejection of other types of grafts. It would be interesting to determine how mesenchymal stem cells elude destruction by the immune system of the host, and whether this is true of other types of stem cells.

Current research areas

It is difficult to identify all relevant research because of the increasing involvement of the biotechnology and pharmaceutical industries, the commercial considerations of which tend to restrict disclosure of the details of work in progress. The following broad areas of research are currently being pursued.

1 Investigation of the origin and properties of stem cells

At present, the provenance of stem cells has been established for only a fraction of the cell types of the adult body. The origins of many tissues from the mesoderm and endoderm, in particular, have yet to be determined. An encouraging development has been the recent identification of a common stem cell for both acinar and endocrine tissues of the pancreas, and of a gene that can affect which of these two outcomes arises. This discovery provides evidence that work on cell lineage control, particularly during the latter part of gastrulation and early in organogenesis, is likely to provide powerful new methods for utilising stem cells for therapeutic purposes.

Much effort is now being directed towards characterizing patterns of gene expression in various types of stem cells so that they can be distinguished from their differentiated progeny. As noted earlier, stem cells are relatively rare post-natally, so significant enrichment for such cells would be required unless fetal material is available. Automated cell sorters can achieve this, providing such cells can be labelled selectively so that they are identifiable by such a machine. Hence, establishing reliable molecular markers for different types of stem cells is an important area of relevant ongoing research.

The sequencing of the human genome will accelerate this process, along with the development of microchip and other technologies, so these investigations will form an important part of the post-genomic challenge. As cell sorting often involves the use of antibodies, further studies of proteins, as well as DNA and RNA, will be required.

2 Re-programming of cells

This entails altering gene expression in readily available specialized cells so that they either re-acquire the properties of stem cells of the same tissue, or switch to the desired type of cell via a poorly-understood process that has variously been termed transdetermination, transdifferentiation or metaplasia. Recently, promising new developments in this area have been reported, but harnessing this approach for therapeutic purposes requires a much better understanding than is currently available of how the differentiated state of cells is maintained.

3 Directing the differentiation of stem cells

Studies are being undertaken on cells that have the potential to form several or many different types of specialized cells. So far, the most advanced work in this area is on haemopoietic stem cells which, through transplantation of the bone marrow in which they are found in adults, have already been used therapeutically for a number of years. Considerable progress is being made in identifying genes involved in directing the differentiation of haemopoietic stem cells to the lymphocytic, rather than other blood cell, lineages. Identification of the full-term umbilical cord, which is discarded at birth, as a source has made haemopoietic stem cells more accessible for study and clinical use.

The other main research direction in this area, both in humans and in other mammals, relates to TS or PS cells derived from blastocysts or early EG cells. Progress has been made in directing the differentiation of both these cells and related embryonal carcinoma cells, as nerve, muscle and blood cells. In both mice and rabbits, such cells have been induced to differentiate as cardiac muscle cells that, after transplantation to a damaged heart, have become incorporated in the myocardium, beating in synchrony with the cells of the host. At present, however, the efficiency of controlling the differentiation of TS or PS cells in vitro is typically limited, so that considerable numbers of uncharacterized cells are usually present in addition to the type that is desired. Cell sorting may therefore be required to obtain a pure population for therapeutic use, requiring further investigation of the antibodies employed for this technique.

More refined use of this system will depend on gaining a better understanding of how differentiation of specific types of cells is controlled at the molecular level. For the production of some types of specialized cells, studies of the embryo indicate the need for a sequence of cellular interactions which may be difficult to reproduce in vitro. In other cases, diffusible molecules that can be simply added to the culture medium may prove effective. The latter approach is already being applied to cells derived from embryos developed from eggs whose nuclei have been replaced by those from adult skin cells in amphibia. In

this work, the dissociated cells are treated with pre-determined concentrations of activin, a transforming growth factor -beta molecule, and then cultured or transplanted back to host embryos. It is expected that this experimental regime will enable functional tissues of many kinds, such as muscle etc, to be derived from a few cells taken form adult skin, and thus inform similar studies in man and other mammals.

It is, however, important to bear two points in mind. One is species differences, as PS cells in the human and other higher primates differ in several important respects from those of mice. The other is that a detailed understanding of the differentiation process exists at present for only a few types of cell.

4 Re-programming of nuclei rather than whole cells

The transplantation of nuclei from specialized cells to eggs, the nuclei of which have been removed, shows that complete re-programming of nuclei can be achieved. The approach that has come to be known as 'therapeutic cloning' has been advocated particularly in relation to anticipated problems of graft rejection. This would entail transplantation of nuclei from cells of the patient into enucleated eggs, which were then activated to develop to the stage when ES cells could be obtained from them. As outlined in the previous section, the differentiation of the resulting ES cells would be directed so as to furnish whatever type of specialized cell the patient required. As the ES cells would thereby carry the nuclear genes of the patient, graft rejection should not be a problem.

This approach, however, raises a number of questions that need to be addressed before it can be considered to be a serious option. First, success rates with nuclei from post-natal sources have been abysmally low (frog, cow, sheep, goat, mouse) or entirely unsuccessful (pig, rabbit). At present, it is not clear whether the problems are purely technical or reflect limitations in the capacity of most nuclei for re-programming. Very thorough analysis of the integrity of the genome of stem cells, or their differentiated derivatives obtained in this way, would have to be undertaken before their use for therapeutic purposes could be contemplated. Furthermore, given the uncertainty about the efficiency of nuclear transplantation and the competing demands of various infertility treatments, it is questionable whether an adequate supply of human oocytes would be available to support an active programme of therapeutic cloning.

Many studies of the potential of nuclei for reprogramming have entailed fusing specialized cells with relatively unspecialized types of cells. However, as the hybrid cells resulting from such fusions are chromosomally abnormal, they do not have any potential therapeutic use. A more refined variant of this approach is to prepare what are essentially nuclear fractions (karyoplasts) from one type of cell, and fuse these with the non-nuclear fractions (cytoplasts) of another type of cell. Use of this approach to investigate whether adult nuclei can be re-programmed by a cytoplast from TS or PS cells would seem a high priority in view of the technical and ethical problems posed by the use of eggs for this purpose.

Conclusions

Most of the techniques described in the previous sections are still in the early stages of development, and the gaps in our knowledge are still very significant. Most of the scientific issues that need to be addressed to exploit stem cells effectively for therapeutic purposes concern fundamental problems in the fields of cell and developmental biology. In particular, these include a better understanding of both the origin of lineages of the various types of differentiated cells, and how their differentiation is both induced and maintained. Differentiation is a multi-stage process that depends on a complex sequence of factors. Much more research will be necessary before we acquire a thorough understanding of these factors, but there may be significant breakthroughs in the future.

The technique of therapeutic cloning is likely to remain inefficient for the foreseeable future, and does raise serious issues about safety, particularly regarding the normality of donor nuclei. If this approach for replacing damaged tissues does work, the cost will be considerable. This may mean that such therapy will only help those individuals who are able to afford an expensive treatment and the majority of patients will be excluded. Therefore, the early applications of these techniques are likely to be offered by private clinics.

Although the same safety concerns would apply, achieving the re-programming of nuclei of adult cells to produce stem cells, without recourse to the oocyte, would seem a better option. However, the feasibility of this approach has yet to be explored. The use of stem cells of embryonic, fetal or adult origin is a more realistic option in the shorter term, although the problem of graft rejection would have to be addressed. We therefore strongly recommend that a working party should be set up to investigate the feasibility of establishing frozen banks of various categories of stem cell that have been both tissue-typed and screened comprehensively for pathogenic viruses.

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ISBN 0 85403 534 6