

Stem Cell Research

Status

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Executive summary and recommendations

Overview of this document

This document arises from the contributions of specialists in various areas of stem cell research, biomedical application and patient advocacy, who – in addition to writing the articles herein – were invited to a consultative meeting held under the auspices of EMBO on 19 April 2006. This summary document provides an introduction to the field of stem cell biology and its terminology for non-specialists and sets current research in the context of scientific knowledge production, relevance to major non-infectious diseases, and economic value. Furthermore, it gives recommendations addressing the policies needed in order to enable stem cell research to achieve its proper potential in Europe.

The areas covered are:

Themes and techniques

- The nature of stem cells and their differentiation into specific cell types
- Embryonic stem cells: potency and potential
- Somatic cell nuclear transfer (SCNT) in the production of embryonic stem cells

Tissue-specific research

- Blood
- Bone
- Cancer (stem cell model)
- Cardiac muscle
- Endothelia
(lining of vessels of the circulatory system)
- Epithelia (skin and other external surfaces)
- Brain and nervous system
- Pancreas
- Skeletal muscle

From theory to therapy

- Evaluating the therapeutic potential of stem cells
- Commercial development of embryonic stem cells: report from a company operating in the USA
- Perspective of an international patient advocacy organisation

Current status

Several applications of stem cells in tried and validated therapies are recognised in humans: from bone marrow transplants through to more recent advances in skin and cornea repair. The cells used in these cases are adult tissue stem cells whose normal function is to maintain and repair tissues throughout life. Stem cells are known to exist in several adult tissues, but they are usually rare. Their exact role, frequency of occurrence, and identity are generally not well understood.

Parallel research on stem cells derived from the earliest stages of embryonic development, has defined a primordial progenitor cell that is capable of producing all tissue types of the adult (pluripotent). Embryonic stem cells (ES cells) are remarkably potent on the one hand, and on the other they are difficult to control at the present state of knowledge.

Because adult tissue stem cells are restricted in the range of cell types they can make, there is great interest in the possibility of converting adult cells into pluripotent stem cells. This could be achieved either *via* the technique of somatic cell nuclear transfer (SCNT) – as used to create Dolly the sheep – or by other methods. Such research is still in its infancy, but it may offer

Adult stem cells are currently used in a number of therapies in humans, but they are rare and not yet well defined...

Embryonic stem cells can reproduce all tissues of the adult, but are currently hard to control...

Researchers are working on making similarly potent cells from adult cells...

The combination of studies on adult and embryonic stem cells is advancing biomedicine...

However, there is little interest from industry in Europe because of the current patent situation...

Stem cell research is beginning to address many important diseases, and some new therapies will move from pre-clinical to clinical trials soon...

Diabetes, atherosclerosis and leukaemia treatment are likely to benefit from such research...

ES cells show great potential in treating degenerative diseases and as pharmaceutical testing platforms...

Found in a growing number of tissues, adult stem cells could be amenable to activation by drugs etc. to carry out innate tissue repair...

Because adult stem cells do not appear to have much capacity for diversifying, researchers are working on "reprogramming" the nucleus to a more primordial state; this has potential for treating serious hereditary diseases...

Stem cell technology will continue to move apace in the USA with the involvement of industry...

great possibilities for regenerative medicine in the future.

Research on both ES cells and adult stem cells is advancing our knowledge of the biology of stem cells in general, as well as providing prospects of therapies for a variety of tissue-specific diseases, including such major illnesses as type 1 diabetes and Parkinson's disease. Research is also increasing our understanding of the biology of cancer, which may arise through mutation of stem cells.

Currently there is relatively little commercial support for, or industry engagement in, stem cell research and development in Europe. A significant inhibitory factor is the European Patent Office's interpretation of the morality clause in the European Biotechnology Directive (adopted into the European Patent Convention), which relates to the patentability of inventions dependent – at some point in their development – on the use of the human embryo. This is recognised as a serious impediment to obtaining full benefit from stem cell research and development.

Prospects

Stem cell research is at the beginning of a development that will likely address many important diseases for society, particularly in the ageing population. Stem cells do not only offer the hope of reconstructive therapies: A better understanding of their biology and the markers that distinguish them from "normal" cells, will likely contribute to better prognosis and finely targeted drug treatment of cancers.

Some potential stem cell therapies are already in pre-clinical tests in animals. In the skin and blood systems stem cell research is already moving into a second phase, in which gene correction in combination with cell therapy is used to target serious heritable diseases. In the coming 2-5 years, more stem cell based therapies are planned to enter clinical trials, notably in the areas of muscle regeneration and bone injury.

In the search for a cure for type 1 diabetes, much attention is now focussed on stem cell sources – embryonic, foetal or adult – that may be used to renew the insulin-producing beta cells that are destroyed in this chronic debilitating disease. In atherosclerosis research, findings on circulating endothelial progenitor cells (cells that form the lining of blood vessels) may contribute to improved prognostic indicators and new

therapeutic strategies. In the blood system there is scope to improve bone marrow and umbilical cord blood transplants. This research will also lead to a better understanding of leukaemia cancer cells, and through that to more accurately targeted drug therapy.

ES cells may ultimately be invaluable in a variety of tissue regeneration scenarios. Trials in rat models of Parkinson's disease already demonstrate the potential of cell replacement in the nervous system. Furthermore, the proliferative potential of ES cells creates the basis for an endless supply of human cells for modelling cellular development and disease, and for pharmaceutical testing. Research on adult and ES cells are interlinked, and will increasingly be mutually beneficial as better expansion and directed differentiation of ES cells are achieved.

As far as adult stem cells are concerned, research also aims to identify and characterise resident stem cells in a growing number of tissues. A better understanding of their normal mechanisms of activation in tissue damage repair will likely open the way to directed control of this innate repair machinery by pharmaceuticals, cytokines and growth factors. There are hints of such developments already in research on brain and neural tissues as well as heart – ultimately it could apply wherever tissue-specific stem cells exist in the adult.

However, there is currently little evidence for the persistence of pluripotent cells in adult tissues. Therefore a major goal of stem cell research is to find ways of "reprogramming" adult cells to a pluripotent state. SCNT is one way of achieving this. The technique may soon be used to create ES cells from a patient for investigation of motor neuron disease (amyotrophic lateral sclerosis). Other serious hereditary degenerative diseases are amenable to this approach – in this context, the potential power is in understanding inherited cellular diseases for which the gene(s) has not yet been identified and/or there is no good model system available. Research into SCNT should also lead to an understanding of the mechanism of reprogramming and accelerate the development of methods that do not require nuclear transfer into enucleated eggs.

Industry increasingly has a crucial role to play in the development of stem cell technologies, and in the United States it is actively doing so in the areas of glial cells (support cells in the nervous system) for spinal cord injury, cardiomyo-

cytes (heart muscle cells) for heart disease, islet cells for diabetes, haematopoietic cells (cells that give rise to all blood cells) for blood diseases, and hepatocytes (liver cells) for pharmaceutical testing.

Concerns, problems and open questions

Although the development of stem cell applications is sometimes presented as involving a choice between ES cell research and adult tissue stem cell research, biomedical advances can be foreseen equally from both approaches. It is known that ES cells are pluripotent, having the capacity to generate any cell type in the body. However, that potency is offset by some lack of certainty as to their stability in a partially or completely differentiated state, and a limited knowledge of the signals that are necessary to achieve definitive differentiation. Adult stem cells, while restricted in the range of cell types that they can generate, are more amenable to controlled differentiation, precisely because they have fewer choices available. However, what can definitively be demonstrated is that research in each field benefits the other at the levels of basic science and application. It is, therefore, wise to support both.

The stem cell is an entity that should be understood and manipulated in the laboratory before reintroduction to the patient. In general, the direct regenerative effect of stem cells is due to the proliferation and differentiation of the cells in the desired tissue. In some cases, however, cell transplantation may have indirect beneficial effects due to anti-inflammatory and growth signals that the introduced cells emit – the so-called “bystander” effect. More tissue-based research is necessary in order to clarify exactly how introduced cells interact with the host tissue, and what the essence of their regenerative power is. On the basis of such research, optimised therapies can be developed. Some types of tissue regeneration likely require the introduction of two or more cell types in order to have optimal clinical benefit. In other cases it may be more effective to administer a mixture of cytokines rather than, or in addition to, introducing cells.

In principle a genetic defect in the tissue to be treated may be repaired using stem cells. Using a patient’s own cells and repairing/replacing the gene in question avoids the problems of immune

rejection of foreign cells. This could provide a longer-term therapy, or even cure. The value of such “genetically corrected” stem cells has been demonstrated at the level of proof of principle. However, it may require the development of alternative methods to the hitherto used viral vectors in order to be accepted for widespread clinical application.

In certain applications (i.e. skeletal muscle and the lining of blood vessels) infusion of stem cells into the circulatory system can be used as the route of administration. However, in the majority of applications of cell-based therapy, introduction of cells directly to the site of repair/regeneration is likely to be necessary. This will require techniques and equipment specifically tailored to each clinical application, and the application of sophisticated imaging technologies to track the introduced cells. Furthermore, several experimental approaches – notably in bone and cartilage repair – rely on the development of suitable “scaffold” materials on which cells can grow prior to implantation. Research on such bio-compatible materials must keep pace with stem cell research, and production must be scaled up to satisfy demand.

In both basic and clinical stem cell research, progress is required in selecting from a tissue only the rare population of true stem cells among a mixture of partially differentiated progenitor cells. Such cell sorting is important in order to increase the efficacy and specificity of the dose of cells finally administered. In many areas of research it remains to be seen whether progenitor cells grown in the laboratory are still capable of differentiating and functioning properly after transplantation. Clearly, consistency of production and quality control are prerequisites for safe clinical application of cell therapies. However, the efficacy of the cells designated for therapy is at least as important as their safety. Cells that appear to pass overly stringent regulatory control may do so at a price: they may lose a subtle component of their potency to regenerate tissue, and upon transplantation they may no longer behave as desired. It is therefore essential to maintain an appropriate perspective and avoid compromising the functionality of stem cells before transplantation. An important consideration is whether the current level of regulation – with the associated bureaucracy and costs – would have allowed the development of life saving stem cell therapies for

Adult and embryonic stem cells have different advantages and disadvantages; research on both is complementary and crucial...

For effective administration, stem cells must be well understood and specially prepared; further research is needed to clarify the role in tissue regeneration of the cells themselves and the chemicals they emit...

Genetically altered stem cells could be used to treat and correct genetic diseases, but this may require new methods for introducing the “repair” gene...

Most therapies will be given directly into the tissue in question, requiring sophisticated administration and imaging technology, and in some cases “scaffolds” of advanced materials to grow the cells...

Obtaining the right cells from a tissue, and ensuring that they remain effective until administration are crucial; efficacy controls are as important as safety controls, and must not be swamped by the latter...

Large investments would be necessary to scale up therapies for general availability; to minimise immune rejection, large numbers of cells would need to be banked; SCNT with patients' own cells needs to be researched further as a complementary strategy...

But legislative obstacles are hindering basic research...

And techniques and products involving the use of a human embryo remain unpatentable in Europe; researchers' main concerns are the techniques for manipulating stem cells and the differentiated cells that result...

The appeals process of the European Patent Office is dealing with the matter, but a resolution is not expected before 2008...

Meanwhile patents on human ES cells are granted elsewhere around the world...

Leaving Europe at a big disadvantage because the situation is so complex and far from positive resolution that there is little industry interest in ES cell R&D...

blood disorders in the 1960s and severe burns in the 1980s.

A further concern at the level of delivery of stem cell therapies is that very significant investments need to be made if such therapies are to be generally available one day. Infrastructures for scale-up, safety and quality control, as well as distribution and storage need attention before therapies start to move out of clinical trials and become generally available therapies. Furthermore, should ES cells prove to be a therapeutic that is applicable to a large number of diseases, the problem of immunological rejection will need to be overcome. This may be tackled by banking a relatively large number of ES cell lines in stem cell banks for histocompatibility matching (as used in organ transplants), combined with immunosuppressive drugs or strategies to induce tolerance. The derivation of ES cells from a patient *via* SCNT or direct reprogramming represent alternative possibilities, but such techniques need to be firmly proven and then developed into an efficient process.

A major concern hanging over the field of stem cell research – more particularly research with human embryonic stem cells (hES cells) – is that there are legislative obstacles to free international collaboration and exchange of materials. The disparate national regulations governing stem cell research in Europe inhibit the field from benefiting from the internationality of approach inherent to scientific advance. In some countries scientists are allowed to pursue research with a limited number of human ES cells, but are faced with the threat of prosecution if they engage in research on other human ES cell lines developed by collaborators in Europe.

In the context of economic value and patents, European stem cell researchers are concerned with the protection of intellectual property relating to the manipulation of established human ES cells and differentiated derivatives, not to their derivation from the human embryo. However, the current stance of the European Patent Office (EPO) is to exclude from patentability all inventions or claims relating to human embryonic stem cells. This position has its roots in rule 23d(c) of the European Patent Convention (the legal framework in which the EPO operates), which stipulates that “European patents may not be granted in respect of biotechnological inventions which, in particular, concern uses of human embryos for industrial or commercial purposes.” According to current EPO interpretation, that

excludes from patentability all claims to associated products necessitating the direct and unavoidable use of a human embryo – including claims on cells derived from an embryo.

Whether this interpretation is correct, and in particular whether inventions using established hES cell lines – being procedurally distant from the original embryo – should be subject to patent exclusion, rests on the verdict of the EPO's enlarged board of appeal. That decision may well not be reached until some time in 2008. To the extent that the final interpretation of the European Patent Convention article 23d(c) might exclude from patentability all applications of, and techniques pertaining to, human ES cells, it would have a major negative impact on future commercial investment in this area in Europe.

This unusual situation – patents on human ES cells are granted elsewhere around the world, notably in the US – and the lack of a verdict at the EPO present the temptation for researchers or companies to apply for national patents in individual European countries instead. But – depending on the particular case and the interpretation of the European Biotechnology Directive – that process risks contravening the very instrument designed to aid harmonization across Europe (the Directive itself), hence potentially precipitating legal proceedings at the European Court of Justice.

In summary, the intellectual property situation is so complex, and so far from a positive resolution, that there is little – if any – incentive for commercial involvement in the development of ES cell applications in Europe. Since patents on human ES cells are already granted in the United States and elsewhere, investors and bioindustry are focussing on those opportunities. This situation renders the European biotechnology sector at a serious disadvantage compared with global competitors, and carries potentially damaging consequences for the economy and healthcare.

Recommendations

In order that stem cell research and development in Europe stand the maximum reasonable chance of fulfilling their potential for advancing health-care, the biological sciences and the economy...

- 1. The prospective value of stem cell research in benefiting healthcare, knowledge production and the economy should be publicly recognised. Human stem cell research should be integrated into the mainstream of biomedical research, including disease modelling, study of cellular degenerative processes, development of pharmaceutical and toxicological screening platforms, and regenerative therapy.
- 2. Research on both adult and embryonic stem cells, being highly complementary, should be fully supported; so too should research into understanding the reprogramming of the nucleus brought about by somatic cell nuclear transfer (SCNT), cell fusion and other techniques.
- 3. Communication and professional education on stem cell research and applications should be promoted with active participation of scientists and clinicians along with ethicists, regulators and patient group representatives.
- 4. Intellectual property relating to the utilisation of human ES cells and cell lines after their derivation from the embryo should be patentable in order to encourage the necessary industry involvement in the translation of stem cell research into clinical applications.
- 5. Regulations relating to stem cell research and applications should be clarified and harmonised, and wherever possible legislative obstacles to free international collaboration between scientists removed.
- 6. Care should be taken that regulatory requirements are not excessively stringent and do not present an unreasonable financial and/or bureaucratic barrier to clinical applications, as currently threatens.
- 7. Efficacy measures and standard operating procedures for clinical use of stem cells should be developed. In establishing clinical practices, efficacy measures should be accorded at least as much significance as that of safety controls.
- 8. Stem cell banks with high levels of quality assurance should be encouraged, and international access by scientists and industry facilitated.
- 9. Greater harmonisation and interlinking of clinical trials and their results across Europe should be promoted in order to facilitate cross-border studies and evaluation of all cell therapies (including, but not limited to stem cell therapies). Such an initiative should be for the benefit of all citizens and underpinned by public funding.
- 10. Investments should be made in technology development to enable large-scale processing of stem cells for applications in pharmaceutical screening, toxicological testing, and cell transplantation.

Introduction

Stem cell research is both an evolution and a revolution in modern biomedicine. It can be regarded as a milestone on a progression of signal findings and developments: from small molecule antimicrobials (e.g. penicillin), antibodies and monoclonal antibodies, to modern genetics, genomics and cell therapy. Indeed, it is possible only because of the multitude of discoveries in biology that precede it. At the same time, it is quite different from anything so far attempted in biomedicine on a global scale. Stem cells – particularly embryonic stem cells (ES cells) – represent part of the post-genomic technology for delivering on the promises of the human genome sequencing project. Commercial companies in the USA already use genomics to derive ES cell lines.

Despite its revolutionary nature, the concept of using stem cells for therapeutic purposes has much in common with the now conventional principle of organ transplantation; but rather than introducing a whole new organ to a patient, only a certain population of cells is given. Furthermore, if these cells are from the same individual, the problem of immune rejection is also avoided. Some established cell therapies relying on stem cells can even be regarded as intermediate between organ transplantation and cell therapy: for example, bone marrow transplants and transplantation of pancreatic islets (groups of so-called β -cells that produce insulin in the pancreas). Transplant technology has allowed medicine to heal people in ways unachievable by mere surgery or medication; the same can be said about the prospects of stem cell technology. Furthermore, stem cells have the potential to treat a broad spectrum of diseases from the common to the rare, and across all age groups (e.g. rheumatoid arthritis, Duchene muscular dystrophy and congenital immune deficiencies).

There are already well-validated clinical applications of stem cells. Thousands of patients have benefited from bone marrow transplants, for example. During the Cold War the fear of nuclear war prompted an acceleration in research to repair human tissues that are particularly prone to radiation damage (renewing tissues that replace themselves continuously throughout life) such as the blood. Experiments in mice showed that following destructive irradiation, the entire blood system could be regenerated *via* bone marrow cell transplantation. The first successful human bone marrow transplant resulting in the long-term survival of the patient (a leukemia sufferer, whose bone marrow had been destroyed by radiotherapy) was performed in 1956 by Dr. E. Donnall Thomas, New York; the proof of principle was turned into a life-saving clinical application. Further signal advances were made by other researchers in 1968 (first bone marrow transplant using a related donor for non-cancer treatment) and 1973 (first bone marrow transplant using an unrelated donor). Today many thousands of lives are also saved by stem cell skin grafts to burns victims, and many hundreds of eyes are saved from blindness with the help of corneal stem cells. In certain cases of corneal injury, traditional transplantation fails simply because the transplant does not regenerate itself as the normal tissue does. Stem cells can overcome this problem.

A significant number of diseases targeted by stem cell therapy are due to a faulty gene that manifests its effect in a particular tissue or sub-population of the patient's cells. Using genetically corrected stem cells to repair faulty genes in the patient appears an attractive alternative to the classical method using viral vectors to deliver the repair gene *in situ*. Genetically corrected stem cells are proving their value in clinical trials

Stem cell technology represents a revolutionary way of exploiting human genome data...

But it is also closely related in principle to well established and successful transplantation technology...

It has already proven itself in several clinical settings, and has the potential to address a broad variety of diseases...

Even to the extent of repairing the genetic defect that gives rise to certain diseases...

Stem cells may be derived from adult and foetal tissue, and from embryos...

But their characteristics can differ greatly depending on their source...

Adult stem cells cannot be considered a replacement for embryonic stem cells...

Pre-clinical testing in animals is crucial for the safety of stem cell-based therapies...

Cells will predominantly be administered directly to the site of repair...

And will most likely be at least partially differentiated beforehand...

Stem cell research also gives insights into intrinsic tissue repair mechanisms, disease processes and normal tissue function...

And could provide the basis for better pharmaceutical testing methods and ways of studying diseases, particularly rare ones...

for at least one seriously debilitating hereditary skin disorder.

Stem cells vary greatly in derivation, proliferative capacity and diversity of adult cell types to which they can give rise.

Stem cell sources:

- Adult stem cells obtained from so-called niches in adult tissue (such cells have been identified in many tissues from brain to muscle)
- Foetal stem cells (e.g. from aborted foetuses or umbilical cord blood)
- Embryonic stem cells:
 - From disused *in vitro* fertilisations
 - *Via* nuclear transfer (also known as “somatic cell nuclear transfer”), in which the nucleus from a normal body cell is placed into a fertilised egg from which the original nucleus has been removed. The environment of the fertilised egg has the effect of ‘re-setting’ the transferred nucleus to a kind of primordial state.

The notion that adult stem cells can replace embryonic stem cells – hence side-stepping the ethical concerns of the latter – is frequently cited. However, the potential of adult stem cells to solve problems should not be overstated, especially since (as is shown in box 1) the nature of adult stem cells from different tissues is not completely understood. Embryonic stem cell research contributes to the understanding of adult stem cell biology and *vice versa*. More research is urgently needed to move the field beyond the uncertain current position regarding the benefits and relative merits of different stem cell applications.

An inevitable and very important part of this research involves the use of animals. From the first experiments on bone marrow transplantation to current day genomics experiments (defining the genetic nature of stem cells and their development), animal experiments have been indispensable. Where therapeutic applications are concerned, no regulatory authority (neither the FDA nor the European EMEA) would consider an application that is not supported by pre-clinical safety and toxicity tests in animals.

In order for a stem cell therapy to enter the clinic, many challenges must be met. Several open questions surround the mode of delivery of the cells. The method, route and site of introduction to a patient are likely to be specific to the

particular stem cell and its purpose. The potential for local delivery to the tissue of interest has been markedly improved by developments in catheter technology, further improving safety. The concept of infusing cells into the blood and allowing them to reach the desired target is probably only of merit in a minority of cases (e.g. damage to the blood vessel lining or other directly accessible parts of the vascular system).

In clinical application, stem cells will most likely be at least partially differentiated before introduction to the patient. The introduction of undifferentiated stem cells would at most be the rare exception. Pre-differentiated cells represent a more controlled material, which is already set on a path to becoming the desired tissue. However, for repair of external skin defects, *in vitro* generated epidermis containing undifferentiated adult stem cells is currently used with success.

Though stem cells are overwhelmingly thought of in terms of a material to be administered as a therapeutic, it is important to realise that they also allow researchers to understand the way in which resident stem cells can be mobilised to repair damage “from within”. Current work in the field of muscular dystrophy and muscular atrophy is such an example. Moreover, stem cells are an experimental resource in themselves, enabling researchers to better understand cancer, developmental biology, pharmacology, degenerative diseases and the maintenance of normal physiological function, to mention a few topics.

Stem cells – because they can be differentiated into a variety of cell types found in our bodies – represent ideal material for the testing of pharmaceuticals: for therapeutic effects on specific cells, for unwanted side effects, and for breakdown by liver cells. In principle, stem cells derived from a person could provide an individual profile for response and toxicity of a given medicine. Such use of stem cells is estimated to be a huge area of application, with a significant impact on the safety of medicines. For studying how different tissues are affected by a particular disease, and in pharmaceutical testing, somatic cell nuclear transfer (SCNT) technology represents an attractive potential way of making the necessary stem cells. SCNT would in principle make possible the controlled production of genetically identical but functionally different cells from an individual. It would also offer an attractive way of studying orphan diseases, for which there are few sufferers and a low availability of clinical samples.

Embryonic stem cells	Adult stem cells
Are a ubiquitous component of the embryo, with a well-understood function and life history.	Are rare, often difficult to identify, of unknown origin, and partially understood function and life history.
Are defined by position in the embryo (the inner cell mass of the blastocyst).	Are defined by a complex list of features such as cell-surface markers, behaviour <i>in vitro</i> , and in some cases, position in the tissue.
Can divide symmetrically indefinitely in culture without changing characteristics.	Can divide few or many times (up to 200 or more) in culture, but not indefinitely.
A single cell can give rise to a colony of genetically identical cells with the same properties as the original cell.	In some tissues, absolutely consistent precursor cells can be identified and cultured; that remains to be shown for all tissues.
Pluripotent: can give rise to all three tissue types of the embryo (endoderm, mesoderm and ectoderm).	Sometimes multipotent: most only give rise to their tissue of origin, but some may be able to give rise to different cell types within the larger groupings of endoderm, mesoderm and ectoderm (so-called plasticity)

Box 1 Crude comparison between embryonic and adult stem cells

In conclusion, stem cells are an enormous – as yet partially tapped – resource of biological knowledge, and through this knowledge a major new hope for improved therapies. Stem cells have already proven their worth in treating certain diseases and types of tissue damage. They have benefited thousands of patients already, and offer tangible prospects of application in other diseases and clinical settings. Furthermore, research on stem cells leads to the accumulation

of crucially important information of relevance to fields of research as diverse as cancer and degenerative diseases. Indications from established clinical application, research and development are that the science and technology of stem cells have the potential to benefit medicine, industry and the larger economy in very significant ways – if correctly supported at the levels of funding and policy.

The nature of stem cells and their differentiation into specific cell types

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Introduction

Stem cells are a pervasive component of embryonic and foetal development, of tissue maintenance and of regeneration and repair. Accordingly stem cells are central to normal human growth and development, and are also a potential source of new cells for the therapeutic regeneration of diseased or damaged tissue. The body is made up of a large number of diversely functioning specialised (i.e. differentiated) cells that are organised into specific tissues and organs. During development and also throughout life, many of these tissues are able to repair themselves after damage. This regeneration and repair depends on reserve populations of cells that divide slowly to maintain their own population but can also proliferate to provide the committed precursors for specific cell differentiation. These stem cell populations are specialised in (that is, committed to) specific directions of differentiation. Although there have been some studies which have shown that stem cells extracted from one tissue have repopulated another these results are by no means unambiguous⁽¹⁾. Nevertheless, highly specialised, tissue-specific stem cells are exactly what are needed for a particular therapy if they could be isolated in sufficient numbers.

It is self-evident that at the earliest stages of development there will be dividing populations of cells which have a very wide range of developmental potential (but not all such cell populations will be proliferative stem cells – i.e. capable of extensive division). It is not self-evident at which, if any, stages along this progression from totipotent to single lineage restriction the cells

may cycle mitotically and keep that developmental fate statically intact. However, such Embryonic Stem Cells (ES) have been isolated originally from mouse embryos and subsequently from a small number of other species including man.

ES cells are pluripotent – that is, they are still at the base of the differentiation tree and have retained their embryonic capacity to give rise to most, probably all, cell types. The potential advantage of ES cells is their ability to be isolated and grown in large numbers coupled with their ability to differentiate into any other cell of the body. This may provide a route to obtain populations of precursor cells, which will allow the therapeutic regeneration of damaged adult tissues for which there is no other endogenous or sufficient source.

Tissue transplantation carries with it the risk of immune reaction and rejection unless the donor cells are closely immunologically matched or (ideally) identical to those of the patient. One seductively enticing scenario (which has done much to power the field) would be to provide a source of ES cells, genetically identical to the patient from which that required specialised precursor cells might be differentiated. This would involve “de-differentiation” of a cell from the patient to an ES state. The only method so far known to work in animal model experiments is nuclear transplantation into an oocyte and establishment of the ES cell line from the early embryo formed. Such somatic cell nuclear transfer (SCNT) technique could be used to generate a patient specific ad hominen ES cell line. This scenario of generating an embryo by nuclear transfer of an adult cell nucleus into an enucleated oocyte, making an ES cell line and using this to generate cells which will repopulate tissues of an adult, has been fully demonstrated in a mouse model.⁽²⁾

1: Wagers AJ, Weissman IL. Plasticity of adult stem cells. *Cell*. 2004 Mar 5;116(5):639-48.

2: Hochedlinger, K., W. M. Rideout, et al. (2004). “Nuclear transplantation, embryonic stem cells and the potential for cell therapy.” *Hematol J 5 Suppl 3*: S114-7.

Current status

Derivation of ES cells from IVF human embryos has been achieved in a repeatable way, and there are numbers of research grade cell lines available. Derivation of human ES cells from nuclear transfer embryos, however, remains unproven. The reports from Hwang and his Korean team have proven to be falsified information. ⁽³⁾ There remains as yet no reliable evidence of derivation of human ES cells from a cloned embryo. The generation of an early cleaving human embryo from nuclear transfer has been reported by others but not in mainstream scientific journals. ^(4,5) Differentiation of mouse ES cells to defined progenitor or differentiated populations (nerve, pancreatic islet etc.) has been demonstrated, as has specific differentiation of human ES cells, and there are cell transplantation therapies using adult stem cells – but not ES cells – either established or in trial.

Prospects

The advent of human ES cells and the ideas for the future potential for therapeutic medicine of using stem and precursor cells is the beginning of a very major change in therapeutic practice, which will address many areas of great importance, particularly in our ageing population. Europe can provide leadership in an ethical application of science to a type of healthcare that is patient-centred and evidence-based. We are seeing the beginning of major changes in medicine that are coming about because of our greatly increased biological and genetic knowledge. Insurance is the sharing of unknown risk. As such, increasingly predictive medicine does not fit comfortably in an insured provision of healthcare. Moreover, the change to patient-specific (such as cellular or genetically selected) therapies may be changing the commercial pharmaceutical model of the blockbuster drug.

For these reasons, Europe needs to be in the vanguard of stem cell research and application. In order to achieve this we need regulation that will facilitate advances and prevent abuses and in which our diverse public is confident. We need, ethical and legal frameworks, which separate considerations of reproductive intervention from laboratory and therapeutic cell biology. ES cells must be viewed as a cell type, and not as an embryo.

It is clear that an adult cell nucleus can be reprogrammed -- that is its differentiation can be reversed -- but we only know one complete method at the moment: nuclear transfer into an oocyte. Nuclei can also be de-differentiated in the context of cell fusion. We are coming to understand most of the components of the stability of the differentiated state and these are all micro reversible. Methods of direct de-differentiation of somatic cells need to be developed. Moreover, as the therapeutic product will be a committed precursor cell population, we need to know much more about inducing and validating specific cell differentiation. It will be important to invest heavily in cell and developmental biology using both model organisms (mainly mice) and human cells in culture.

- Research on ES cells, adult stem cells and progenitor cells heralds a possible revolution in the treatment of diseases, especially in the ageing population and a major contribution to personalised medicine.
- Regulations and ethical/legal frameworks need to be constructed in such a way to allow Europe to take advantage of the potentials for stem cell applications.

Cell developmental biology in animal model systems and human cells will be important in elucidating the reprogramming of the cell nucleus in SCNT, differentiation and the reversal of differentiation.

Problems, concerns and open questions

The debacle of the Hwang team serves to highlight both the practical and ethical problems of a SCNT approach. The ethical sourcing of sufficient oocytes may be a major practical block. Derivation of an embryo by cell nuclear transfer needs to be an efficient process, and much development work needs to take place before this can be so.

In order to provide therapeutic cells the source needs to be histocompatible with the patient. The patient specific *ad hominem* approach, be it by “cloning” or another method of de-differentiation will provide this perfectly, but there are other potentially more practicable ways forward. If it is possible to establish a very large bank of

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4: Cibelli JB, Lanza RP, West MD, Ezzell C. The first human cloned embryo.
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genetically diverse embryonic stem cells most patients could be provided with a near perfect match. Another approach would be to have a much smaller bank of cells which are pre-characterised and which could be tolerated in transplantation by many patients. The calculation of the number of cell lines needed for a minimally acceptable match is surprisingly small if their genotypes were very specifically chosen.⁽⁶⁾ Such a very carefully designed bank might need to be made from genetically chosen IVF embryos from genetically selected individuals i.e. made to order. This alone could present a huge ethical problem, potentially bigger than using SCNT⁽⁷⁾. In order to be available for therapeutic use these cell lines have to be established, validated and maintained under Good Manufacturing Practice Regulations.

- In the event that SCNT becomes practical, the ethical sourcing of sufficient human oocytes (eggs) would present ethical concerns.
- The likely necessary creation of banks containing genotypically carefully chosen allogenic (non-self) stem cells would present ethical problems equivalent or larger to those of SCNT
- Good manufacturing practices and regulations would have to be validated and established.

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Embryonic stem cells: potency and potential

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Introduction

Embryonic stem cells (ES cells) are unique biological entities that have the ability both to reproduce themselves endlessly and to give rise to all specialised cell types of the body. The capacity to form all cell types is called “pluripotency”. This property is normally restricted to cells that only exist for a few days in the early embryo before formation of the initial body plan. ES cells, however, preserve pluripotency even after massive expansion in the laboratory. Therefore, they can in principle provide a continuous supply of specialised cells for basic research, disease modelling, drug testing, and possibly future cell replacement therapies.

Current Status

Our ability to produce ES cells arose out of fundamental research into the properties of a type of tumour, teratocarcinoma, that contains many specialised tissues such as hair, teeth and gut⁽¹⁾. Teratocarcinomas also contain un specialised cells that were shown in the 1970s to be the cancer stem cells. These stem cells can individually give rise to all the specialised cells in the tumour. Therefore they are pluripotent. Furthermore they can be grown in the laboratory. At the same time it was found that if early mouse embryos are taken out of the uterus and planted into other tissues they form teratocarcinomas. These observations stimulated efforts to produce pluripotent stem cells directly from embryos.

This was successful first in mice in 1981 and then in primates including human in the 1990s.

ES cells are produced from early embryos called blastocysts. Blastocysts are the stage before implantation into the uterus or formation of specialised tissues. They contain up to 100 un specialised cells that are pluripotent and will act as founders for the entire body if the embryo implants. Pluripotent cells in the embryo do not maintain themselves after implantation but turn into specialised cells, a process called differentiation. In the laboratory, however, it is possible to induce a state called self-renewal in which the cells multiply without differentiation. ES cell self-renewal depends on specific signals called growth factors. This is an artificial situation and if mouse ES cells are placed back into blastocysts, they cease self-renewal and re-enter normal development, differentiating on cue into all cell types of the developing foetus. Resulting animals can have functional ES cell derived progeny throughout their tissues and are called chimaeras. However, although ES cells can contribute to tissues and organs of the developing mouse, they can only do this with the support of cells provided by the recipient blastocyst. Therefore they are not totipotent.

Human ES cells are produced by similar methods to mouse ES cells using supernumerary blastocysts originally intended for infertility treatment or diseased blastocysts identified by pre-implantation genetic diagnosis. With informed consent such embryos may be donated for research in those countries that permit human ES cell derivation. Clonal human ES cells can differentiate into a broad range of cell types, meaning that they are pluripotent. Intriguingly, human ES cells are reported to require different self-renewal factors from mouse ES cells.

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Furthermore, for ethical reasons it should remain unknown whether human ES cells can contribute to chimaeras. It is perhaps unfortunate, therefore, that the cells from the two species have been given the same name before a common identity has been established.⁽²⁾

- Embryonic stem cells are “pluripotent” - capable of generating all cell types found in the body.
- Embryonic stem cells are produced in the laboratory by special treatment of cells removed from blastocysts.
- Production of human embryonic stem cells involves the use of blastocysts discarded from infertility treatments or diagnosed by genetic screening as carriers of a lethal disease gene.

Prospects

Differentiation involves a stable change in the activity of genes to give a cell specialised properties, such as contractility for heart cells or electrical activity for nerve cells. A pluripotent cell can differentiate down many different pathways. A key challenge for ES cell scientists therefore is to understand how to control differentiation. Guided by discoveries in basic developmental biology and armed with post-genomic information and technologies, researchers are acquiring increasing knowledge of the mechanisms that maintain self-renewal of mouse ES cells or direct their differentiation.⁽³⁾ Protocols and selection techniques have been devised to produce relatively pure populations of particular cell types. Importantly, these differentiated derivatives do not carry the potential risks of undifferentiated ES cells to form tumours.

Differences between mouse and human in the basic biology of ES cells and early development present challenges, but it is reasonable to anticipate well-controlled, scaleable, production of differentiated cell populations from human ES cells in the next few years. This will provide basic researchers and the biopharmaceutical industry with a major new bioresource for discovery and drug development. Directed differentiation into cell types of clinical relevance, such as dopaminergic neurons or pancreatic beta cells, should also

provide the platform for rigorous pre-clinical evaluation of cell replacement therapies.

A further opportunity provided by ES cells is the production and characterisation of tissue stem cells that may be very rare *in vivo*. This may lead to methods for isolating such stem cells directly from adult tissue or even to development of drugs that can activate these tissue stem cells for repair in the body.

ES cells are critical for the above developments because there is little evidence that pluripotent cells exist in the adult body. However, use of ES cells in transplantation would trigger rejection by the patient's immune system. This could be combated by immunosuppressive drugs, but these are costly and have damaging side-effects. An ambitious goal for researchers, therefore, would be to find methods of converting adult cells into pluripotent cells. It is already known that this can be achieved by nuclear transfer into oocytes or by fusion with ES cells. Furthermore, in mice developing germ cells can be converted under particular conditions into pluripotent stem cells similar to ES cells.⁽⁴⁾ The next challenge is to identify the proteins that mediate such “reprogramming” and attempt to convert adult tissue cells directly.

- Researchers will take increasing control of the expansion and directed differentiation of human embryonic stem cells to produce new bioresources.
- Embryonic stem cell bioresources will lead to greater understanding of tissue stem cells, and enable major advances in modelling development and disease, identification of new drugs, and cell transplantation.
- Experimentation to enable creation of pluripotent stem cells directly from cells taken from our bodies will be a focus of increasing research efforts.

Problems, concerns and open questions

Human ES cell research is still in its infancy and the scientific challenges in harnessing the potential of these cells should not be glossed over. A realistic timescale for new therapies may be

decades rather than years. For example, human ES cells are genetically diverse and it remains to be seen whether they can be standardised in the same fashion as genetically uniform mouse ES cells. The genetic stability of ES cells during long-term expansion is also uncertain and will require comprehensive monitoring prior to any clinical applications. Sophisticated bioprocessing techniques will be required to enable large scale production of functionally mature phenotypes suitable for biopharmaceutical screening. Complex regulatory requirements for cell therapies may impede clinical translation and greatly increase the costs. Novel mechanisms will be required for funding multi-centre clinical trials in the absence of major industry partners. These hurdles should be overcome by a combination of individual scientific creativity and European-wide co-ordination and investment.

However, a divisive political climate is currently a major barrier to the advance of stem cell research in Europe. Most notably, legislation in some countries prohibits researchers from studying human ES cell lines derived after an arbitrary date. It is a criminal offence for scientists from those countries to collaborate fully with other European researchers on human ES cell work. The European research base in stem cell biology is consequently in danger of fragmentation and individual researchers are under threats of legal action.

A second roadblock obstructs biotechnological exploitation of ES cell research in Europe. The European Patent Office (EPO) has currently suspended all patent applications involving human ES cells. EPO has adopted a rigid interpretation of the “morality clause” in the EU Directive on Biotechnology which states that “use of human embryos for industrial or commercial purposes” is excluded from patentability⁽⁵⁾. Apart from the fact that ES cells are not embryos, the stance of the EPO ignores the opinion of the European Group on Ethics that patents should be allowed on modified human stem cells or processes involving human stem cells, whatever their source.

Although some European countries have broken rank to allow national patents concerning human ES cells, the position of the EPO places Europe collectively at a significant disadvantage compared with North America or Asia where ES cell patent applications are routinely granted.

- Co-ordinated cross-disciplinary programmes are necessary to address the broad scientific, technical, clinical and regulatory challenges of moving stem cells from the bench to the patient in a safe, effective, and affordable manner.
- New funding mechanisms will be needed to support clinical trials in cell replacement therapy.
- Restrictive national legislation and failures to grant Patent protection undermine European research morale and competitiveness, jeopardising future economic and healthcare benefits from stem cell research

Conclusions

ES cells provide unmatched opportunities for applying post-genomic technologies to understand cellular development, functional differentiation and disease. Out of this should emerge medical benefits in the form of new biomarkers, improved drugs, and ultimately cell replacement therapies. Crucially, ES cells also hold the key to understanding pluripotency which might eventually enable scientists to convert one type of specialised cell into another cell type for treatment of a disease or injury in the same patient. For Europe to participate fully in, and reap the rewards of, stem cell research will require supra-national cooperation in both research and regulation.

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Somatic cell nuclear transfer (SCNT): prospects in disease research and treatment

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Introduction

New opportunities in biology and medicine will be provided by the ability to derive embryonic stem cells (ES cells) from artificial embryos created *in vitro* by a technique called somatic cell nuclear transfer (SCNT), and to control their differentiation into all the cell types of the adult body. SCNT involves removing the genetic information from an unfertilised egg and replacing it with the genetic information from a cell taken from the body (or “soma” – hence “somatic”) of the person under investigation.

In the case of someone suffering from an inherited disease the resulting “embryo” will have the inherited genetic characteristics of the patient; so too will stem cells derived from the developing embryo. The stem cells may then be artificially differentiated into the cell type(s) affected in the disease, and the resulting cells will display the pathology of the original patient. Once the method is developed, it will be possible to study human genetic diseases in entirely new ways.

This approach has the potential to provide opportunities – not available *via* any other approach – to study inherited diseases in which the genetic cause has not been identified. The family of diseases that are variously known as motor neuron disease (MND), amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease is one such case, and this will be used to illustrate the opportunities.

Current status

The approach to studying inherited human diseases using a clonally expanded and *in vitro*-differentiated population of stem cells involves three steps: 1. the use of somatic cell nuclear transfer (SCNT) to produce a single cell resembling a fertilised human egg, i.e. “embryo” with the inherited characteristics of the patient suffering from the disease; 2. derivation of ES cells from that cell; 3. culture from that cell line of the specific type of cell that is affected in the disease. While the second and third steps have been achieved using embryos resulting from *in vitro* fertilisation (IVF), there is at present no unequivocal report of the derivation of stem cells from a human embryo produced *via* SCNT (step 2).

Failure to derive stem cell lines from SCNT embryos may reflect the limitations of the present procedures for SCNT. ES cell lines are obtained from embryos after 6 days of development, when they are at the blastocyst stage (hollow ball of cells from which the recognisable form of the embryo takes shape). Two laboratories have described the production of human blastocysts by SCNT. At present it is not possible to understand the cause of the failure to isolate ES cells from them, but experiments to produce animals *via* SCNT show that such embryos have a reduced developmental potential. It may be that modifications to the procedures of SCNT are required for primates, including human.

- ES stem cell lines can be obtained from IVF embryos, and differentiated into different adult cell types.

- SCNT embryos have been demonstrated to be capable (in a few cases) of developing into a blastocyst.
- Methods have not yet been established for the production of ES cells from SCNT embryos.

Prospects

The ability to produce stem cells from SCNT embryos will provide new opportunities to study inherited diseases, such as ALS, a relentlessly progressive muscle wasting disease that causes the death of 130,000 people worldwide each year. Degeneration of motor neurones is the common cause of this fatal condition, but the causes of the degeneration are not understood. In a small proportion of cases the disease occurs repeatedly within a family, but it seems likely that in general several genetic and environmental factors contribute to the pathogenesis of ALS. Experiments in mice suggest that toxic effects of an abnormal protein cause damage to both motor neurons and neighbouring cells, but a detailed understanding is still lacking.

In order to understand the mechanisms by which the abnormal protein causes the disease it would be extremely useful to be able to study nerve cells from patients with ALS in the laboratory. Unfortunately this is not possible with cells taken directly from living patients, because the cells that are affected are deep within the central nervous system. Furthermore, even if extracted, it is unlikely that they could be maintained in culture and expanded to the numbers needed to study. Finally, by the time the patient dies there may have been many secondary changes resulting from the illness or natural ageing.

Suitable stem cell lines could be obtained from SCNT embryos in such a way that they have an inherited form of the disease. Such a methodology would make it possible, for the first time, to study the development of ALS in nerve cells equivalent to those of a patient with ALS. Comparisons could be made with nerve cells from healthy embryos.

Following analyses of the proteins produced in the “diseased” cells, it would be possible to develop rapid methods for screening potential drugs in order to identify compounds able to stabilise the condition of the patient and prevent progression of the disease. First it is necessary to understand the causes of the disease and identify

a change that may be used as a test in assessing compounds. Using high through-put screening systems it would then be possible to assess several hundred drugs comparatively cheaply. By contrast, at the present time drugs are tested in experimental animals. At the same cost it is only possible to assess a handful of drugs in a year compared with hundreds in high through-put cell-based screens.

The same approach could be used to study any inherited human disease. The advantage is greatest if the genetic error that causes the disease is not known. Naturally, it is also essential that the affected cell types can be produced in the laboratory from ES cells. Other candidate conditions for study include neurodegenerative diseases, psychiatric diseases, abnormalities of the heart that may cause sudden death (cardiomyopathy), and some forms of cancer.

But it is not only in the research and treatment of identified diseases that adult cell types resulting from SCNT would be of use: the screening of drugs for efficacy and side-effects on various tissues of the body would benefit enormously from such readily available and genetically identical cells. In the era of “personalised medicine” cells representing different patient “genotypes” could be produced *en masse* and used for a more realistic test of a drug’s action across the diversity of patient types in society.

- Differentiated cells from SCNT-made ES cells would introduce new opportunities for studying inherited human disease, and for researching suitable drugs to treat them.
- Cells with the characteristics of those in patients with inherited disease will open up new ways of studying inherited diseases and developing drugs. Examples are ALS, Parkinson’s disease and some cancers.
- SCNT could theoretically produce large numbers of identical cells of various tissues for efficacy and safety testing of drugs across a range of patient “types” in the population at large, contributing significantly to “personalised medicine” for everyone.

Problems, concerns and open questions

The opportunities available through SCNT will complement those provided by different approaches. In a small proportion of cases the genetic error that causes a disease has already been identified. If the error has been identified, it may be introduced into existing ES cell lines by standard methods of molecular biology to create cells that are expected to have the characteristics of the disease. Such modified cells can then be contrasted with cells of the original line, which are identical except for the precise change associated with the disease. This approach is simple and direct, but only possible in the small proportion of cases in which the mutation has been identified. In the case of ALS the proportion is 2 per cent of patients. In this situation a very precise direct comparison can be made between nerve cells from the ES cell line with or without the genetic error.

In cases in which the error has been identified, molecular tests can be used to identify embryos that have inherited the disease as the result of one of their parents having an inherited form (We inherit one copy of a gene from each parent). Usually only one of the two copies of the gene is damaged in the case of inherited cases of ALS. Hence, only half the embryos produced by a patient are expected to have inherited the disease. In this case, cells can be taken from each embryo and molecular techniques used to discover if the embryo has inherited the damaged gene in the process known as Preimplantation Genetic Diagnosis. Indirectly, these tests identify those embryos that have inherited the disease and these would be destroyed if they were not used for research. This approach can be used to obtain cells for any disease for which the causative mutation is known.

SCNT provides opportunities when the cause of the disease is not known. However, the present methods of cloning are inefficient, although they are repeatable and used by many laboratories around the world. This low overall efficiency reflects a failure of current procedures to change the function of the genetic information from that appropriate for an adult cell to that required for normal embryonic development. It is not known whether similar abnormalities in gene function would occur in ES cells derived from SCNT. In developing and assessing

the use of SCNT for these research purposes, it is essential that comparisons be made to discover whether or not errors in cell function introduced by SCNT mask those that are due to the disease. Such tests may involve carrying out SCNT from ES cell lines carrying known genetic errors before producing new ES cell lines. It would then be possible to compare the manifestation of the genetic error between conventional ES cell lines and those produced by SCNT.

- The success rates of SCNT in producing human embryos that appear (substantially) normal are currently low. A better understanding is required of how exactly the transferred nucleus becomes “re-set” to an embryonic state.
- More research is required to establish methods of producing ES cells from SCNT embryos (or of optimising SCNT formation, if it is at that stage that failure is determined).
- Should ES cells be reliably produced *via* SCNT, appropriate comparisons must be made between conventionally derived ES cells and those from SCNT to rule out side effects due only to the procedure of SCNT.

Conclusions

The possibilities that arise if one envisages the reproducible creation of stem cell lines from SCNT-derived embryos are very significant indeed. They include not only improvements in research and treatment of serious degenerative inherited diseases, but generally applicable methodologies for the pharmaceutical industry that could improve the lives of millions. SCNT-derived cells may in certain cases also facilitate a reduction in the number of animals used to research a condition or test drugs against a disease. The culturing of ES cells from IVF embryos, and the establishment of stable cell lines, has already been achieved, and are no longer technical challenges. It has also been demonstrated that several tissues can be produced by artificially differentiating ES cells in the laboratory (with appropriate growth factors, for example). The step from SCNT to adult tissue cell that currently represents a challenge is the formation of definitive ES cells from the SCNT-derived embryo. The research to solve that obstacle is of crucial importance in achieving the considerable potential illustrated above.

Background/further reading

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Somatic stem cells of the blood: Haematopoietic stem cells

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Introduction

The stem cells that form the adult blood, haematopoietic stem cells (HSC), are the most widely studied and clinically applied cell differentiation and transplantation system. Research in this field has been ongoing for several decades and has contributed to improved cell replacement therapies for blood related genetic deficiencies and malignancies. Current challenges in this field include: 1) *Ex vivo* expansion of HSCs for clinical use in autologous (self-derived) and allogenic (non-self) transplantations; 2) Knowledge of the stem cell molecular program for efficient manipulation of normal and leukaemic stem cells; 3) Stimulation of HSC generation from embryonic stem (ES)/precursor cells.

Current status

Enormous numbers of mature blood cells such as red blood cells, macrophages, lymphocytes and platelets are produced daily from HSCs through an extensive cellular differentiation hierarchy. HSCs are stringently defined by their ability to generate all blood lineages for long periods of time after transplantation into haematopoietic deficient recipients. Transplantation of HSCs was the first applied human stem cell replacement therapy.⁽¹⁾ Hundreds of thousands of patients have been successfully transplanted with donor HSCs from bone marrow, the adult tissue that harbours them. More recently, umbilical cord blood has also been successfully used for blood replacement therapy.⁽¹⁾

Graft-versus-host disease is much less frequent in patients receiving umbilical cord blood stem cell transplants, and these cells are easily accessible. However, the numbers of HSCs obtained from these tissues are limiting. Thus, as the best characterized cell differentiation system with clinical relevance, HSCs have been the focus of intense fundamental research.

Cellular, molecular and developmental research approaches have increased our understanding of the processes by which HSCs are generated, maintained, expanded and differentiated.^(2, 3) For example, the differentiation of HSCs into erythrocytes (red blood cells), granulocytes, macrophages and lymphocytes (white blood cells) is the result of stimulation by haematopoietic growth factors. Such growth factors have been identified and are routinely used in the clinic to stimulate haematopoiesis and to mobilise HSCs into the circulation. However, much less is known about self-renewal factors: Self-renewal is the process by which the HSC divides to produce two progeny, one progeny that differentiates into blood while the other cell retains stem cell potential. To date, no growth factors or growth factor combinations have been identified that faithfully promote the expansion of HSCs (i.e. the production of two stem cell progeny).

Since HSCs normally reside in particular environments within the body, the non-haematopoietic cells in these regions are of interest. These non-haematopoietic cells are derived from mesenchymal stem cells that differentiate into osteocytes (bone), adipocytes (fat), chondrocytes (cartilage), smooth muscle and vascular cells (blood vessels). These so-called stromal cells provide the signals necessary for the maintenance of HSCs throughout adult life. It is thought that there are only a defined number of these environ-

1. Hakim Nadey S and Paplois Vasilos E. (2003) History of Organ and Cell Transplantation, *Imperial College Press*, pp 304. and *Leukemia and Lymphoma Society*

mental niches and that HSC numbers are controlled by the numbers of, and factors produced within, these niches. Stromal cell lines from the bone marrow have been generated for the *ex vivo* laboratory study of the molecular interactions between HSCs and their surrounding environment. Knowledge of the molecular programs of HSCs, as well as the stromal cells within the niches, is of great importance for improvement of blood therapies.

Developmental environments and factors are a current intense focus of haematopoietic research.^(2,3) It is thought that HSCs are generated only during embryonic development and that no further generation occurs in the adult. Recent cell transplantation research in animal models has identified the originating source of HSCs as the cells of the developing embryonic blood vessels, such as the dorsal aorta and the umbilical artery. Several other tissues including the yolk sac and placenta may also contribute to the HSC pool found in the adult. HSC generation in the human embryo appears to be very similar to that of the mouse embryo.⁽³⁾ Within the first four weeks of conception, HSCs are found in the dorsal aorta, and slightly later in the yolk sac. They appear to be at least as potent as adult bone marrow and cord blood HSCs. Molecular manipulation of the genetic and epigenetic programs that direct the generation and expansion of HSCs in development should provide further important insights into the regulation of HSCs and may therefore lead to improved blood replacement therapies.

- HSCs produce all adult blood cells.
- HSCs are produced in multiple sites in the embryo and are thought to contribute to the adult blood system.
- Stromal cell niches support the growth of HSCs through the production of factors.
- Genetic and epigenetic programming of HSCs is beginning to be explored.

Prospects

Health related prospects for the use of information from HSC research include the ability to *de novo* produce HSCs in the adult from cells

of other lineages. Clearly, the demonstrated first development of HSCs from the embryonic vasculature implicates a particular molecular state (genetic and epigenetic) in the generation process. Elucidation of the program of these embryonic precursors, early HSCs and the cells of the embryonic stromal environment and comparisons with the programs of HSCs and cells of the stromal environment of the adult could suggest signalling pathways for stimulation or inhibition through small targeted drugs. Already known developmental signalling pathways such as the Notch, Hedgehog and Wnt pathways have been shown to affect haematopoietic cell growth. Thus, further cellular and molecular insights are yet gained into formation and expansion of HSCs during embryonic stages, and their potential contribution to the adult haematopoietic system. Interestingly, it is as yet unknown in mammals whether the HSCs generated during embryonic stages do indeed migrate and colonize the adult bone marrow to provide life-long haematopoiesis. It remains possible that rare endothelial cells in parts of the adult vasculature, perhaps the abundant vessels of the human placenta retain potential to generate haematopoietic cells. If this is the case, prospects for further expansion and induction of these vascular cells for therapeutic application are encouraging.

The availability of unlimited numbers of ES cells for differentiation to desired lineages has raised the possibility for use of ES cells in blood replacement therapies as an alternative to adult bone marrow or umbilical cord blood haematopoietic stem cells.⁽³⁾ Additionally, universal donor strains of ES cell derived HSCs would alleviate the problems inherent in obtaining closely matched donor stem cells. Since the first demonstration of haematopoietic differentiation of mouse ES cells over 20 years ago, many insights into the embryonic development of the mouse haematopoietic system have been applied to ES cell haematopoietic differentiation. Through the dedicated efforts of a few laboratories, the improvement of ES cell culture conditions has resulted in an enhanced ability to produce cells of the erythroid lineage (precursors of red blood cells), myeloid lineage (precursors of white blood cells with non-specific, non-adaptive, immune functions) and lymphoid lineage (precursors of B and T lymphocytes of the specific adaptive immune system), and to identify a common vascular and haematopoietic precursor.⁽⁴⁾

2. Dzierzak E. (2005) The emergence of definitive hematopoietic stem cells in the mammal. *Current Opinion in Hematology*, 12:197-2002.

3. Hematopoietic stem cell development: Review Issue (2005 Ed: Yoder MC. *Experimental Hematology*, 33.

Additionally, the haematopoietic differentiation of human ES cells has been achieved.⁽⁵⁾ These studies have furthered our understanding of some of the genetic programs directing the differentiation of the developmentally early haematopoietic cells.

Most interestingly, many advances have been made recently in the treatment of blood malignancies/leukaemias with the drug Gleevec. However, while this drug affects the cells that form in large part the leukaemia, small populations of leukaemia-inducing cells survive, and in time lead to the reappearance of the leukaemia. These “leukaemia stem cells” are interesting targets for novel drug treatments, which could be revealed through knowledge of the genetic and epigenetic programs in normal and leukaemic HSCs.

Realistic prospects or outcomes of research are:

- Stimulation of the developmental reprogram directing *de novo* HSC generation from vascular precursors
- ES cell derived HSCs in unlimited numbers and for universal engraftment in blood replacement therapies
- Targeted drug treatment of “leukaemia stem cells” for elimination of the persistent self-renewing cell source of leukaemia

Problems, concerns and open questions

Disappointingly, to date, haematopoietic differentiation of mouse and human ES cells has not achieved the production of potent transplantable adult HSCs.⁽⁵⁾ More studies of the *in vivo* development of HSCs will be necessary to provide clues concerning the directed differentiation of ES cells to HSCs. If the simultaneous differentiation of surrounding developing embryonic tissues is required for HSC induction (as appears to be the case in the mid-gestation dorsal aorta), multidimensional ES cell differentiation culture systems will need to be developed in which other lineages of cells are also induced. Additional studies will add insight into how HSCs can be generated from ES cells by manipulating developmental programs and additionally provide

new opportunities for obtaining large numbers of HSCs from other easily accessible tissues, such as the placenta. Moreover, these studies will further our understanding of how HSCs are generated from the embryonic vasculature.

- HSC generation from ES cells and/or vascular cells will most likely need a complex environment

Conclusions

The haematopoietic system is a dynamic, highly proliferative and complex differentiation system with many levels of regulation. Faulty regulation of HSC self-renewal can lead to 1) over proliferation, as in leukaemia or 2) lack of stem cell maintenance. An increasing knowledge of the process of self-renewal, as well as the developmental signals and environment leading to the generation of the HSCs, is essential for directing the generation and expansion of HSCs from adult and/or embryonic tissue sources or ES cells for the treatment of blood related diseases.

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Stem cell research in bone

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Introduction

Each year over 800 000 bone grafts are performed globally with a total market of around 300 million euros. 27 million Europeans undergo dental surgery requiring bone augmentation for success. Osteoporosis affects 44 million American citizens, with an anticipated equal market size in Europe. The total cost for treating these patients amounts to over 14 billion euros a year. Bone disease, either of surgical or medical interest, is emerging as a major health problem in the western world. Stem cell research in bone provides an innovative, rational, and challenging avenue, promising major scientific and applicative reward.^(1,2)

Current status

The concept of a skeletal stem cell is not original; it dates back to the late sixties. More recently, skeletal stem cells have risen to centre stage as a specific kind of post-natal stem cell, and have been renamed 'mesenchymal stem cells'. Contained in the fraction of the bone marrow that does not give rise to blood cells (called the stromal tissue), skeletal stem cells can be isolated in culture from small samples of adult bone marrow, obtained *via* simple extraction procedures. Skeletal stem cells (SSCs) can be culture-expanded and induced to generate mature cells, which can form bone tissue (the main constituent of individual bones), cartilage (the main constituent of joint surfaces in the bones), adipose tissue (a component of the bone marrow filling bone cavities, and of the soft tissue around the bones),

fibrous tissue (the main constituent of tendons and ligaments), and the tissue required to support haematopoiesis (haematopoietic stroma, which creates the environment for the making of blood cells within the marrow cavities).

Thus, all tissues that together make the skeleton can be generated by a single skeletal stem cell. This can be demonstrated using appropriate transplantation models, in which culture-expanded cell strains originating from single SSCs generate one or more skeletal tissue *in vivo*. Initially designed as proof of principle studies using immunocompromised mice as recipients, transplantation studies have evolved into pre-clinical models and subsequently into pilot clinical studies. Focus has been on the use of SSCs for bone regeneration. To this end, clinical trials are either underway or planned worldwide. The use of SSCs for bone repair *via* tissue engineering approaches may be considered clinically feasible – if not today, then at least in the near future.

Currently, the most common approach to bone tissue engineering is based on the generation of a cell-biomaterial construct that is then locally transplanted. The cell component of the construct is obtained through *in vitro* culture of SSCs isolated from the patient's own bone marrow. The cell strain obtained in this way is then combined with a scaffold, typically consisting of a mineral phase (hydroxyapatite, tricalcium phosphate, calcium carbonate, or combinations thereof). Cells adhere to the mineral phase, which in turn facilitates the deposition of new bone by the cells loaded onto the scaffold. Ideally, the scaffold should be amenable to resorption by the host over time, leaving behind only the newly formed bone.

- Skeletal (mesenchymal) stem cells can easily be isolated from the post-natal bone marrow.

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- Skeletal stem cells give rise to bone, cartilage, fat, fibrous tissue, and haematopoietic stroma.
- The ability of SSCs to give rise to specific skeletal tissues has been tested, and can be exploited to regenerate bone – and possibly other tissues in the future.

Prospects

We can expect to see the results of ongoing clinical trials for bone tissue engineering using SSCs in the next two years. The results of these studies will provide additional evidence of the feasibility, safety and efficacy of the approach. Meanwhile, it is expected that these studies will highlight specific problems. These will include dealing with the diversity of future approaches in the surgical arena, and the need to work out specific strategies for different applications (e.g., segmental reconstruction versus bone augmentation versus spinal fusion etc).

Equally important prospects lie in the area of cell therapy using skeletal stem cells. While the scale of the clinical problem, and related market, is enormous for bone tissue engineering, individual diseases theoretically amenable to cure through cell therapy are relatively rare. However, the severity of the clinical, social and human problem that they represent will suffice to justify intense efforts. Just as the notion of stem cells in haematopoiesis allowed researchers to envisage a cure for diseases that appeared to be incurable such as leukaemia, so the greatest appeal and challenge of skeletal stem cells lies in the prospect of curing crippling and at times lethal diseases of the skeleton. Specific problems must be tackled through intensive and targeted research before this aspect can reach an applicable horizon. Current therapeutic approaches to diseases such as osteogenesis imperfecta (OI) and fibrous dysplasia cannot provide a cure. However, pursuing the innovative avenue of stem cell based therapy for these diseases is a medical need more than an investigative option.

Important prospects are also associated with some less apparent properties of SSCs. The ability of SSCs to generate the stromal framework for the production of blood cells has major theoretical and applicative implications that link the field of SSCs with the field of haematopoiesis

and haematological diseases. This is an emerging area of interest that is likely to see important advances of relevance both to the skeleton and to haematopoiesis.⁽³⁾

- Clinical trials testing the use of SSCs for bone repair are underway.
- SSCs make it theoretically possible to treat severe skeletal diseases that currently await a cure.
- SSCs and their progeny are functionally related to hematopoietic (blood-forming) cells.

Problems, concerns and open questions

In the area of bone tissue engineering, important advances are needed and expected in the area of biomaterials. The materials employed in the first round of studies (both preclinical and clinical) were selected on the basis of their ability to facilitate bone formation by cells that usually perform that function *in vivo* (osteoconductive materials). The use of stem cells for bone tissue engineering poses specific challenges, and materials employed as scaffolds will need to be tailored to maintain the characteristic properties of stem cells in the long-term following *in vivo* transplantation. Studies are underway to design and test ‘advanced generation’ materials, including those designed to release bioactive factors in a controlled way.

The use of SSCs for repair of other skeletal tissues, such as articular cartilage (which covers the surfaces of joints), seems less immediate, and still requires substantial pre-clinical work. Again, the size of the clinical problem and related market is very large. Whereas it is easy to obtain genuine cartilage from SSCs (using both *in vitro* experiments, and some *in vivo* models), further research is needed to identify better biomaterials, and to ensure ultimate stability of the tissue.

In the area of cell therapy, advances are expected in technologies designed to: a) modify stem cells prior to their use *in vivo*; and b) deliver stem cells in ways other than direct, local implantation. Advances in both areas are needed for the conception and design of studies using stem cells for therapy of genetic diseases. This

is also important for other diseases that affect the entire skeleton, rather than a single bone or a single region of a bone. To genetically modify stem cells, basic tools have been developed over the past few years (such as viral vectors) and they need to be adapted to use in specific cell systems. In addition, systems must be developed whereby single genes can not only be transferred into stem cells, but also *silenced* in stem cells. This would be necessary for addressing the treatment of specific skeletal diseases in which excessive function of a gene product – rather than lack of a gene product – causes the abnormality in bone structure and function. Devising effective ways to deliver stem cells to all sites in the skeleton is important for treating diseases that affect the whole skeleton. At present, there is no solid evidence that one can deliver SSCs to the whole skeleton *via* the bloodstream, i.e. using an intravenous injection.

In the area of stem cell biology that is directly relevant to clinical use, advances are needed and expected in the purification of the genuine stem cell fraction comprised in the cell population that can be currently explanted and grown in culture. This population includes a hierarchy of stem and progenitor cells with variable potential for cell replication and maturation into specific types of skeletal cells. The availability of purified stem cell populations will permit the design of strategies that may avoid a cell culture phase prior to clinical use. This would greatly simplify the clinical use of SSCs, and possibly improve their efficacy.

- Advanced generation, stem cell-friendly scaffold materials for tissue engineering must be developed.
- Strategies for genetic correction of SSCs must be developed and validated.
- Strategies for systemic delivery of SSCs must be developed and validated.

Conclusions

Stem cell research in the field of skeletal disease opens important new perspectives in multiple areas of medicine and orthopaedic surgery. In orthopaedic surgery, it provides the option of using stem cells for generating the tissues

required to repair bone defects (e.g. to replace resected segments of a bone; to increase bone mass at a specific site; or to bridge defects resulting from fracture non-union, etc – bone tissue engineering. Beyond the boundaries of orthopaedic surgery, the notion that skeletal tissues originate from stem cells opens the perspective of using stem cells for the cure of non-curable diseases, such as crippling genetic diseases of the skeleton. In such cases, stem cell therapy can be combined with genetic correction. Furthermore, the notion that growth and life-long turnover of the skeleton is fundamentally dependent on the biology of skeletal stem cells provides a perspective on skeletal diseases in general. It influences the design of new drugs, and the very understanding of disease mechanisms. This sets skeletal stem cells (SSCs) in the context of targets for pharmacological intervention in a wide range of bone disorders, including diseases of very high prevalence and social and economical impact.

Stem Cells and Cancer

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Introduction

The cancer stem cell represents a useful model – increasingly supported by experimental findings – to explain and investigate cancer. In general, cancer is thought to arise from normal tissues along a multi-step progression from precursor lesions to increasingly more invasive (malignant) stages. Local and distant metastases arise from these primary malignant lesions and still represent one of the major causes of morbidity and mortality among cancer patients throughout the industrialized world. Along this sequence of events, a stepwise accumulation of genetic alterations in specific cancer genes is considered the driving force in tumor initiation, progression and metastasis.

A well-defined number of cellular changes, such as self-sufficiency in growth signals, resistance to programmed cell death (apoptosis), insensitivity to growth-inhibitory signals, limitless replicative potential, and the capacity for inducing growth of new blood vessels (angiogenesis), are thought to represent essential requirements for the cancer cell to grow and invade distant sites.⁽¹⁾ However, although formally correct, this model takes little account of other essential characteristics of human cancers, namely their pronounced cellular heterogeneity (many different cell types are often present within the tumour mass) and the putative role played by a subpopulation of cells, the cancer stem cells (CSCs), in driving tumour growth and determining local invasion into surrounding tissues and distant metastasis.^(2,3)

Tumours are not autonomously-acting proliferation machines, but are very heterogeneous, both in their morphological and functional aspects. In fact, an individual tumour may show distinct areas of proliferation, cell cycle arrest, epithelial differentiation, cell adhesion and dissemination. According to this more dynamic CSC model (**figure 1**), the majority of tumour types arise from within stem cell niches characterised by a tightly co-ordinated balance between self-renewal, migration, proliferation, differentiation and apoptosis. Mutations in genes known to be responsible for this balance in normal tissues result in the formation of a partially differentiated and heterogeneous tumour mass that, upon additional mutations and under the positive influence of micro-environmental factors, progresses towards malignancy.

Tumour cells are shed from this heterogeneous mass into the micro-environment. However, they will reflect the heterogeneity of the primary tumour and only few, the *migrating* cancer stem cells, will retain the necessary plasticity to undergo trans-differentiation and enable their migration and homing in distal organs.⁽³⁾ Accordingly, aggressive cancer progression has been correlated with the loss of epithelial identity and the acquisition of a migratory phenotype. This phenomenon, referred to as *epithelial to mesenchymal transition* (EMT), is considered a crucial event in malignancy. Additional steps enabling dissemination and metastasis may be reversible (mesenchymal to epithelial transition, MET), and thus cannot solely be explained by irreversible genetic alterations, indicating the existence of a dynamic component to human tumour progression and of a regulatory role for the tumour environment.

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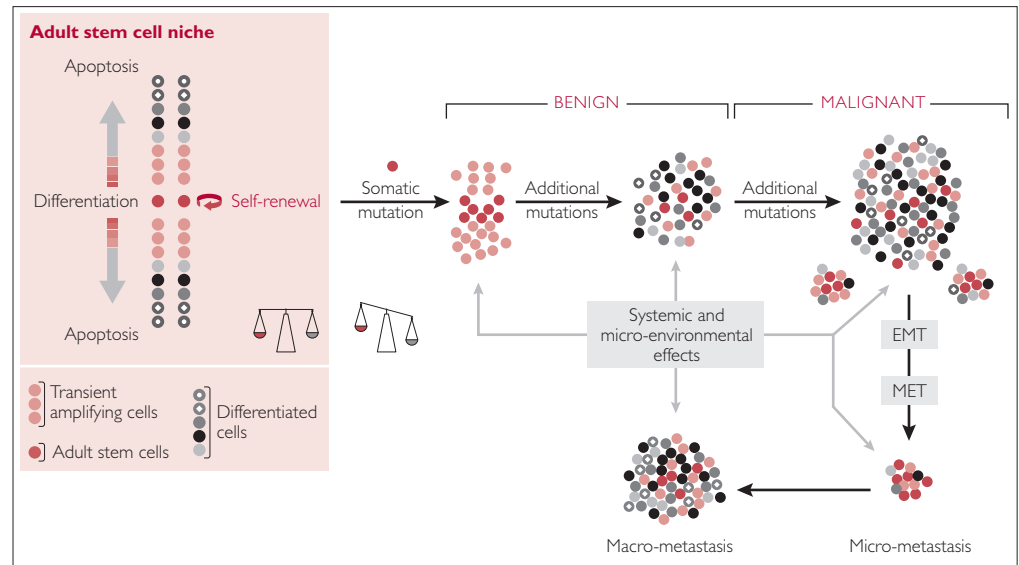


Fig. 1 Schematic representations of the cancer stem cell hypothesis in tumor formation and progression to malignancy. In the adult stem cell niche, pluripotent stem cells together with more committed progenitors and fully differentiated (specialized) cells are kept in equilibrium. Gene mutations leading to cancer alter this equilibrium between stem cells and more specialized ones. This disequilibrium results in excessive cellular proliferation and/or to lack of programmed cell death (apoptosis) thus leading to the formation of a heterogeneous cellular mass (benign tumor). Additional gene mutations underlie progression to more malignant tumor stages. Different cellular types will detach from the primary tumor, reflecting its heterogeneous cellular composition. Cancer stem cells, because of their plasticity and capacity to trans-differentiate, will successfully move across the body and invade distant organs by forming micro-metastases (enriched in cancer stem cells) and eventually macro-metastases which recapitulate the primary tumor in terms of cellular composition and heterogeneity.

To summarize:

- Tumour heterogeneity is not explained by current genetic models for tumour initiation and progression to malignancy and metastasis.
- CSCs arise from their normal counterparts within adult stem cell niches.
- CSCs represent a minor, though highly relevant, sub-population within the tumour mass.

Current status

An important area of research is tumour-specific CSC surface markers underlying molecular and cellular defects. The concept of cancer stem cells was first proposed more than a century ago but only recently has it gained new momentum with the latest advances in stem cell biology. Normal stem cells are commonly defined by two main intrinsic features: the capacity of perpetuating themselves (through self-renewal), and that of acquiring and exerting specialized

functions (through differentiation). Self-renewal and differentiation are inherent to the stem cell's ability to divide 'asymmetrically', meaning that each cellular division can generate both pluripotent (stem-like) and committed (differentiated) daughter cells in a strictly regulated fashion.

Apart from their embryonic counterpart, 'adult' stem cells exist, albeit at low level, in virtually all organs of our body and play essential roles in preserving and replenishing tissues in our adult body. Moreover, specific stem cell niches are expanded upon hormonal stimuli (e.g. the mammary gland during pregnancy) or recruited to repair tissue damage (e.g. during wound healing). Within these adult stem cell niches, it is of utmost importance that self-renewal and differentiation, but also cell migration and programmed cell death, are strictly kept in equilibrium so as to avoid either cellular loss (tissue waste due to more differentiation than self-renewal) or gain (tissue growth or neoplasia, due to more self-renewal than differentiation).

Cancer stem cells are likely to arise from their long-lived normal counterparts through muta-

Cancer Type	CSC-specific markers	Reference
Leukemia	CD34 ⁺ /CD38 ⁻	<i>Nat Med</i> 3 : 730 (1997)
Breast	CD44 ⁺ /CD24 ^{low lin-}	<i>PNAS</i> 100 : 3983 (2003)
Brain	CD133 ⁺	<i>Nature</i> 432 : 396 (2004)
Myeloma	CD138 ⁻	<i>Blood</i> 103 : 2332 (2004)
Prostate	CD44 ⁺ /α ₂ β ₁ ^{hi} /CD133 ⁺	<i>Cancer Res</i> 65 : 10946 (2005)
Lung	Sca-1 ⁺ /CD45 ⁻ /Pecam ⁻	<i>Cell</i> 121 : 823 (2005)

Tab. 1 Prospectively identified cancer stem cells and the corresponding markers

tion events affecting signal transduction pathways (e.g. Wnt, Hedgehog, and Notch) known to regulate this finely regulated balance. The latter can be achieved, for example, by simply favouring symmetric vs. asymmetric cell division or by insensitivity to growth-inhibitory signals from the surrounding stem cell niche.

Hence, according to the CSC model, a small sub-population of cells retains stem-like properties and is responsible for tumour growth (by self-renewal) and heterogeneity (by differentiation). Experimental evidence for the existence of cancer stem cells has been delivered for several tumour types including leukaemias, multiple myeloma, breast, brain, prostate and lung cancer (table 1). In this approach, human tumours are dissociated to single-cell suspensions, sorted by different cell surface markers, and transplanted into immunodeficient (NOD/SCID) recipient animals.

Cancer stem cells are hereby defined by their capacity to recapitulate tumorigenesis even when transplanted at low multiplicity in an experimental model. For example, 1 in 10⁵ acute myeloid leukaemia (AML) cells express the cell surface markers CD34⁺CD38⁺ and can recapitulate the histological heterogeneity of the disease when transplanted in SCID animals, whereas CD34⁺CD38⁺ leukaemic cells cannot, even at higher multiplicities. Likewise, human breast cancers encompass a subpopulation (1-10 per cent) of CD44⁺CD24^{low lin-} cells. These putative breast CSCs can form tumours in NOD/SCID mice when as few as 200 cells are transplanted. Notably, many cell surface markers are

shared by CSCs isolated from different cancer types, possibly indicating activation of common signal transduction pathways. Deregulation of the Wnt signal transduction pathway for example, has been shown to be a very early event in colon, breast, skin and haematopoietic malignancies and is likely to activate self-renewal and ‘stemness’ in the corresponding tissue-specific niches^(4, 5)

To summarize:

- Self-renewal and differentiation represent the main CSCs’ features: the former drives tumour formation and growth whereas the latter underlies tumour heterogeneity.
- Deregulation of signalling pathways known to modulate self-renewal and differentiation during development and in adult stem cell niches underlies CSC onset and invasive behaviour.
- Cell-sorting by specific combination of cell surface markers and transplantation in immune-deficient mice has allowed the prospective identification and isolation of CSCs from different human cancer types.

Prospects

Research on cancer stem cells offers prospects for improvements in cancer prognosis and treatment. The demonstration of the existence in several human malignancies of a minority of cancer stem cells capable of reproducing the heteroge-

neity and malignancy of the human disease in experimental animals has several implications for cancer prognosis and treatment. Expression profiling is currently regarded as the most promising tool for cancer prognosis and response to treatment. However, if tumour onset and invasive behaviour are driven by a minority of CSCs, their relative number within the tumour mass may correlate with the patient's risk of developing local and distal metastases. Hence, expression profiling of total tumours by microarrays encompassing the complete set of human genes is unlikely to detect CSC-specific expression signatures as they are diluted within a majority of heterogeneous cellular types. Likewise, whole tumour expression profiles are likely to be of less value to design tailor-made therapeutic approaches.

The identification of CSC-specific signatures by expression profiling of purified CSCs will allow their supervised bioinformatic analysis from whole tumour profiles and the refinement of clinical prediction of metastatic behaviour and response to treatment. Also, it will facilitate the identification of novel CSC-based therapeutic targets. Upon conventional chemotherapy or radiation therapy, tumour shrinkage is likely to result from death of differentiated tumour cells. However, if CSCs represent a minority of cancer cells and if, as reported in the literature, they are characterised by an intrinsic resistance to radiation and chemical agents, they are likely to escape adjuvant therapy and underlie cancer relapse. Therefore, the development of therapies specifically targeted against CSCs is likely to result in considerable improvement of the cancer patient's long-term survival.

The cancer stem cell hypothesis also suggests novel prospects for the detection of circulating cancer cells after surgical removal of the primary malignancy. In general, circulating cancer cells in blood and bone-marrow are known to be present at various multiplicities in cancer patients. Circulating cancer cells however, will reflect the heterogeneity of the primary tumour mass and only a minority will succeed in the metastasis of a distal organ. CSCs, because of their intrinsic plasticity and capacity of transdifferentiate upon stimuli from their direct environment, represent a clinically highly relevant sub-population of migrating cancer cells capable of reproducing

the primary lesion at distal locations. The identification of CSC surface markers for specific cancer types (**table 1**) will allow the detection and quantification of migrating CSCs in body fluids, blood and bone marrow, and guide post-surgical clinical management and surveillance of the cancer patient.

To summarize:

- Whole tumour expression profiles are likely to encompass heterogeneous cellular types thus possibly masking the clinically relevant CSC sub-population.
- Definition of CSC-specific gene signatures will improve our capacity to predict cancer prognosis and response to treatment based on expression profiles of whole tumours.
- The same CSC expression profiles will facilitate the identification of therapeutic targets for tailor-made intervention.

Conclusions

The cancer stem cell hypothesis represents a truly innovative concept in cancer biology with profound fundamental and clinical implications. Research on cancer stem cells will run in parallel with the research on normal embryonic and adult stem cells. Central to both lines of investigation is the identification of cell surface markers for their prospective identification from healthy and diseased tissues. The subsequent molecular characterisation of the purified stem cells by genomic and proteomic analysis will lay the foundation for the elucidation of the molecular and cellular mechanisms that regulate self-renewal and differentiation in homeostasis and cancer. These advances in our understanding of cancer stem cell biology will open new avenues for the improvement of cancer risk assessment, prognosis, surveillance, prevention and targeted therapy. To this end, both fundamental and applied research should be encouraged, combining genetic, cellular and molecular analysis of *in vitro* and *in vivo* CSC experimental models with the prospective detection, purification and analysis of CSCs from surgical specimens and body fluid from cancer patients.

Repairing the damaged heart: Stem cell research in acute heart attack and chronic coronary artery disease

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Introduction

Therapeutic successes in the area of stem cell research have opened up many new avenues for treating cardiovascular diseases, especially with respect to the prevention of the development of cardiac failure due to acute heart attack (acute myocardial infarction caused by sudden and prolonged oxygen starvation) or chronic coronary artery disease (gradual oxygen starvation due to cumulative narrowing of the coronary artery). In Germany alone, nearly 330,000 people per year suffer an acute myocardial infarction – classic heart attack. Currently the delivery of bone marrow-derived stem cells *via* the coronary artery (intracoronary), the left ventricle (transendocardial) as well as directly into the heart muscle during cardiac bypass surgery (intramyocardial) is being investigated for the treatment of acute heart attack and chronic coronary artery disease.

All application modes pursue the same objective of regenerating damaged myocardium (heart muscle), whether it be *via* the transdifferentiation of autologous (self-derived) bone marrow-derived stem cells into cardiac cells, *via* the cytokine-mediated repair of dying cardiac muscle cells, or *via* the enrichment of endogenous cardiac stem cells in the infarcted cardiac muscle. These differing mechanisms of action may play very different roles in various cardiac diseases, a fact that can have a decisive effect on the individual nature of their treatment.

Current status

Bone marrow-derived stem cells are able to divide and – depending on their environment – transform into various functional cardiac cells (e.g. endothelial cells, smooth muscle cells and cardiomyocytes – heart muscle cells), though such an effect may only occur at a low level, according to the latest scientific research – a point that is still debated. Despite that, the therapeutic application of stem cells, and the release of their intracellular cytokines and growth factors results in preventing the cardiac cells at the border zone of the infarction from undergoing “apoptotic” cell death. Furthermore, through the local application of stem cells one can achieve a high concentration of stem cell-derived cytokines in the infarcted zone. Cytokines then regulate the migration of endogenous cardiac stem cells from their niches in the heart (e.g. from the atrium and left ventricular apex) to the damaged areas of myocardium.

The concept of regeneration was first confirmed *via* an *in vivo* mouse model of cardiac infarction in 2001⁽¹⁾ by microscopically studying stained sections of heart (a well-established technique known as histology). The old dogma that the heart lacks an intrinsic repair mechanism was made redundant, and the concept of muscle-growth (neomyogenesis) and vessel-growth (neovasculogenesis) was confirmed. In this context, numerous animal experiments and a lesser number of clinical studies in humans suffering from acute heart attack or chronic coronary artery disease have been performed. These studies confirm the concept of myocardial regeneration after application of autologous bone marrow-derived stem cells, using several investigative methods: histologically, immunohistologically (using antibodies to stain tissue),

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2. Strauer BE, Brehm M, Zeus T, *et al.* (2001) Myocardial regeneration after intracoronary transplantation of human autologous stem cells following acute myocardial infarction. *Dtsch med Wschr.* 2001; 126:932-938.

molecular-biologically and finally clinically. However, the molecular-biological and cellular-biological mechanisms are still a matter of intense research, and the *in vitro* and *in vivo* monitoring of applied bone marrow-derived stem cells are of particular interest in better understanding the regenerative processes that occur within the cardiac muscle.

- Bone marrow-derived cells can differentiate into cardiac specific cells (e.g. endothelial cells, smooth vascular muscle cells and cardiomyocytes).
- Observed repair to damaged myocardium could be due to such transdifferentiation, as well as the migration and differentiation of resident cardiac stem cells in response to factors released by the newly introduced cells.
- Various clinical studies have confirmed that autologous bone marrow-derived stem cells taken from the patient herself/himself can repair the damaged heart, hence improving cardiac function, perfusion and metabolism in a number of cardiac disease states.

Prospects for research

In treating cardiac diseases it is important to evaluate three different approaches for applying stem cells to achieve myocardial repair: (I) autologous bone marrow-derived stem cell transplantation *via* different application routes, preferably by the intracoronary technique, (II) stimulated myogenesis and vasculogenesis from endogenous resident cardiac stem cells or progenitor cells, and (III) cytokine-mobilized bone marrow stem cells that home in on the damaged myocardium. Acute heart attack and chronic coronary artery disease have benefited from autologous bone marrow-derived stem cell transplantation.

Most progress has been made with acute heart attack, and has proceeded without any major complications over the last five years. Strauer *et al.* were the first to inject autologous bone marrow-derived stem cells during routine catheterization in a reopened coronary artery (left anterior descending coronary artery, LAD) in a patient with anterior wall infarction in March 2001. The outcome was a definitive recovery in anterior wall motion, anterior wall perfusion and

global pump function of the heart.⁽²⁾ In larger clinical studies a benefit of using autologous bone marrow cells for patients with acute infarction has been confirmed both after 3 months and after 24-36 months. In a small minority of studies this effect was not manifested so clearly: here the cells were given during the acute phase within the first 24 hours after the acute infarct, at a time when the degradation of the dying cardiomyocytes was in full swing, and the inflammatory response has to be taken into account.

Differing results have been obtained in the long-term follow up after stem cell transplantation. In one study, an improved pump function was recorded in the majority of patients, persisting even after 3 years. In another study carried out by magnetic resonance tomography (MRT), only a temporary effect was recorded after 3 months, and after 12 months there was no improvement compared to the non-cell transplanted control group. However, in one multi-centre double-blind placebo-controlled study a significantly improved regional and global pump function of the heart (as measured *via* left ventricular angiogram) was recorded after 4 months in the stem cell-treated patient group compared with the control group (which received no stem cells).

The great benefit of bone marrow derived stem cell therapies for myocardial repair is not merely the replacement of damaged heart tissue (e.g. cardiomyocytes, endothelial cells and smooth muscle), but also the functional working together of the new heart muscle cells with the existing tissue. Bone marrow-derived stem cells may indirectly mediate additional beneficial effects through the stimulation of local, resident, cardiac stem cells. Transplanted bone marrow-derived stem cells can produce cardiostrophic cytokines (growth factors, chemoattractant factors), which may support the survival of dying heart muscle cells or modulate the immune response that occurs upon damage to the heart.

An attractive alternative to transplantation of autologous bone marrow-derived stem cells is to activate the differentiation of resident endogenous cardiac stem cells or cardiac progenitor cells into new cardiac cells. Resident stem cells are found in so-called "niches" in the cardiac tissue. Such an approach would have the advantages of being potentially non-invasive, and would only use the patient's own resident stem cells. At

first glance this may appear extremely challenging, since it involves several steps, including cell migration, proliferation and differentiation, all of which would have to function for a successful therapy to occur. However, it certainly appears that the adult heart muscle has the capacity to repair itself. In experimental studies it has been demonstrated that resident cardiac stem cells can be attracted by certain cytokines (cardiotrophic factors) when injected into the heart.

Perhaps the least challenging approach to therapeutic myogenesis (muscle (re)generation) is to enhance the process by mobilizing bone marrow stem cells with the help of systemic application of so-called chemoattractant factors such as G-CSF or GM-CSF. This increases the mobilisation into the peripheral blood of haematopoietic stem cells, mesenchymal stem cells and cardiac committed progenitor cells from the bone marrow. The circulating stem cells can then migrate into the damaged area of the heart. Such an approach – which may indeed prove beneficial in treating heart diseases – was first put forward by Anversa and coworkers.⁽³⁾ The systemic application of chemoattractant factors (chemokines) such as the mobilizing granulocyte colony stimulating factor (G-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF) promoted (i) the migration of bone marrow-derived stem cells into the infarcted heart muscle, and (ii) the proliferation of bone marrow-derived stem/progenitor cells in the heart muscle for repair and functional recovery of the heart after a heart attack. Moreover, in human studies only a slight beneficial effect after G-CSF injections has been demonstrated in patients with acute heart attack, although some investigators have demonstrated an increase in re-narrowing in the infarct-related artery that is not insignificant. It is important to point out here, however, that myocardial replacement might involve regions where muscle growth (myogenesis) does not occur at appreciable levels under normal conditions.

- Several studies have demonstrated that the transplantation of autologous bone marrow-derived stem cell is beneficial in acute heart attack and in chronic coronary artery disease.
- Stem cell-derived heart muscle cells and endothelial cells reduce clinical symptoms such as angina pectoris and dyspnoea (diffi-

cult or laboured breathing), and increase daily activity as well as quality of life in humans with heart diseases.

- Pharmaceutical stimulation of heart muscle growth from endogenous cardiac stem cells may offer a non-invasive approach for myocardial repair.
- Cytokine-induced mobilisation of stem cells from the bone marrow may offer an alternative technique for myocardial regeneration, but the possibility of a clinically relevant coronary re-narrowing currently weighs against such an approach.

Problems, concerns and open questions

The main challenge for developing any stem cell based therapy for heart diseases is the control of cell migration, proliferation and differentiation *ex vivo* as well as *in vivo*, i.e. the achievement of a high proportion of cardiac committed progenitor cells without any other unwanted cell types. This is important for improving the clinical effect achievable to date, and also for transplanting these cells into elderly patients who have a reduced stem cell reservoir in their bone marrow. The evaluation of the experimental and clinical effects in animal models or clinical trials is difficult, because different mechanisms are involved in the different myocardial diseases.

- Cardiac stem cell differentiation needs to be controlled in order to ensure that the desired cell types are generated.
- Generation of unwanted cell types must be prevented.
- Clinical studies for tracking stem cells to evaluate their cellular fate (exact location and identity) are important.

Conclusions

The once conventional dogma that the heart does not have the capacity to repair itself is now redundant. Several clinical trials have demonstrated the beneficial effect of replacement ther-

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apy after stem cell transplantation in coronary artery disease.⁽⁴⁾ Additional heart diseases may be treated with increased knowledge of stem cell/progenitor cell migration processes, proliferation

pathways, together with the realisation of cell fusion processes and the effects of growth hormones and other chemical signals released in the neighborhood of the regenerating cardiac tissue.

Stem cell research in vascular endothelia

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Introduction

The inner lining of the vessel wall, the endothelium, plays a crucial role in prevention of atherosclerosis and the initiation of new blood vessel formation after vessel blockage and oxygen starvation to a tissue (ischaemia). The integrity of the endothelial monolayer appears to be maintained by circulating endothelial progenitor cells, which accelerate repair of the lining (re-endothelialisation) and limit atherosclerotic lesion formation. Circulating progenitor cells additionally home in on sites of injury, and contribute to new blood vessel formation.

Current status

Stem/progenitor cells in atherosclerosis

The integrity and functional activity of the endothelial monolayer plays a crucial role in the process of atherosclerotic lesion formation. Injury to the endothelial monolayer by mechanical removal of the endothelium (e.g. by expanding the vessel with a balloon catheter or inserting a semi-rigid stent), or inflammatory activation of the endothelial cells, induce a cascade of pro-inflammatory events resulting in infiltration of immune cells and smooth muscle cell proliferation. These processes culminate in the formation of atherosclerotic lesions, may result in plaque rupture and finally myocardial infarction (acute heart attack), which is still the leading cause of death in the western world. The maintenance of

the endothelial integrity, therefore, is of crucial importance to prevent the initial processes in acute myocardial infarction and coronary heart disease.

The turn-over of endothelial cells was previously believed to be very low. However, increasing evidence suggests that risk factors for coronary artery disease increase the programmed death (apoptosis) of endothelial cells (EC), thereby leading to a disturbance of the endothelial monolayer. Recent insights additionally suggest that the injured endothelial monolayer may be regenerated by circulating endothelial progenitor cells⁽¹⁾. Accordingly, implanted Dacron grafts were shown to be rapidly recovered by a certain population of bone marrow-derived haematopoietic (blood-forming) stem cells.

In humans, the surface of devices inserted into the left ventricle to assist heart function can become covered by an even more immature subset of such cells. Additionally, several studies have demonstrated that circulating endothelial precursor cells can home in on denuded parts of the artery after balloon catheter injury. The incorporated cells have been shown to derive from the bone marrow. Enhanced incorporation of certain bone marrow-derived cells is associated with accelerated endothelial repair, and a reduction in the re-narrowing of vessels following clinical intervention. Overall, these studies implicate circulating endothelial progenitor cells in significantly contributing to re-endothelialisation.

Of note is the following observation: the number of circulating endothelial progenitor cells, which might have an anti-atherogenic activity, is significantly down-regulated in patients with coronary artery disease. Classic risk factors for atherosclerosis such as age and diabetes reduced the number of circulating progenitor

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cells indicating that individuals at risk for coronary artery disease have an impaired endothelial repair capacity. This hypothesis is supported by recent findings demonstrating that measurement of circulating endothelial progenitor cells predicts the prognosis of patients with coronary artery disease ⁽²⁾.

- Circulating bone marrow-derived cells can contribute to regenerating the endothelial monolayer after injury, and improve vascular healing.
- Patients with coronary artery disease show an impaired number and function of circulating endothelial progenitor cells.
- Quantitative measurement of circulating endothelial progenitor cells may be useful in monitoring patients at risk of atherosclerosis.

Prospects

Based on the preliminary findings that circulating endothelial progenitor cells contribute to endothelial repair processes and have an anti-atherosclerosis effect, one may envisage defining therapeutic strategies to increase the number of these circulating progenitor cells. Moreover, the measurement of these cells may be used as “cellular” biomarker to predict the prognosis of patients at risk for coronary artery disease.

Problems, concerns and open questions

Ongoing research programmes are addressing the following questions related to the identification and contribution of endothelial progenitor cells in atherosclerosis:

- Characterisation and identification of endothelial progenitor cells: Various studies provide compelling evidence that endothelial progenitor cells can be derived from haematopoietic stem cells expressing the marker proteins CD133 or CD34, and used to identify and measure endothelial progenitor cells in humans (e.g. CD34/KDR, CD133/KDR). However, that does not exclude the presence of endothelial progenitor cells deriving from

other sources within the bone marrow (e.g. mesenchymal stem cells, SP cells) or from tissue resident stem cells. Moreover, myeloid intermediates (cells that mature into white blood cells) might have the capacity to contribute to endothelial repair. Definition and characterisation of these endothelial progenitor cells is ongoing. ⁽³⁾

- The double-edged role of endothelial progenitor cells: By improving neovascularisation (formation of new blood vessels) endothelial progenitor cells may also contribute to blood vessel formation in atherosclerotic plaques, thereby potentially destabilising the plaques – a negative consequence. Although, clinical trials and animal data do not support a profound pro-atherosclerotic effect of endogenous or applied progenitor cells, careful evaluation of putative side effects have to be considered in future studies.

Stem/progenitor cells in new blood vessel formation

Experimental studies indicate that circulating endothelial progenitor cells home in on sites of injury and contribute to neovascularisation, thereby, facilitating blood supply to the tissue and supporting repair processes. Consistent with these observations, infusion of progenitor cells from different sources (e.g. peripheral blood, bone marrow, vessel-associated cells, fat tissue, heart tissue etc) has been shown to improve the recovery after ischaemia (blood-starvation of a tissue). Based on these experimental findings, clinical phase I trials were initiated in 2001 to test whether cell therapy may exert a beneficial effect in patients with acute myocardial infarction or peripheral vascular disease ⁽⁴⁾. These initial clinical pilot trials indicated that infusion (by catheter-based technology) or injection of bone marrow-derived or circulating blood-derived progenitor cells improves the blood supply to the heart or to the legs in patients with ischaemia. The consequences are improved heart function or longer pain-free walking distance, respectively. Meanwhile, these initial clinical studies have been confirmed by larger randomised double-blind trials, confirming an effect of cell therapy on the blood supply to the heart.

Whereas an increase of new blood vessel formation is an important treatment option for patients with acute or chronic ischaemia,

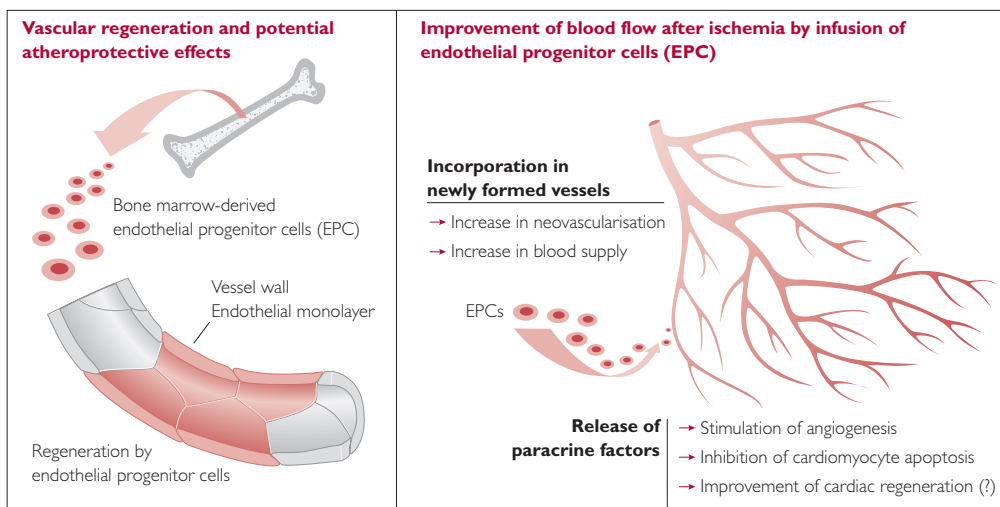


Fig. 1 Putative therapeutic benefits of EPC in the cardiovascular system

an enhancement of blood vessel formation in tumours by circulating progenitor cells may prove detrimental. Interestingly, studies in mice lacking the capacity to mobilise endothelial and haematopoietic stems have revealed reduced tumour growth, supporting the concept that circulating progenitor cells facilitate blood vessel formation in tumours, and hence tumour growth. Although the contribution of progenitor cells to the tumour vasculature is variable, blocking the recruitment of progenitor cell mobilisation or recruitment might be a novel therapeutic option for cancer treatment.

- Infusion of bone marrow-derived or circulating blood-derived progenitor cells improves the formation of new blood vessels and blood supply to ischaemic tissues in experimental studies and clinical phase II/III trials.
- The contribution of endothelial progenitor cells in blood vessel formation in tumours may depend on the type of tumour. The measurement of circulating endothelial progenitor cells might be useful for monitoring responses to anti-tumour therapy.

Prospects

The use of cell therapy for the treatment of ischaemic diseases is under clinical investigation and needs to be confirmed in larger scale clinical trials with hard end-points. So far only bone marrow-derived or circulating blood-derived

progenitor cells have been used for therapeutic angiogenesis (blood vessel formation) studies in patients. Additional adult cell stem/progenitor cell populations will be tested in comparison, in order to define the most efficient stem/progenitor cell for treatment of ischaemic disease. Enhancement strategies to increase cell number (e.g. improvement of cell survival, proliferation), cell homing (e.g. increasing adhesiveness of the cells to the target tissue), and priming of the target tissue to facilitate cell up-take, may be developed to improve cell therapy strategies.

Ample evidence suggests that circulating progenitor cells play a role in blood vessel formation (neovascularisation) in tumours⁽⁵⁾. Further studies are required to clarify whether interference with circulating progenitor cell mobilisation or recruitment might be useful as a therapeutic approach. Alternatively, the measurement of circulating progenitor cells might be suitable for monitoring the responses of individuals after anti-tumour therapies⁽⁵⁾.

Problems, concerns and open questions

The major obstacle at present relates to the huge differences in incorporation rates of circulating progenitor cells in the vascular endothelium. Experimental studies have described an endothelial incorporation rate of bone marrow-derived progenitor cells from 0 to 100 per cent of newly formed blood vessels in ischaemia and tumour models. The extent of incorporation

5. Schneider, M., Tjwa, M. and Carmeliet, P. (2005) A surrogate marker to monitor angiogenesis at last. *Cancer Cell*, 7, 3-4.

may crucially depend on the experimental model (e.g. extent of injury, tumour type etc) and the source of progenitor cells used. However, the therapeutic effect of cell therapy of ischaemic diseases may not only depend on the physical incorporation of the cells into the endothelial lining. It might also be due to growth factors released by the progenitor cells, which in turn promote the formation of new blood vessels and tissue repair in a paracrine manner (i.e. acting locally on neighbouring tissue).

Progenitor cells also can be incorporated in the vessel wall (perivascular localisation) rather than in the endothelial monolayer, thereby supporting vessel stability and maturation. It is unclear at present whether the improvement in new blood vessel formation induced by stem/progenitor cell therapy indeed depends on the “stemness” of the cells or might also be achievable by other cell types, including those that give rise to white blood cells (myeloid lineage).

Stem cell research in Epithelia

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Introduction

Regenerative medicine, which is aimed at the permanent restoration of damaged tissues and/or organs, is tightly linked to stem cell biology. Regenerative medicine includes cell therapy (a clinical setting where stem cells are isolated, usually cultivated and transplanted back onto patients) and gene therapy (a clinical setting where stem cells are also genetically modified to cure a genetic disease). Self-renewing tissues (such as blood and epithelia) contain a population of stem cells that are responsible for their generation and continuous regeneration. The entire integrity of the tissue and its repair depend on these cells.

Current status

Exceptional progress has recently been made in understanding how epithelial stem cells develop into tissues such as skin. A type of adult stem cell from the epidermis (the outer protective layer of our skin, which possesses no blood vessels), and known as a “holoclone”, has already shown great therapeutic promise. Firstly, holoclones are multipotent – i.e. they can (re)generate all of the cell types of their tissue of origin. Furthermore, they are able to restore permanently the epithelium when grafted onto patients with massive epithelial damage/defects. This is partly a product of their phenomenal self-renewal properties and resilience: human holoclones can be rescued from skin and regenerated years after the original grafting. These very special cells can resist the normal process of chromosome shortening that leads to ageing in normal cells, and they have a

tremendous capacity to proliferate. A single epidermal holoclone can double enough times to produce the skin surface area of an adult human being (8×10^{10} cells). Under appropriate conditions, such human keratinocyte stem cells (which give rise to the tough layer of keratin in our skin) can thus be maintained in culture and are currently used in many cell therapy protocols, as outlined below.

Human epidermal keratinocytes can be grown in the lab to give sheets of so-called stratified epithelium (the outer layer of our skin), these have the characteristics of real skin. Keratinocytes cultured from the particular patient to be treated (so-called autologous keratinocytes) have been used worldwide to regenerate a functional epidermis in patients suffering from massive full-thickness burns. Human epidermis is renewed monthly. Permanent epidermal regeneration has been achieved in these patients – over 20 years follow-up, hence over 200 renewing cycles. This technology has proven to be life-saving.

The corneal epithelium of the eye has yielded a great wealth of information about keratinocyte stem cells. Depending on their exact location in the cornea or adjacent tissue, they have different characteristics and properties. So-called transiently amplifying (TA) cells continuously migrate into the cornea from locations sometimes millimeters away, the equivalent of pushing one's way from one end of a crowded football stadium to the other – without legs!

Chemical burns to the eye result in a loss of a special group of cells at the boundary between the cornea (clear part) and the sclera (white part) of the eye – so-called limbal cells. Surprisingly

the damage is repaired by cells from a region of the conjunctiva (the thin membrane covering the external surface of the eye). This “abnormal” wound healing causes formation of new blood vessels, chronic inflammation and scarring; this can seriously reduce the sight of the affected eye, and even cause complete blindness. Allogeneic (from non-self donors) corneal grafts are not successful in these patients unless limbal cells taken from the uninjured eye are grafted at the same time. Such an approach has been successful (longest follow-up: five years post operation) in giving back normal vision to patients who could not have been helped by conventional surgery.

The penile urethra, the tube in which urine flows through the penis, is lined with a multi-layered epithelium. In a congenital condition known as “hypospadias”, the urethra ends either on the ventral surface of the penis, and in extreme cases (around 20 per cent) at its base, i.e. the penile urethra is totally absent. Treatment requires reconstruction of the penile urethra, usually with autografts (grafts of tissue from the patient himself) made by folding back adjacent skin, or transferring skin taken from the foreskin, other parts of the body or even bladder lining. This is sometimes problematic, leading to hair growth, sebaceous secretion, calcification, and even openings in the urethra. However, epithelial cells taken from the end of the urethra can be grown in the lab and then used to rebuild the missing section of urethra. Fourteen years after such treatment, the urethral epithelium of such patients continues to be normal. This shows that even when the end of the urethra is incorrectly located; it still contains stem cells capable of permanently regenerating the epithelium at a different site.

Patients with non-worsening vitiligo or piebaldism (skin with large irregular patches of lacking pigmentation) have been successfully treated with their own melanocytes (pigment producing skin cells) grown in the lab. When a culture of the patient’s melanocytes mixed with keratinocytes is applied to a shallow wound made in an affected area of skin, the healed epidermis becomes populated by melanocytes and these melanocytes persist for at least seven years. Normal human melanocytes alone grow and divide poorly in culture, but when grown together with keratinocytes they produce large enough numbers to transfer them to the large areas affected by vitiligo. Thus, keratinocytes

play an indispensable accessory role in the restoration of the pigmentation.

- Stem cells capable of being grown healthily to enormous numbers in the lab can be obtained from various epithelial tissues.
- There is substantial evidence that cultured epithelial stem cells can regenerate/repair a tissue when transplanted back to patients.
- Frequently this approach achieves results unattainable by conventional grafting surgery, and can be life-saving (e.g. in burn victims).

Prospects

The natural development of the research on keratinocyte stem cells would be: (i) to use them in cell therapy procedures aimed at the regeneration of other stratified epithelia e.g. mouth, bladder, vagina and rectum; (ii) to develop ways of repairing genetic defects in the cells before returning them to the patient (so-called *ex vivo* gene therapy), hence treating genetic epithelial diseases, such as Epidermolysis Bullosa (EB). EB is a group of devastating inheritable disorders characterised by fragile skin (at a deep level). There are broadly speaking three types of EB, depending on the level at which the lack of tissue integrity occurs. Genetic analysis has led to the identification of mutations in at least 10 distinct genes expressed within the cutaneous basement membrane, largely explaining the spectrum of severity observed in EB. Inherited EB affects approximately 30,000 individuals in Europe and about 400,000-500,000 people worldwide. The severity of the clinical manifestations depends on the type of EB, and can include continuous blistering of the skin as a result of minor trauma or temperature change, nail loss, alopecia (hair loss), milia (white bumps on the skin), blistering in or around the mouth and throat, oesophageal lesions and respiratory difficulties. Mortality is as high as 87 per cent in the first year of life for infants with the lethal form of juvenile EB. No cures for EB have been developed so far, and currently a phase I/II clinical trial aimed at the *ex vivo* gene therapy of juvenile EB is ongoing. The goal is to validate *ex vivo* procedures for genetic correction of epidermal stem cells in a clinical setting, and to analyse critical concerns such as (i) the overall safety of the treatment, (ii)

the long-term survival of the genetically modified cells, (iii) immune responses against the new (repair) gene product, (iv) the persistence of expression of the new gene. The application of gene therapy protocols to limbal keratinocytes in the eye could lead to the correction of genetic disorders affecting the corneal surface, such as corneal dystrophies (developmental disorders).

- Keratinocyte stem cells could well be used to repair/regenerate other multi-layered epithelia.
- The combined approach of gene therapy and stem cell therapy holds great promise for treating at least one severe disorder of the deep skin in infants.
- In the future, developments in genetic modification of keratinocyte stem cells could open up new perspectives in other fields of regenerative medicine.

Problems, concerns and open questions

It has been about 30 years since the discovery of a method of artificially growing large number of human epidermal keratinocytes from a small skin biopsy. The procedure was often highly successful, but the failure rate remained high. Success depends firstly on the quality of the cultures used to prepare the grafts. This means that the cultures should contain a sufficient number of the keratinocyte stem cells essential for long-term epidermal renewal. Once this criterion is met – and only then – success rests in the hands of the surgeon. In the absence of an adequate number of stem cells, failures of epidermal regeneration are inevitable, and will entail not only suffering of the patients and possible loss of life, but also general confusion as to what results are to be expected.

In the field of pharmaceuticals products, quality and safety requirements are often laid down by agencies. This is understandable, since a drug is usually a well-defined chemical product. A living cell, however, is a complex entity. Hence, in the field of regenerative medicine, quality and safety should be considered separated issues. Obviously it is undesirable to transmit diseases or to expose patients to toxic compounds through cell cultures, so appropriate safety controls are manda-

tory. However, appropriate quality controls that ensure the preservation of stem cell characteristics in culture should be compulsory as well. Safety controls can be identical for the cultivation of all types of cells, but different quality controls must be developed for different cell types.

The cultivation of keratinocyte stem cells by newly established culture facilities, the proposal of a new culture system and/or of a new carrier for autologous keratinocytes destined to permanent restoration of massive epithelial defects, should be dependent on: (i) the direct demonstration of the presence of holoclones in culture, (ii) the periodical clonal analysis of a reference strain of keratinocytes (both in terms of ability to replicate unchanged, and in growth potential), (iii) the evaluation of the percentage of aborted colonies during cultivation, (iv) when applicable (as in the case on human cultures from corneal tissue), evaluation of the expression of specific holoclone markers. These basic “quality controls” should eliminate one important hitherto uncontrolled variable in the evaluation of the clinical performance of epithelial cultures.

- Safety of stem cells is a pre-requisite for their application in medicine, but quality is equally important, and this measure must be controlled in ways specific to particular cells.
- New culture facilities and methods must pass such controls first before they serve clinical applications.

Conclusions

Epithelium-derived stem cells have already demonstrated their value in repairing congenital defects and injuries, some of which are completely beyond the reach of conventional tissue/cell grafting. Proof of principle is currently being tested for *ex vivo* gene therapy using stem cells, i.e. genetically repairing the patient's defect in his/her own cultured cells, before grafting them back in order to repair the lesion. The prospects for increased application in the areas already addressed, and in other areas of epithelial tissue (re)construction, are good. To ensure safety to patients, cell type-specific quality controls should be imposed by regulatory agencies, and new culture facilities and methods should be scrutinised carefully according to these controls.

Stem cell research in brain and nervous system

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Introduction

Research on stem cells in the developing and adult nervous system is a very active research field. Many research groups study the development of the nervous system, and the realisation of the maintenance of stem cells in the adult brain and the continuous generation of neurones has attracted increasing interest the last decade. There are two conceptually different ways one may envisage utilising stem cells for neural repair: transplantation of stem cell derived cells or stimulation of neurogenesis from endogenous stem cells. These are complementary approaches, the simultaneous pursuit of which is expected to produce synergies. Furthermore, it is likely that different diseases may be treated utilising different strategies (using transplanted cells and/or endogenous potential).

Current status

The nervous system is formed as a hollow tube, initially made out of neural stem cells capable of generating both neurones and specialised supporting cells. Neural stem cells are immature cells that have the potential to generate the main cell types of the central nervous system: neurones, astrocytes and oligodendrocytes.^(1, 2) Another key feature is their capacity to divide to give rise to new stem cells, i.e. self-renewal capacity, thus enabling the persistence and activity of the system over long time. The neural stem cells also give rise to a variety of non-neural cells such as, for example, muscle cells, cartilage cells, bone cells and pigment cells

during early development by emigration of so called neural crest cells from the neural tube. A large body of knowledge regarding the function of different cell types and the molecular control of stem cells during nervous system development has been gained from the studies of, for example, fruit flies, mice, monkeys and human embryos. To date we have substantial knowledge regarding the development of neural stem cells and what signals that control their generation of a variety of different cell types. Some of the discovered developmental signals have successfully been imposed on embryonic stem cells in culture to give rise to desired cell types that have proved functional after transplantation in animal models of human neurological diseases. The study of nervous system development must thus be seen as very successful in terms of aiding in the development of cell transplantation strategies (see further below).

It was not until the 1990s, with the introduction of novel techniques and the unequivocal demonstration by many laboratories of new neurones produced in the adult brain that the dogma of no neural regeneration after birth fell and the concept of adult neurogenesis gained full acceptance. Today we know that new neurones are generated throughout life in discrete regions of the brain. Most neurones, however, do not appear to turn over. In a seminal study in 1998 Eriksson and colleagues for the first time demonstrated neurogenesis in the adult human hippocampus by BrdU labelling, but the knowledge regarding the extent and potential affects on this process in pathologies is still very limited in man. The neurones generated in adulthood derive from stem or progenitor cells. Stem cells are notoriously difficult to identify because of their immature phenotype and lack of specific markers. Many studies have attempted to iden-

1. Gage FH. Mammalian neural stem cells. *Science* 2000;287:1433-1438.

2. McKay R. Stem cells in the central nervous system. *Science* 1997;276:6671.

tify stem cells in the adult brain, but much of the data are contradictory. This is largely due to the lack of methods to identify stem cells, and there is a need for the development of novel strategies for the visualisation of the distinct steps in a cellular lineage *in vivo*.

- Neural stem cells give rise to neurones and supporting cells.
- Understanding the development of the nervous system has helped direct the differentiation of stem cells for therapeutic applications.
- Neurones are generated from stem cells in discrete areas of the adult brain.

Prospects

There are two conceptually different routes for the use of stem cells for neural repair: cell transplantation and stimulated neurogenesis from endogenous stem or progenitor cells. Several neurological diseases have been suggested to benefit from cell transplantation, but most progress has been made in Parkinson's disease. Patients have been transplanted with grafts of ventral midbrain tissue from aborted human foetuses containing dopaminergic neurones, the predominantly affected neuronal type in Parkinson's disease, with promising results⁽³⁾. In some studies, the patients have benefited substantially from the grafts, whereas other studies have been less encouraging or even demonstrated negative side effects, hence indicating the need to develop the strategy further. Even when optimised, there are, however, substantial hurdles for the use of foetal grafts in terms of clinical feasibility. Stem cells could, in theory, be a source of unlimited supply of neurones for transplantation⁽⁴⁾. One could in this case consider several sources of stem cells, and the most studied thus far in such a context are embryonic stem cells (ES cells) and foetal neural stem cells. Studies in experimental animals using ES cells or foetal neural stem cells have lent support to the concept of stem cell based cell replacement therapy for Parkinson's disease⁽⁴⁾.

Perhaps the most intuitive benefit of stem cell based therapies for neural repair is the replacement of lost neurones. Neural stem cells may, however, mediate beneficial effects in indirect

ways by affecting the resident cells. Transplanted neural stem cells can produce neurotrophic factors, which may support the survival of neurones or have immunomodulatory effects. Moreover, stem cell transplantation in animal models of metabolic disorders suggests that the stem cells, or their progeny, can substantially reduce the accumulation of toxic products.

An attractive alternative to cell transplantation is to induce resident stem or progenitor cells to produce new cells. This approach would have the advantage of potentially being non-invasive and using the patients own cells, without the need for immunosuppression. This may at a first glance appear very challenging, as there are several steps, including cell proliferation, differentiation and migration, which would need to work. However, it appears that the adult brain may retain many of the necessary instructive signals. In addition to that, several commonly used pharmaceuticals prescribed in psychiatry actually stimulate neurogenesis, which may partially account for their therapeutic effect.

Perhaps the least challenging approach to therapeutic neurogenesis is to enhance this process in the normally neurogenic regions. Characterisation of the molecular pathways that normally control different steps in the generation of neurones in the adult brain have resulted in insights as to how this process can be enhanced. A dramatic indication that such an approach may indeed be beneficial in neurological pathology was first provided by Nakatomi et al. They suggested that the delivery of the mitogens epidermal growth factor (EGF) and fibroblast growth factor (FGF) promoted the proliferation of stem/progenitors by the lateral ventricle resulting in neuronal replacement and functional recovery after stroke⁽⁵⁾. Importantly, the neuronal replacement appeared to include regions where neurogenesis does not occur at appreciable levels under normal conditions.

- Several studies suggest that cell transplantation may be beneficial in Parkinson's disease.
- ES cell derived neurones reduce symptoms in animal models of neurological disease.
- Pharmaceutical stimulation of neurogenesis from endogenous stem cells may offer a non-invasive approach for neural repair.

3. Björklund A, Lindvall O. Cell replacement therapies for central nervous system disorders. *Nature Neuroscience* 2000;3:537-544.

4. Lindvall O, Kokaia Z, Martinez-Serrano A. Stem cell therapy for human neurodegenerative disorders-how to make it work. *Nat Med* 2004;10 Suppl:S42-50.

5. Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O, Kawahara N, et al. Regeneration of hippocampal pyramidal neurones after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell* 2002;110:429-441.

Problems, concerns and open questions

The main challenge for the development of any stem cell based cell therapy is the control of cell differentiation, i.e. to make sure that the desired cell type, rather than all the other cells in the stem cell's repertoire, is generated. This is not only important in order to obtain a clinical effect, but also to avoid the development of rapidly proliferating cell types and tumour formation.

Many of the challenges in stem cell research are common to several fields. The evaluation of effects in injury models in experimental animals or in clinical trials is often extraordinarily difficult in neurological diseases. This is because of the often large inter-individual variation in spontaneous recovery after, for example, stroke, and the very slow dynamics of brain plasticity. An indication of this is the unusually high costs and large proportion of failed clinical studies in neurology.

- Neural stem cell differentiation needs to be controlled in order to generate the desired cell types.
- Generation of unwanted cell types must be avoided.
- Clinical studies in neurology are difficult and costly.

Conclusions

The brain has traditionally been viewed as a static organ with little possibility of repair. Rapidly increasing knowledge on nervous system development – which has been translated to protocols for the directed differentiation of stem cells for transplantation – together with the realisation of the existence of stem cells in the adult brain, has resulted in increasing optimism that replacement therapies may be developed for neurological diseases.

Stem cell research in pancreas

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Introduction

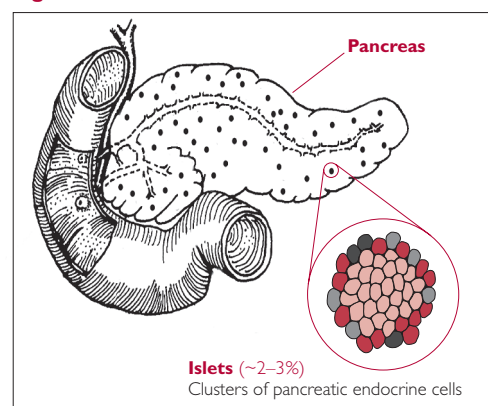
In the European Union, more than 30 million people suffer from diabetes (mainly type 2), which can lead to serious and costly complications such as cardiovascular disease, stroke, kidney failure, blindness and amputation. The figure is predicted to rise to nearly 50 million by 2030. The two most commonly known forms of diabetes – type 1, or juvenile onset diabetes, and type 2, or adult onset diabetes – are increasing at an alarming pace. In type 1 diabetes the insulin producing β -cells are destroyed by the immune system, leading to partial or complete deficiency of β -cells. Type 2 diabetes is the result of insulin resistance and β -cell failure, and is closely linked to obesity. In severe and advanced forms of type 2 diabetes there is also a loss of β -cells mass. Hence, type 1 diabetics and patients with severe forms of type 2 diabetes may benefit significantly from cell replacement therapy through transplantation of normal, functional, insulin producing β -cells. The recent development of new protocols, in particular the so-called Edmonton protocol⁽¹⁾, to prevent the rejection and to improve the viability of transplanted pancreatic β -cells – or rather islets (see below and **figure 1**) – has validated the principle of this approach in restoring the number of functional β -cells required to normalise blood glucose levels, and thus to cure diabetes. This therapy is, however, not yet practical on a large scale because of the shortage of human islets.

The pancreas is a mixed exocrine and endocrine organ (secretes substances into the alimentary canal, and into the blood) in which the exocrine tissue, which produces and secretes a variety of digestive enzymes, makes up the major part of the organ. The pancreatic endocrine cells (~2-3 per cent of the entire pancreas) come in four different types – one being the insulin

producing β -cells – and these cluster into so-called islets of Langerhans (or “islets” for short) (**figure 1**). The β -cells control the concentration of glucose in the blood by secreting insulin in response to increased blood sugar levels. However, the different hormone producing cells of the pancreas form an integrated entity, or “mini-organ”, which ultimately ensures a fine-tuned regulation of blood glucose levels in response to physiological changes.

- Diabetes develops as a consequence of β -cell loss and/or β -cell failure and increases dramatically world wide.
- The insulin producing β -cells cluster with the other pancreatic endocrine cells into small “mini-organs” called islets that constitute a mere 2-3 per cent of the entire pancreas
- Islet transplantation is a promising therapy for the treatment of diabetes but there is shortage of human islets

Fig. 1 Pancreas islets



1. Shapiro, A.M. et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med.* 343:230-238, 2000.

Hormones produced by endocrine pancreas

- α -cells → Glucagon
- β -cells → Insulin
- δ -cells → Somatostatin
- PP-cells → Pancreatic polypeptide

Current status

All pancreatic cell types are of so-called endodermal origin (originating from the embryonal tissue layer that gives rise to the lungs, digestive tract and related organs). The morphogenesis and structural development of the pancreas are well characterized, and studies of pancreatic gene expression have identified several factors that mark different stages of pancreas development and pancreatic cell differentiation.⁽²⁾ The genetic dissection of pancreas development in mouse has provided valuable information on basic mechanisms of pancreatic organogenesis, exocrine and endocrine cell differentiation, β -cell function, and maintenance of normal blood sugar levels⁽²⁾. Several of the identified factors have also been linked – by expression or function – to human pancreatic development and β -cell function, providing evidence for an evolutionary conserved cascade of factors controlling pancreas development and β -cell function.

One attractive approach to generate sufficient numbers of transplantable cells is to generate functional β -cells from stem and/or progenitor cells *in vitro*. An alternative approach would be to try to stimulate β -cell replication or neogenesis (*de novo* formation) *in vivo*. Several different approaches⁽³⁻⁴⁾ to generate new insulin producing β -cells have been or are being pursued:

- Embryonic stem (ES) cells
- Bone marrow stem cells
- Adult pancreatic stem cells
- Transdifferentiation of adult stem cells
- Adult β -cells

ES-cells have unquestionably the highest self-renewal capacity and pluripotency of all stem cells, making them a prime candidate for stem cell-based therapies. The use of ES cells for the generation of pancreatic cell types has, however, been hampered by the difficulties in ensuring the generation of definitive endoderm from which the pancreas later forms. The recent progress with both mouse and human ES cells in achieving this goal represents an important step towards the prospect of generating pancreatic cell types from ES cells.

The initial claims that bone marrow stem cells – which are derived from a developmental cell layer called mesoderm (giving rise to muscle,

bone and blood) – could differentiate into other lineages, including insulin-producing cells, have been largely refuted by several independent investigators. The differentiation of bone marrow stem cells into non-mesodermal lineages is at best questionable.

The adult pancreas appears to possess some capacity, albeit limited, to regenerate in response to diseases such as diabetes and pancreatitis, or various types of tissue injury. These observations have given rise to the concept of an adult pancreatic stem cell. The existence of a stem cell in the adult pancreas remains elusive however, and a recent study (in mice) provides evidence that the formation of β -cells *in vivo* in adult mice following removal of the pancreas is rather the result of β -cell replication. Transdifferentiation of adult non- β cells has also been suggested as alternative mechanism by which β -cell neogenesis can occur.

The liver is developmentally related to the pancreas, and the liver has a significant regeneration capacity. Consequently, several investigators have looked into the possibility of trans-differentiating liver cells into pancreatic cells, both in mice and humans. With this approach limited expression of some pancreatic genes both *in vivo* and *in vitro* has been achieved. The efficacy and reproducibility of this approach need to be further investigated and the transdifferentiated cells need to be rigorously characterised at the molecular and functional level.

An alternative adult source for β -cells might be the adult β -cells themselves. Studies performed by independent investigators suggests that isolated β -cells can be expanded following dedifferentiation and then induced to re-differentiate back to a β -cell like state. However, the resulting cells express very low levels of insulin and it is still unclear what the true origin of the expanding cells is. Again, the efficacy and reproducibility of this approach also need to be rigorously analysed.

- Genetic dissection of pancreas development in mouse has generated key information regarding factors controlling pancreatic cell differentiation.
- Several different stem cell sources for the generation of β -cells are currently being explored.

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- The recent breakthrough in obtaining definitive endoderm from ES-cells represents an important step towards generating β -cells from ES-cells.

Future prospects and open questions

The use of stem cells for the generation of insulin-producing β -cells is of great interest, but it will remain fiction rather than fact until we can efficiently and reproducibly ensure that stable, fully functional cells can be generated *in vitro* or *in vivo*. Today *bona fide* β -cells have not yet been successfully obtained from stem or progenitor cells. The current ongoing stem cells research, and in particular the recent developments regarding *in vitro* differentiation of ES cells, is encouraging, but the prospect of fully *in vitro* or *in vivo* differentiated β -cells is still for the future. Given the tight interactions of the different pancreatic endocrine cells and the finely tuned regulation of hormonal secretion in response to variations in blood sugar levels, an important question is whether the generation of insulin producing β -cells *per se* will be sufficient to ensure glucose homeostasis, or whether we need to recreate fully integrated islets containing all pancreatic endocrine cells.

An equally important issue for the prospect of curing diabetes by cell replacement therapy is immune rejection. As for any transplantation therapy, both type 1 and 2 diabetics face alloimmunity, i.e. immune rejection of foreign cells or organs. In addition, the primary cause of type 1 diabetes is autoimmunity: the body's immune system turns against its own pancreatic β -cells. That means that even β -cells derived from the patient's own progenitor or stem cells are susceptible to attack and destruction after re-implantation. Hence, the future success of therapies based on mere cell replacement will require the development of improved immunosuppressive drugs.

- In stem cell-based transplantation therapy for diabetics it is not clear whether β -cells alone will suffice or whether complete, functional islets need to be recreated.
- Even if autologous transplantation became a reality, the problem of how to protect the new β -cells from autoimmune destruction needs to be solved.

Conclusions

Cell replacement therapy is an appealing approach for the treatment and cure of diabetes. Proof-of-concept concerning the prospect of curing diabetes already exists: islets transplantations have enabled numerous patients to stop injecting themselves with insulin. For this to become a fully functional therapy, further optimisation of the immunosuppression protocol, and increased availability of transplantable insulin producing cells are required. In general, the use of stem or progenitor cells as a source for β -cell replacement therapy would offer a near inexhaustible source of transplantable cells. However, this depends upon two important points: i) the use of appropriate markers that allow the classification of distinct stages of cell differentiation (insulin is just one of many key markers that defines a functional β -cells), and ii) information regarding key signalling factors that – in a sequential manner – operate at the different stages of differentiation to ultimately guide the cell towards a mature β -cell. As already mentioned, studies of pancreatic development in animal models have generated information that is of relevance for both these aspects, and will continue to do so. Information thus obtained needs to be integrated with our current knowledge regarding pancreatic development, β -cell differentiation and function, to ensure that stringent criteria regarding marker gene expression and functionality are used in the evaluation of stem and progenitor cell derived β -cells. No matter which other criteria are fulfilled, to be clinically useful as a replacement for current therapies, the cells should secrete fully processed insulin in response to physiological concentrations of glucose.

- *In vitro* differentiation of stem or progenitor cells offers an attractive source for the generation of insulin producing β -cells.
- Stem cell derived β -cells need to be rigorously characterised to assure functionality; insulin expression alone does not make a β -cell. Identification of factors that direct the generation of true β -cells during development will be critical for the prospect of generating β -cells from stem cells.

Stem cell research in skeletal muscle

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Introduction

Diseases that specifically affect skeletal muscle—as opposed to diseases in which muscle is collaterally affected, are often associated with progressive destruction of the muscle fibers themselves, and in the most severe cases, progressive replacement of the muscle tissue with scar and fat. This leads to progressive and irreversible paralysis and ultimately death of the patient. As is the case in muscular dystrophies⁽¹⁾, a diverse group of diseases; the most common and devastating of which is Duchenne Muscular Dystrophy (DMD), no effective therapy exists.

Like many of the other muscular dystrophies, DMD is caused by mutations of genes encoding proteins that link the muscle cell cytoskeleton to the supportive structures outside the cell. These proteins form a composite unit with other proteins, and lack of a single component leads to destruction of the whole functional unit (to various extents in different forms of dystrophy) and hence to fragility of the muscle membrane. During contraction, defects of the membrane cause calcium to enter muscle fibers that are either damaged or die. These fibers are initially repaired or replaced by resident “satellite” cells (reservoir progenitors), but these cells have the same genetic defect and thus produce new fibers that also degenerate. With time the population of satellite cells is exhausted, no further fiber regeneration occurs and the tissue is progressively replaced by scar and fat. Muscle fiber death

is associated with chronic inflammation, and anti-inflammatory steroids are currently the only therapy available. However, their positive effect is modest and their side effects can be serious. Stem cell research involving repair of the faulty gene before re-introduction to the patient could provide a glimpse of hope for future generations of DMD sufferers.

Current status

Until recently, only one myogenic progenitor cell (the precursor to the mature muscle fiber) had been clearly identified and partially characterized in post-natal skeletal muscle. First described in 1961, satellite cells were identified and so named because they occupy a position “satellite” to the muscle fiber, between the muscle membrane and the basal lamina that surrounds every fiber.⁽²⁾ In adult healthy muscle, these cells are in a resting phase, very small and with a condensed nucleus. If muscle is injured, they are rapidly activated and begin to divide to generate a progeny that repair damaged fibers and/or generate new fibers to replace those that have degenerated. However, part of the progeny, does not differentiate, and resumes a position as satellite cells, thus ensuring the possibility of further regeneration in case of repeated damage. This possibility is not infinite: during the course of muscular dystrophies, continued muscle fiber damage and degeneration causes a continuous activation of satellite cells. The pool is progressively exhausted until no further muscle regeneration can occur.

In 1998 it was reported that bone marrow, which is known to host blood progenitor cells, also contains cells that, at very low frequency, may participate in skeletal muscle regeneration and contribute to muscle fiber repair or regeneration⁽³⁾. The nature of these cells was initially

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– and still remains – somewhat elusive, but in the following years the scientific literature was flooded with reports of unorthodox differentiation of progenitor cells of one tissue into differentiated cells of distant and unrelated tissues (e.g. blood stem cells were differentiated into neurons, and *vice versa*). This phenomenon, termed “plasticity” had a strong impact even outside the scientific community: in fact the possibility of inducing differentiation of a stem cell from an unaffected tissue into a cell type affected in a given disease, opened a new therapeutic perspective and questioned the need to invest in embryonic stem cell research. The prospect of avoiding the heated ethical controversy that embryonic stem cells elicit was short-lived. In reality, plasticity is a very rare event, often – though not always – due to cell fusion. Until the molecular mechanisms are elucidated and the biology of these cells is understood, plasticity will be irrelevant from a therapeutic point of view.

Nevertheless, during the past few years evidence has accumulated that different progenitor cells, isolated from tissues unrelated to muscle, such as blood vessels, adipose tissue, sinovium and nervous tissue, are able to differentiate into skeletal muscle cells (for a recent review⁴). This differentiation occurs at low frequency (usually less than 10 per cent of the total cell population) and needs to be induced by signals released by differentiating *bona fide* myogenic (muscle forming) cells, or by drugs that modify the genetic instructions.

The possible developmental significance of this “unorthodox” muscle differentiation is complex and not yet well understood: its discussion is beyond the scope of this article.

- Muscle development, maintenance and growth are well understood, and the role of satellite stem cells in renewal and repair has been clarified.
- Satellite cells replace damaged muscle fibers, but in certain muscular dystrophies, fibers are destroyed with such a high frequency, that the pool of satellite cells is sooner or later exhausted.
- Stem cells from other tissues may have some value as a therapy, but their differentiation is a complex task and requires further studies.

Prospects

Currently cell therapy for muscular dystrophy can be envisioned either using satellite cells or one of the above “unorthodox” progenitors whose positive attributes have to be considered carefully. Where possible, cells may be derived from the same patient (autologous) or from a healthy donor (heterologous): in the first case the defective gene must be corrected or replaced to make transplantation effective; in the second case, gene correction would not be necessary, but the patient would require immune suppression to prevent rejection of the foreign cells.

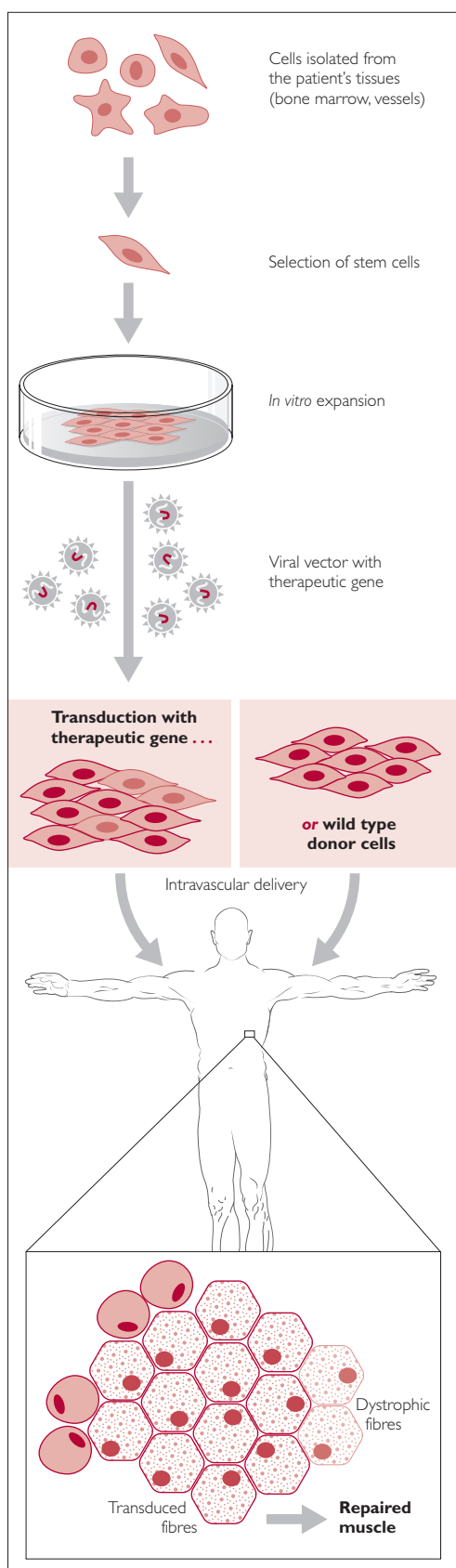
In both cases, to optimize the chances of success for stem cell for therapy of muscular dystrophy it would be necessary a) to isolate cells from an easily accessible anatomical site; b) to grow them up in the lab without loss of self-renewal and muscle differentiation ability; c) to efficiently introduce the repairing gene (for autologous cells); d) to reach the diseased muscle through the blood circulation. A theoretical scheme of this protocol is reported in (figure 1). Cells may be isolated from biopsies of skeletal muscle (satellite cells but also vessels), fat, bone marrow, sinovium and dermis.

Satellite cells from DMD patients would obviously be the first choice but there are problems that seriously limit this possibility: they are already exhausted in DMD patients, cannot cross the vessel wall – and thus cannot be delivered systemically – and furthermore, they cannot migrate from the site of intra-muscular injection, hence requiring many thousands of injections. For all the other cell types it is imperative to show efficient muscle differentiation *in vitro* and *in vivo*, after transplantation into immune deficient, dystrophic mice. For the non standard myogenic progenitors, recent evidence of efficacy came from a study utilizing vessel-associated progenitors in a mouse model of limb-girdle muscular dystrophy.⁽⁵⁾ More recently, human cells from adipose tissue and bone marrow have been shown to reconstitute dystrophic mouse muscle, but the extent of this reconstitution remains to be quantified.

Stem cell research is likely to produce a clearer and more comprehensive picture of the identity, biological features and lineage relationships of satellite cells and other non-standard muscle cell progenitors. Simultaneously, cell therapy protocols – which will benefit from ongoing basic

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research – are moving to large animal models, such as the dystrophic dog. This will set (perhaps in a couple of years) the stage for clinical trials in dystrophic patients. Inevitably, the results will initially be modest (improvement, rather than cure), because the methods will require optimisation. In the following five to ten years, and assuming appropriate funding (muscular dystrophies are rare and of little interest to Companies), optimisation of the protocols will progressively increase clinical outcome, and a complete “cure” of such untreatable and devastating disease may be in sight.

- Unless current limitations of satellite cells are solved, the best candidates to treat muscular Duchenne Muscular Dystrophy are other mesoderm stem cells, either resident in muscle or (less likely) derived from other tissues.
- The origin of these stem cells, their relationship with satellite cells, their characteristics and their ability to form skeletal muscle must be better understood in order to better assess their usefulness in treatment.
- Large animal trials will be crucial in allowing the step to the first clinical trials in patients suffering from DMD.

Problems, concerns and open questions

For an efficacious treatment of DMD, it remains to be calculated how many viable cells can be isolated from a tissue biopsy; almost certainly this number will not be suitable for direct transplantation (possibly after gene correction), hence, cell amplification in culture would be required to achieve the number of cells (billions) necessary to treat the most important muscles in the patient. During this process cells may reach senescence (old-age) i.e. failure to further proliferate and also to differentiate. This may be the case for satellite cells from a dystrophic patient, since they may have already spent their proliferation potency during the cycles of regeneration that occur *in vivo*.

For cells derived from patients, efficient gene correction will be necessary, and this may be prob-

Fig. 1
Stem cells for the cell therapy of muscular dystrophies

lematic for the very large dystrophin gene, which cannot be fitted into viral gene-transfer vectors. However, alternative molecular strategies – such as mini-genes or methods to skip the mutation during transcription – appear promising. Vectors derived from lentiviruses seem very efficient in correcting diseased cells, but their use is still pending approval from regulatory agencies. Finally, delivery to skeletal muscle tissue appears to be the major technical problems to be solved especially for satellite cells which cannot cross the blood vessel wall.

- A major unknown is whether cells taken from the patient and grown in the lab will be fit enough to grow and differentiate properly in the patient.
- Alternative methods to the commonly used viral vectors will be needed to transfer the repair gene into the patients' cells.
- Directing the “gene-repaired” stem/progenitor cells to the skeletal muscle *via* the circulation appears to be the method of choice for efficient delivery.

Conclusions

Biomedical research has given us an understanding of the nature of muscle repair and regeneration to the extent that experiments with muscle-related stem cells can be initiated. The ultimate aim is to provide a therapy or even a complete cure for seriously debilitating diseases such as muscular dystrophies. More work is necessary to understand the origins and differentiation of the different types of muscle stem cells in order to assess their ultimate usefulness in cell therapy of muscular dystrophies in humans. Repairing the defective gene in these cells will be a feasible possibility, and coupled with culturing the cells to high numbers in the lab, may provide the first proof of principle in patients in the next few years or so. There still are many obstacles to overcome, but the possibility of combining future stem cell therapy with novel pharmacological approaches should lead to a cautious optimism that clinical efficacy may be reached in a not too distant future.

Evaluating the therapeutic potential of stem cells

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Introduction

Over the past five years attention has been focused sharply on stem cells and the extraordinary potential that they offer in treating a number of currently intractable human diseases. The list is long: heart disease, arthritis, spinal cord injury, stroke, Alzheimer's disease, Parkinson's disease, diabetes, cancer and immune disorders. There is much evidence from pre-clinical studies pointing to the effectiveness of stem cell delivery. A few small human studies have also been published, mainly on patients with heart failure, which suggest also that stem cells offer new hope to patients. At this point, there is a particularly optimistic perspective about stem cell therapy and the likelihood that it will be effective in a broad spectrum of diseases.

This optimism may be premature, because experience with stem cells in humans is still very limited. Many research projects are underway in different parts of the world and many governments have made a decision to invest very large proportions of Research and Development (R&D) budgets in stem cell research. Now is a good time to set stem cell therapy in a realistic perspective and examine current scientific and clinical approaches. Stem cell technology offers many opportunities for large scale commercial development but there are obstacles yet to be overcome in the delivery of a successful cellular product.

Several characteristics of stem cells make them unique in comparison with other mammalian

cells. Firstly, they exist as unspecialised cells lacking tissue-specific characteristics, and they maintain that undifferentiated phenotype until exposed to appropriate signals. Secondly, they have the capacity for (extensive) self-renewal. Thirdly, under the influence of specific biological signals they can differentiate into specialized cells with distinct characteristics/function. Mesenchymal stem cells (MSCs) in the bone marrow conform to this definition. These cells, as their name implies, are the precursors of cells of mesenchymal lineage (a definition of a tissue layer in the developing embryo), including cartilage, bone, fat, muscle and tendon. They are easily isolated from bone marrow and adipose (fat) tissue and from a number of other sources. At this point we have an incomplete understanding of the regulation of differentiation, commitment and plasticity of this cell population. We can identify a number of the signals that activate the cells to differentiate along specific cell pathways and we can describe the phenotype of the fully differentiated cells, but we understand little of the intermediate steps. Nor do we understand transdifferentiation (the changing of one cell type into another), or the ability of cells to differentiate horizontally from one lineage to another. Furthermore, there is little clarity surrounding the niche, or tissue-specific microenvironment, in which the cells reside. Despite the lack of understanding of these cells and their natural history, it is very likely that they have therapeutic potential in a broad variety of clinical applications. MSCs can easily be isolated from a small sample of marrow and grown in the laboratory. The disadvantage of MSCs and other adult stem cells is that they have limited differentiation potential compared with ES cells. The advantage is that they present no ethical dilemma.

Current Status

There are numerous reports in the scientific literature describing the results of experiments on stem cell delivery in animal models of disease. In many cases the studies are well conducted, carefully interpreted and subject to the sort of stringent review that we expect. In many cases also results suggestive of possible benefits in humans are reported. Recent published reports point to some functional improvement when stem cells from bone marrow are delivered to the injured spinal cord in rats. Other studies suggest that, in a stroke model in rats, there is also good recovery when stem cells are given. Studies on the effect of delivering stem cells in rat and mouse models respectively of heart/vascular disease and arthritis are underway. Preliminary unpublished results indicate positive results.

All of this research contributes to a rapidly growing stem cell database, and it is critical that this work is supported and allowed to continue. There is a need for caution, however, and there is a long road ahead. As with any therapy in development, we need to have a sensible awareness of the issues surrounding stem cells. Good results in rats do not necessarily translate into good results with humans. Furthermore, we know virtually nothing about the long-term effects of stem cells in an immune-competent host. There is the possibility that stem cells may form tumours, or wrong tissue in the wrong place in the host (ectopic tissue). To date, there is very little evidence that these concerns represent real problems, but we have to be careful to continue research with long-term studies designed to evaluate the chronic effects of stem cell therapy.

A further area of interest is in researching the question of stem cell exhaustion in certain diseases, especially of the elderly. Stem cells exist in adult tissues as a repair mechanism. They are mobilised and become active following tissue injury as a result of trauma or disease. When a bone is fractured, the stem cells in the marrow migrate to the site of injury, differentiate into bone cells and participate in the repair process. Some degenerative diseases, where the ability to repair damaged tissues is reduced, may arise because these individuals have reduced populations of stem cells, or because they function poorly. For instance, it has been shown that individuals with chronic osteoarthritis, who need total joint replacement surgery, have severely

compromised bone marrow stem cells. Other workers have shown that, in mice susceptible to atherosclerosis, the stem cell population in the blood is limited. These observations have led us to the idea that certain of these degenerative diseases may be caused by stem cell exhaustion. For reasons unknown to us at this time, such individuals have a depleted or poorly functioning reservoir of stem cells. This theory, although preliminary, is attractive and may help us to understand more about the underlying disease mechanisms. If the theory is correct, it would suggest that stem cell therapy, delivered early in life, would reduce susceptibility to degenerative disease later on.

- Many respectable studies demonstrate benefits of stem cell therapy in animal models in a variety of human diseases. Such therapy has demonstrated promise in a few applications in humans, but the field is at a very early stage of development.
- Differentiation and plasticity (potential to become other cell types) are only partially understood, as is the long-term stability of transplanted stem cell populations.
- The theory of stem cell exhaustion, although requiring much more research, suggests an explanation for certain degenerative diseases at the level of cellular renewal.
- Future prospects, problems, concerns and open questions

Assuming that the early data that we now see are confirmed, and assuming no major adverse events associated with stem cell delivery, it is likely that over the next 5-10 years we will see the development of major commercial enterprises in the area of adult stem cells. Some have already started in the US and in Europe, but they are still small or medium in size. If stem cells continue to be successful in reversing the effects of arthritis or heart disease, millions of patients will potentially be treated each year.

Prior to this, some formidable obstacles have to be overcome. We do not yet fully understand how cell manufacturing technology will cope with markets of this magnitude. Furthermore, current methods for long-term storage and preservation of stem cells require very low tempera-

tures, usually liquid nitrogen. This gives rise to logistical issues and a need for available expertise and equipment at the clinical site. For the moment, this may restrict stem cell procedures to major medical centres.

The ultimate goal has to be universal delivery of stem cell therapy, and for this to happen the technology has to become simpler and methods of delivery more accessible. Another serious obstacle relates to the reliance on foetal bovine serum (FBS) as the growth medium used to culture cells in the laboratory. Two problems arise immediately: (1) the risk of transmission of infectious agents and (2) industry dependence on a limited and geographically restricted commodity. There have been many efforts in the past to develop serum-independent culture conditions but it is still an uncertain area. Until this is achieved the widespread use of stem cell technology will be hindered.

- Manufacturing methods and long-term storage capabilities (involving expertise at clinical sites) need to be scaled up for a potentially large demand.
- For universal delivery of stem cell-based therapies, the technology must be simplified, and delivery methods made more accessible.
- A reliable, safe and plentiful nutritive substrate (medium) must to be identified for production of therapeutic grade cells.

Conclusions

The field of stem cell research will need to move at a fast pace so that new therapies can be made available to patients as quickly as possible. Conversely, if the results turn out to be disappointing, which is unlikely, policy makers and funding bodies will need to be aware of this sooner rather than later. The most pressing issue is to support high quality, accelerated research programs that will enhance European competitiveness in stem cell research and lead to clear and unambiguous conclusions.

Clearly, the pace of research needs to be accelerated, and the funding for high quality projects assessing therapeutic benefit of stem cells increased. Furthermore, translational medicine approaches need to be incorporated more

fully into stem cell research. Many human trials remain to be completed and it is hoped that the results of these will be as promising as the early studies. Early success in small, often poorly controlled studies makes the large placebo-controlled, fully blinded, multicentre studies all the more important. There are many reasons to be optimistic about stem cell therapy in the future, but a lot more research and investigation will be needed for success to be achieved in the clinic.

Commercial development of embryonic stem cells: report from a company operating in the US

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Introduction

The goal of human embryonic stem cell-based therapies is to restore organ function lost to chronic degenerative diseases or injury. Geron intends to establish living cells as tomorrow's pharmaceuticals by means of introducing functional cells derived from human embryonic stem cells (hESCs) into the affected organ. We have been working in the hESC field for over 10 years, funding the original derivation of hESCs at the University of Wisconsin in 1998 and subsequently developing multiple therapeutic product candidates for diverse diseases.

Stem cells generally are self-renewing primitive cells that can develop into functional, differentiated cells. Human embryonic stem cells, which are derived from very early stage embryos called blastocysts, are unique because:

- they are pluripotent, which means they can develop into all cells and tissues in the body, and
- they self-renew and proliferate indefinitely in the undifferentiated state when cultured under appropriate conditions.

The ability of hESCs to divide indefinitely in the undifferentiated state without losing pluripotency is a unique characteristic that distinguishes them from all other stem cells discovered to date in humans. It has been demonstrated that hESCs express telomerase continuously, a characteristic of immortal cells⁽¹⁾. Other stem cells such as blood or gut stem cells express telomerase at very low levels or only periodically; they therefore

age, limiting their use in research or therapeutic applications. hESCs can be expanded in culture indefinitely and hence can be banked and quality tested for scaled product manufacture⁽²⁾.

We have developed methods to grow, maintain, and scale-up production of undifferentiated hESCs using feeder cell-free, chemically defined culture medium⁽³⁾. We have also developed scalable manufacturing procedures to differentiate hESCs to therapeutically relevant cell types^(4, 5, 6, 7, 8, 9, 11, 12). We are now testing six different therapeutic cell types in animal models. In five of these cell types, we have preliminary results suggesting efficacy as evidenced by durable engraftment or improvement of organ function in the treated animals.

Current Status

GRNOPC1, glial progenitor cells for spinal cord injury

The major neural cells of the central nervous system typically do not regenerate after injury. If a nerve cell is damaged due to disease or injury, there is no treatment at present to restore lost function. Millions of patients worldwide suffer from injury to the nervous system or disorders associated with its degeneration. In the case of spinal cord injuries, patients are often left partly or wholly paralyzed because nerve and supporting cells in the spinal cord have been damaged and cannot regenerate. Such patients are permanently disabled, often institutionalised and may require life support.

We have derived oligodendroglial progenitors from hESCs in culture and have begun testing them in animal models to determine whether they can restore normal neural function. Using our serum and feeder-free culture system, we are now producing our first hESC-derived prod-

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uct, GRNOPC1, oligodendroglial progenitor cells for use in treating acute spinal cord injury. Results from a proof-of-concept study - a collaboration between academia and industry - were published in the *Journal of Neuroscience*⁽¹⁰⁾ 2005. The studies showed that the GRNOPC1 cell product, when injected directly in the spinal cord of rats with spinal cord injury, re-myelinated the injured nerve axons, resulting in functional improvement of locomotor activity of the injured animals compared to controls. We are currently completing our preclinical IND-enabling studies and expect to begin testing these cells in patients with acute spinal cord injury in 2007.

GRNCM1, cardiomyocytes for heart disease

Heart muscle cells (cardiomyocytes) do not regenerate during adult life. When heart muscle is damaged by injury or decreased blood flow, functional contracting heart muscle is replaced with non-functional scar tissue. Congestive heart failure, a common consequence of heart muscle or valve damage, affects approximately 5.0 million people in the United States. This year, it is estimated that about 1.2 million people will have a heart attack, which is the primary cause of heart muscle damage.

We have derived human cardiomyocytes from hESCs and observed their normal contractile function and response to cardiac drugs.⁽⁴⁾ We have transplanted these cells into animal models, and to date the cells appear to survive in the myocardium in infarcted animals, as well as restoring cardiac function in animals with induced myocardial infarctions.⁽¹³⁾

GRNIPC1, islet cells for diabetes

It is estimated that there are as many as one million Americans suffering from type 1 Diabetes (Insulin Dependent Diabetes Mellitus). Normally, certain cells in the pancreas, called the islet β -cells, produce insulin which promotes the uptake of the sugar glucose by cells in the human body. Degeneration of pancreatic islet β -cells results in a lack of insulin in the bloodstream which results in diabetes. Although diabetics can be treated with daily injections of insulin, these injections enable only intermittent glucose control. As a result, patients with diabetes suffer chronic degeneration of many organs, including the eye, kidney, nerves and blood vessels.

In some cases, patients with diabetes have been treated with islet β -cell transplantation. However, poor availability of suitable sources for islet β -cell transplantation and the complications of the required co-administration of immunosuppressive drugs make this approach impractical as a treatment for the growing numbers of individuals suffering from diabetes.

We have derived insulin-producing islet β -cells from hESCs and are working to improve the yield of islet cells and characterise their secretion of insulin in response to glucose.⁽¹²⁾ We have transplanted the islets into animal models of diabetes and to date the cells showed the presence of c-peptide (insulin)-producing cells three months after transplantation. Human c-peptide was also found in the serum of these transplanted animals after challenge with high glucose.

Haematopoietic cells to prevent immune rejection

The haematologic system (the circulating cells of blood) is one of the rare tissues of the human body that can replenish itself throughout life. Although complex and expensive, the use of bone marrow transplantation is increasing worldwide. A major unresolved problem in the procedure is the lack of availability of suitably matched marrow donors, which severely limits the numbers of patients who can undergo the transplant. We have derived haematopoietic stem cells from hESCs⁵, and tests of these cells in animal models of bone marrow transplantation show stable engraftment of the cells.⁽¹¹⁾ Haematopoietic stem cells (HSCs), or certain subsets of dendritic cells, produced from hESCs may find use not only in haematopoietic transplantation therapies, but also in procedures designed to prevent immune rejection of other hESC-derived transplanted cells. Employing the principles of tolerance induction, hESC-derived HSCs or hESC-derived dendritic cells, as well as the particular hESC-derived therapeutic cells, would each be differentiated from the same hESC line. Co-administration of the hESC-derived tolerogenic cells may allow "education" of the recipient's immune system to accept the therapeutic cells without rejection.

Hepatocytes for drug discovery and liver failure

Many prospective new drugs fail in clinical trials because of toxicity to the liver or because of

poor uptake, distribution or elimination of the active compound in the human body. Much of the efficacy and safety of a drug will depend on how that drug is metabolised into an active or inactive form, and on the toxic metabolites that might be generated in the process. Hepatocytes, the major cells of the liver, metabolise most compounds and thereby can be used to predict many pharmacological characteristics of a drug.

There are no completely effective systems available today to accurately predict the metabolism or toxicity of a compound in human livers. Rat and mouse metabolism models only approximate human metabolism. The development of several drugs has been terminated late in human clinical trials because rodent systems utilised early in the development process failed to predict that the drug would be toxic to humans. Human hepatocyte cell lines available today do not have the same attributes as their normal counterparts in the body and must be transformed in order to maintain their capacity to proliferate in culture. Access to fresh primary human liver tissue for use in toxicity studies is very limited and substantial variability can be observed depending on the individual donor, the time and process of collection and the culture conditions for the experiments.

We are developing methods to derive standardised functional hepatocytes (liver cells) from hESCs to address the significant unmet need for a reliable predictor of the metabolism, biodistribution and toxicity of drug development candidates.⁽⁸⁾ These cells would provide a consistent source of normal human liver cells that can reliably predict how a new drug will affect, and be metabolised by, the livers of the patients who take it. We are also evaluating the use of these cells in animal models of liver failure.

Problems concerns and open questions

■ Because of U.S. governmental funding constraints, hESC product development in the United States is being advanced primarily by industry, rather than academia. Unlike other paradigm changing technologies such as rDNA or monoclonal antibodies which were thoroughly explored in academic labs prior to development by industry, hESC research and product development is well underway within the American biotechnology sector. Increas-

ing Academic-Industry liaison and applying Framework Programme funding for academic research on hESCs in the EU are urgent priorities needed to prevent the European academic sector from falling permanently behind the industrial sector, as is occurring in the United States.

- The European Patent Office (EPO) position on the unpatentability of hESCs is at odds with most of the world, - even the United States - because of morality objections. This position must be reversed quickly if the EU is serious about attracting an industrial hESC presence in its territory. The Enlarged Board of Appeal of the EPO should resolve this issue expeditiously.
- A divisive EU political climate has prevented the emergence of a technical infrastructure to support, characterise and disseminate hESC technology in Europe. There should be (1) a central resource for characterizing and maintaining cell lines (a hESC cell bank); (2) mechanisms established for sharing know-how, cell lines and reagents and enhancing inter-laboratory collaboration between and within all member nations of the EU; (3) a funding resource devoted for training workshops and for providing fellowships to train scientists on hESC biology; and (4) standards established for regulatory approvals, clinical development and product registration across the EU, rather than country by country.
- A public education program across the EU on the positive impact of stem cell-based therapies for chronic disease should be implemented in order to avoid a GM food-type backlash.

Conclusions

Human Embryonic Stem Cells offer a potentially major breakthrough in our battle against chronic disease and injury. The technology offers for the first time, a low cost, scalable way to reliably produce large numbers of living replacement cells to restore function of nearly every organ in the body affected by chronic disease or injury. We need policies and regulations that will enable this potential to be realised in the near term. The social and economics costs of chronic disease in our aging population demand nothing less.

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Perspective of an international patient advocacy organization

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Introduction

The Juvenile Diabetes Research Foundation (JDRF) is a US-based nonprofit, nongovernmental foundation. It was founded more than 30 years ago by parents of children with type 1 diabetes, and its mission has been constant - to find a cure for type 1 diabetes and its complications through research support. JDRF funds diabetes research all over the world, and is the world's leading nonprofit financier of diabetes research. In 2005 JDRF committed more than USD 98 million to support researchers in 19 countries (about USD 25 million in Europe). In addition to raising money for research, JDRF also plays an important role in public advocacy for issues important to people with diabetes.

Current status

Type 1 diabetes is an autoimmune disease where the body's immune system destroys the insulin-producing beta cells in the pancreas. People with type 1 diabetes require daily injections of insulin. Insulin injections are not however a cure, since even perfectly treated individuals may still experience complications of diabetes. Type 1 diabetes accounts for 5 to 10 per cent of all diagnosed cases of diabetes, but is the leading cause of diabetes in children⁽¹⁾. The International Diabetes Federation estimated that in 2003, 48.4 million Europeans had diabetes (including type 1 and type 2), 7.8 per cent of the population at the time.⁽²⁾ This compares with

7 per cent of the American population estimated to have diabetes in 2005 (20.8 million adults and children)⁽³⁾. The economic burden of diabetes is significant; annual direct healthcare costs worldwide was estimated (in 2003) to be at least USD 153 billion internationally. In Europe, EU figures indicate that diabetes complications represent 5-10 per cent of total healthcare spending⁽⁴⁾. Worldwide, the numbers of people with diabetes are expected to increase, with corresponding increases in healthcare costs.

JDRF was one of the first non-profit foundations worldwide to publicly support human embryonic stem cell (hESC) research⁽⁵⁾, providing a position paper and public statement in December 1998. The initial motivation came from the belief that hESC research could lead to the discovery of new ways to develop unlimited supplies of insulin-producing beta cells⁽⁶⁾, with the hope that everyone with the disease can be treated. This view was significantly enhanced in July 2000 by the announcement from Edmonton, Canada⁽⁷⁾, of successful human islet transplantation in 7 persons with type 1 diabetes, disabling hypoglycemia unawareness and difficult-to-treat disease. These dramatic results firmly established the proof of principle that islet transplantation can significantly ameliorate type 1 diabetes and thus provided the scientific basis to hope that cell replacement therapy could have a role in treating or curing type 1 diabetes.

Since that time JDRF has built a research portfolio that supports both human and animal stem cell research, and research that uses stem cell lines from embryonic, fetal and adult sources. JDRF funding has supported the derivation of new hESC lines, the characterization of lines according to internationally-accepted criteria, ensuring the availability of hESC lines to researchers, as well as research leading to β -cells

1. <http://diabetes.niddk.nih.gov/dm/pubs/overview/index.htm>

2. International Diabetes Federation Diabetes Atlas, <http://www.idf.org/home/index.cfm?node=6>

3. <http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.htm#7>

4. <http://www.europarl.eu.int/activities/expert/>

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6. Otonkoski, T., *et al.* (2005). Stem cells in the treatment of diabetes. *Ann. Med.*, 37:513-520.

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9. <http://www.camradvocacy.org/default.aspx>

10. <http://camr.ctsg.com/>

11. Davenport, R.J. (2005). Drumming up dollars for stem cell research. *Cell* 123: 1169-1172.

12. <http://stemcell.harvard.edu/index.jsp>

13. <http://mednews.stanford.edu/stem-cell-institute.html>

14. <http://www.hopkinsmedicine.org/press/2001/JANUARY/010130.HTM>

15. <http://www.nih.gov/news/fundingresearchareas.htm>

and other cell replacement therapies for diabetes and its complications.

JDRF has further leveraged its funding through international partnerships in stem cell research relevant to its mission. JDRF's partners outside the United States to support stem cell research include funding agencies in Australia, Canada, Finland, France, Sweden, Singapore, and the United Kingdom. JDRF is among the participant organizations of the International Stem Cell Forum (ISCF), which was formed to encourage international collaboration and funding support for stem cell research. Since JDRF funding is not restricted by national boundaries, we have been able to provide support for multi-national cooperative efforts. In just one example, JDRF funds collaborations between two European Union-funded programs, the European Consortium for Stem Cell Research (EuroStemCell); and the Beta Cell Therapy Consortium. The main purpose of the collaboration is to enhance coordination and data exchange between stem cell experts and experts in beta cell biology. In the United States, JDRF has a close working relationship with the National Institutes of Health Stem Cell Task Force to facilitate research that can be supported by the federal government, and to disseminate information among the research community. JDRF works closely with the International Society for Stem Cell Research and also works with stem cell networks around the world, including those in Canada, Australia, and Europe.

To ensure the ethical conduct of this research in 2000, JDRF formed a Stem Cell Oversight Committee of leading researchers, policy makers, ethicists and lay volunteers, charged with providing a "second level" of review (in addition to scientific peer review) for human stem cell research applications. The Oversight Committee was also a practical response to the challenge of varying regulations for this research throughout the world. It was not meant to substitute for institutional ethical review, or regional or national legislation, but rather to ensure that the research is well justified, and subject to appropriate oversight. The Oversight Committee also advises JDRF's Board of Directors on ethical considerations of stem cell research.

In concert with the organisation's efforts to provide funding for stem cell research, JDRF volunteers have participated in numerous forums for public education, dialogue and dis-

semination of information about human stem cell research and somatic cell nuclear transfer. JDRF representatives have participated in panels to draft guidelines for human embryonic stem cell research⁽⁸⁾, made presentations at various educational forums, and testified before the United States Congress.

In 2001, JDRF partnered with the American Society for Cell Biology to form the Coalition for the Advancement of Medical Research (CAMR)⁽⁹⁾, made up of patient organizations, universities, scientific societies, and foundations, to advocate protecting and expanding opportunities for federal funding of biomedical research involving human embryonic stem cells. JDRF's Vice President for Government Relations, Larry Soler, was CAMR's first president. Meetings with Members of Congress by JDRF volunteers and their children with type 1 diabetes are crucial in helping to inform lawmakers. In 2005, CAMR and its members helped to pass H.R.810, a congressional bill to enhance federal support for stem cell research.

CAMR members have also been active at the local (state) level⁽¹⁰⁾, initiating campaigns in support of proactive stem cell research legislation, as well as opposing potentially harmful bills⁽¹¹⁾. Public advocacy has been important in persuading state legislatures to allocate resources for stem cell research (**table 1**), a unique role for states in support of biomedical research.

Table 1 States that have appropriated funding for stem cell research

California	US\$ 3 Billion over 10 years
Connecticut	US\$ 100 Million over 10 years
Illinois	US\$ 10 Million in grants awarded in 2006
New Jersey	US\$ 10.5 Million initially budgeted in 2005
Maryland	US\$ 20 Million proposed

In addition, private donors have supported the establishment of Stem Cell Institutes at institutions like Harvard⁽¹²⁾ and Stanford⁽¹³⁾ Universities. The gift of more than USD 58 million to The Johns Hopkins University School of Medicine established its Institute for Cell Engineering (ICE).⁽¹⁴⁾ The Starr Foundation awarded USD 50 million to three New York City biomedical

research institutions -- The Rockefeller University, Weill Medical College of Cornell University, and Memorial Sloan-Kettering Cancer Center (MSKCC) -- to develop new resources and expertise in stem cell research. Thus, despite the restrictive nature of federal funding for human embryonic stem cell research in the United States (NIH estimated spending on human embryonic stem cell research was USD 40 million in 2005)⁽¹⁵⁾, both the individual states and the private sector philanthropic community have and will continue to make significant contributions to enable this important research.

Despite the major support for hESC research in the United States, questions about federal regulations for the conduct of this research as well as the lack of national ethical guidelines have led to tremendous uncertainties, especially concerning collaborations involving investigators from states with different laws. Recently published IOM guidelines represent a start, given that research institutions or states can adopt or adapt those guidelines to their individual needs.

A diversity of ethical and legal regulation and oversight of stem cell research is also the current situation in Europe. Recently⁽¹⁶⁾, a consortium of investigators has articulated a set of principles for

ensuring transnational collaboration in stem cell research. Among the recommendations was the importance of providing clarity in laws or regulations, and building flexibility in these in order to accommodate advances in the science.

Conclusions

JDRF's focus on finding a cure for type 1 diabetes and its complications enabled it to take an early leadership role in supporting hESC research, both in terms of direct funding for research, as well as advocacy efforts. JDRF has partnered with other, likeminded organisations. The partnership of funding organisations has provided important support for stem cell research, allowing the development of research resources (such as new cell lines and cell line repositories), the exchange of information (such as the international characterisation effort) among scientists, and the nurturing of research collaborations. A coalition of advocacy groups is important to ensure maximum opportunity for hESC research, adequate public oversight, and appropriate just distribution of the benefits from this research.

16. The Hinxton Group Consensus Statement, <http://mbbnet.umn.edu/scmap.html>

17. <http://www.washingtonpost.com/wpdyn/content/custom/2005/08/12/CU2005081200827.html>

A glossary for stem cell biology

- **Austin Smith**
- EuroStemCell European Consortium for Stem Cell Research
- <http://www.eurostemcell.org>

Stem cell biology is in a phase of dynamic expansion and formation of new connections with a broad range of basic and applied disciplines. The field is simultaneously exposed to public and political scrutiny. A common language for the stem cell community is an important tool for coherent exposition to these diverse audiences, not least because certain terms in stem cell vocabulary are used differently in other fields.

Stem cell Cell that can continuously produce unaltered daughters and has the ability to produce daughter cells with different, more restricted, properties.

Self-renewal Cycles of division that repeatedly generate at least one daughter equivalent to the mother cell with latent capacity for differentiation. The defining property of stem cells.

Commitment Engagement in a programme leading to differentiation. For a stem cell, exit from self-renewal.

Potency Range of commitment options available to a cell:

Totipotent self-sufficient to form entire organism; capacity of zygote and of plant meristem cells, not demonstrated for any vertebrate stem cell.

Pluripotent all cell lineages of the body including germ cells plus some or even all extraembryonic cell types;⁽¹⁾ example embryonic stem cells.

Multipotent multiple lineages that constitute an entire tissue or tissues; example haematopoietic stem cell.

Oligopotent two or more lineages within a tissue; example neural stem cell that may make a subset of neurons in the brain.

Unipotent a single lineage; example spermatogonial stem cells.

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8. Warner, J. K., Wang, J. C., Hope, K. J., Jin, L. & Dick, J. E. Concepts of human leukemic development. *Oncogene* 23, 7164-77 (2004).

Clonal analysis Investigation of properties of single cells. Essential for formal demonstration of self-renewal and potency.

Embryonic stem cell Derived *in vitro* from the pluripotent cells in the pre-gastrulation embryo.

Tissue stem cell Derived from, or resident in, a foetal or adult tissue, with potency limited to cells of that tissue. Sustain turnover and repair throughout life in some tissues.

Founder, ancestor, or precursor cell General terms for cell without self-renewal ability that contributes to tissue formation, including in some cases generating tissue stem cells⁽²⁾.

Progenitor cell Generic term for any dividing cell with differentiation capacity. Includes putative stem cells in which self-renewal has not yet been demonstrated.

Transit-amplifying cell Multiplying stem cell progeny fated for differentiation. Initially may not be fully committed and may retain self-renewal⁽³⁾.

Asymmetric division Generation of distinct fates in progeny from a single mitosis: oriented division may position daughter cells in different microenvironments; or, intrinsic determinants may be segregated into only one daughter⁽⁴⁾. Observed in some but not all stem cells and can occur in other progenitor cells.

Immortal strand Hypothesis of selective retention of parental DNA strands during asymmetric self-renewal. Potential mechanism to protect stem cells from replication associated mutations⁽⁵⁾.

Niche Cellular microenvironment providing support and stimuli necessary to sustain self-renewal^(6,7).

Stem cell homeostasis Persistence of tissue stem cell pool throughout life. Requires balancing symmetric self-renewal and differentiative divisions at the population level, or sustained asymmetric self-renewal.

Long-term reconstitution Lifelong maintenance of renewing tissue by transplanted cells. The definitive assay for haematopoietic, epidermal and spermatogonial stem cells, but transplantation may not be applicable to all tissues.

Label-retaining cell Candidate adult tissue stem cell on assumption of slow division rate and/or immortal strand retention⁽³⁾, ⁽⁷⁾. Interpret with caution.

Cancer stem cell Self-renewing cell responsible for sustaining a cancer and for producing differentiated progeny that form the bulk of the cancer^(8,9). Cancer stem cells identified in leukaemias and certain solid tumours constitute critical therapeutic targets.

Cancer cell of origin Precancerous cell that gives rise to a cancer stem cell⁽⁸⁾. May be a mutated stem cell, or a progenitor that has acquired self-renewal capacity through mutation⁽⁹⁾.

Cancer initiating cell General term that encompasses both cell of origin and cancer stem cell.

Regenerative medicine Reconstruction of diseased or injured tissue by activation of endogenous cells or by cell transplantation.

Cell replacement therapy Reconstitution of tissue by functional incorporation of transplanted stem cell progeny. Distinct from “bystander” trophic, anti-inflammatory, or immunomodulatory effects of introduced cells.

***In vitro* stem cell.** Self-renewal *ex vivo* in cells that do not overtly behave as stem cells *in vivo*. Occurs due to liberation from inductive commitment signals or by creation of a synthetic stem cell state⁽¹⁰⁾.

Lineage priming Promiscuous expression in stem cells of genes associated with specific differentiation programmes.

Reprogramming Increase in potency. Occurs naturally in regenerative organisms (“dedifferentiation”). Induced experimentally in mammalian cells by nuclear transfer, cell fusion, or genetic manipulation.

Plasticity Notion that tissue stem cells may broaden potency in response to physiological demands or insults.

Stemness Notion that different stem cells are regulated by common genes and mechanisms.

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- EXECUTIVE SUMMARY AND INTRODUCTION
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