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Stem Cells and the Philosopher's Stone

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Abstract Stem cell biology is now one of the most exciting and rapidly advancing areas of scientific endeavor. Promises of cures of a wide variety of diseases by specific replacement of damaged or malfunctional tissues by use of totipotent or multipotent stem cells is on the horizon in clinical practice. Stem cells derived from the embryo and from adult tissues have been shown to have extensive potentials for self-renewal and differentiation. In addition, the plasticities of phenotype exhibited in vivo by some of these cell populations challenge the doctrine of irreversibility of cell commitment after particular developmental stages. This brief review considers certain aspects of these recent findings of the many unexpected potentials of stem cells to differentiate into alternative processes, and their potential value for use in tissue reconstruction procedures are prominent areas that require further study. Rigorous investigation of these topics will lead to realistic approaches in the future for stem cell therapy in a variety of human diseases and other clinical problems. J. Cell. Biochem. Suppl. 38: 13–19, 2002. © 2002 Wiley-Liss, Inc.

Key words: stem cells; embryonic stem cells; adult stem cells; differentiation; plasticity

Contemporary concepts, that are being put forward by numerous researchers, regarding the possibilities for using new methods for construction or repair of the constituent organs and tissues of mammalian organisms, appear to approach the wizardry of Harry Potter [Rowling, 1997]. To transmute a somatic cell into the variety of cell types needed for tissue regeneration and reconstruction in vertebrates now appears imaginable and this potential health-giving ability equates to the attainment of the alchemists' quest for gold and the elixir of life. In fact, tissues that were formerly considered not to be capable of extensive regeneration, such as brain, spinal cord, and cardiac muscle, now appear to be capable of reconstruction functionally, to some extent at least, by "stem cell" populations. There have been a number of claims made regarding the developmental potential of these primitive cells and a confusing picture is emerging as to the physiological relevance of some of the apparent modulations of phenotype observed by some experimenters. In addition, the idea of obligatory specificity of cell lineage commitment in postnatal cells is now under question. In animals, cells become progressively more restricted in development and the number of cell types that they spawn. However, recent evidence suggests that a variety of progenitor cells, and even end-stage cells, can be reprogrammed by extracellular or intracellular signals to yield multipotential stem cells, with more extensive and restored differentiation potential. As has been pointed out recently, this invites definition of stem cells not by their participation in in vivo tissue formation but by in vitro characteristics of extensive expansion and clonogenicity [Robey, 2000].

This brief review will consider and question some of these issues perceived in recent experiments performed particularly with adult stem cells, and their possible relevance to normal and abnormal physiological processes and future reconstructive procedures in cell therapy and tissue engineering. Clarity in these aspects of stem cell research is essential for identification of the future of such agents for extensive clinical benefit. A number of recent reviews consider various aspects of vertebrate stem cell biology and these and other issues are discussed further here. Pluripotent embryonic stem cells generate all cell types including the specific stem cells

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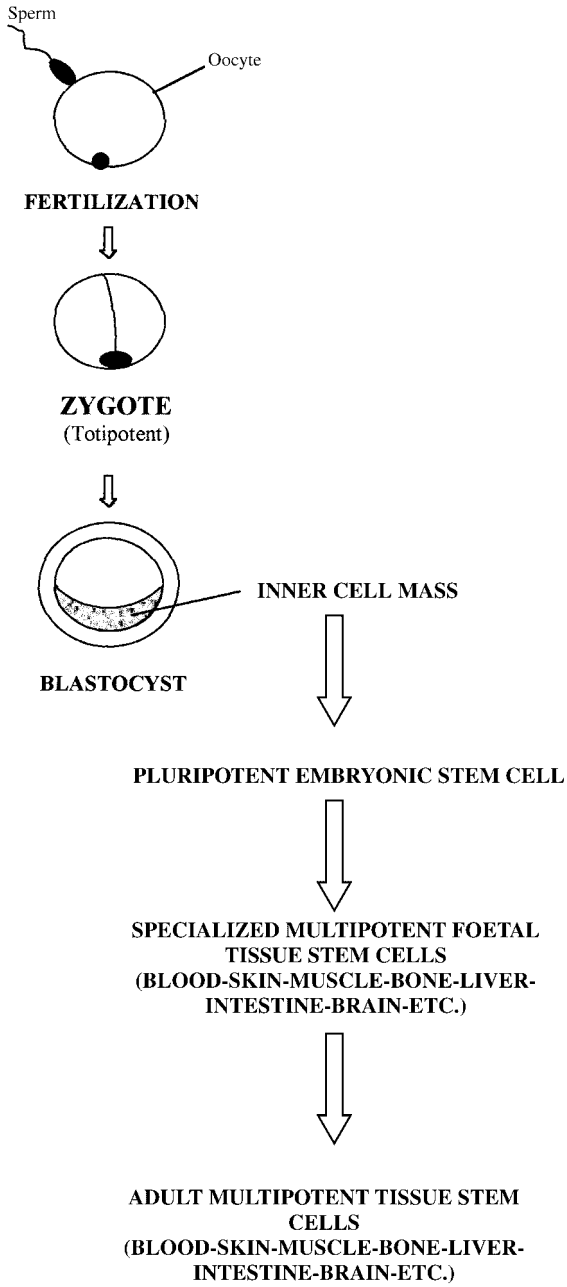

Fig. 1. Derivation of stem cells from the fertilized zygote. Early zygotic cells are totipotent and give rise to a hollow sphere of cells, the blastocyst. The inner cluster of cells of the blastocyst is termed the inner cell mass and the constituent cells are pluripotent, giving rise to almost all tissues of the body. These cells gives rise to multipotent stem cells with more restricted development into mature cells of more than one particular functional type. In in vitro culture, pluripotent stem cell lines have been obtained from the inner cell mass and from primordial germ cells that migrate to the embryonic gonads. Pluripotent stem cells may also be established from somatic cell-derived blastocysts following the use of somatic cell nuclear transfer.

residing in particular embryonic tissues, and that are known to persist in some adult tissues (Fig. 1).

Stem cell biology is now a hot topic both politically and scientifically and extensive research is being undertaken internationally in numerous laboratories. Not all can be mentioned in this brief review and selection of reports for reference in no way relates to the value or significance of those not mentioned.

Stem Cell Concept

The stem cell concept has been in existence for almost a century [Robey, 2000] but has only recently become the pinnacle of attention in general public debate. This is not only because of the potential derivation of these cells from human embryos and the resultant, contentious, ethical issues, but also because of the possibilities for using these and adult stem cells in an array of clinical procedures to treat disease and improve health and the quality of life.

A very general definition of stem cells is that they are unique cells in that their development is asymmetric and they both self-renew and also give rise to cell types different to themselves [Wolpert, 1988]. Such cells may be expected in embryonic development as maximal growth and tissue differentiation occurs, but they also occur in adult tissues. Initially, studies on the most rapidly generating organs by germ cell biologists and haematologists lead to development of the general concepts that have existed to the present day. In the adult, functional stem cells as defined by Lajtha [1967] were considered to exist only in organs that regenerate appreciably during life, for example the blood, intestine, cartilage, bone, and skin, as they serve to regenerate themselves and the specific tissue necessary to maintain physiological function. Currently "stem cells" are considered present even in tissues of the adult cardiovascular and central nervous systems, that practically do not turn over or repair to great extents, even after extensive progeny cell removal or tissue damage. Are these really functional stem cells in vivo that replace damaged or diseased tissues, or are they cells that retain proliferative potential that are just being manipulated and exposed to extraordinary conditions, either in vitro or in vivo, that affect the genome by increasing scientific versatility? Is this the reason for the suggestions that organ-specific stem cells can cross lineage boundaries, transdifferentiate into a

variety of other cell types [Anderson et al., 2001] and also exhibit extensive plasticity [Temple, 2001]? So in terms of stem cell potential, "can anything make anything [Morrison, 2001]? Whatever the causes of the phenomenology seen by increasing numbers of investigators in this field, new definitions of the potentials of particular stem cells are urgently required, the physiological relevance identified and the practical value of the cellular manipulations for clinical benefit determined.

CELL THERAPY

The development of cell therapy-based procedures clinically for healing of large skin losses [Gentzkow et al., 1996] and repair of cartilage defects [Brittberg et al., 1994] heralded the advent of a new era in tissue reconstruction from isolated or amplified cell populations. The observed plasticity of marrow fibroblast differentiation into cell phenotypes known to be part of this stromal fibroblastic cell lineage (bone, cartilage, fat, fibrous tissue, reticular tissue, and muscle) indicated the possibilities for use of these cells to augment, replace and repair these diseased and damaged tissues [Oreffo and Triffitt, 1999]. For example, genetically marked human cells were seen to home locally to bone surfaces after injection subcutaneously in calvarial sites, or intravenously, in immunocompromised mice, and participate in the functional osteoblast activity on bone surfaces [Pereira et al., 1995; Hou et al., 1999; Oreffo et al., 2001]. There are two main factors that expanded the interest in using such progenitor cells not only for potential skeletal reconstructions, but also for delivery of beneficial genes to selected skeletal and non-skeletal tissue sites. These were the ease of culture and amplification of these cells and the wide organ distributions and their observed survival in a variety of tissues after systemic injection [Hou et al., 1999; Dahir et al., 2000; Thalmeier et al., 2001].

With the dramatic increases globally in life expectancy, a variety of diseases are having increased impact on human populations. These include cardiovascular, neurological, musculoskeletal, hepatic and other specific diseases and general malignancies. For example, in the skeletal area incidences of diseases such as osteoporosis and osteoarthritis are rising rapidly and so are the resultant requirements for new and more adequate methods of replacing skeletal mass and refurbishing bone and joint structures. Additionally, a number of other genetic and metabolic conditions affecting the skeletal tissues of younger individuals require even more effective replacement of missing or damaged tissues. Genetic conditions such as osteogenesis imperfecta (OI) produce life-long crippling in some patients and treatment of the skeletal defect is dependent on strengthening and correction by mechanical orthopaedic procedures. Any future advances in strengthening the major load-bearing parts of the skeleton by other methods in this condition would provide significant improvements in prognosis. Such attempts of marrow cell-based transplantation therapy are being made in the clinic, some say prematurely as little evidence of functional patient benefit has been rigorously confirmed to date [Horwitz et al., 2001]. Obviously more attempts in experimental animal systems in the musculoskeletal and other areas need to be pursued to increase the functional creation of new tissue in in vivo situations and clarify the value of cell-based therapy for any tissue repair. This may include altering the environment of the transplanted cell by genetic engineering to expose the donor cell transiently to factors needed for growth and functional activity as well as correction of any heritable defects.

Recent Evidence of Surprising and Extensive Stem Cell Potentials

Only a few years ago, it was concluded that there was incontrovertible evidence that in postnatal life the haemopoietic and stromal fibroblastic systems were distinct and had separate cellular origins from an early stage in foetal development. Like the stromal system, there is now evidence that the haemopoietic system may have some degree of plasticity and is not entirely uni-directional and inflexible in the differentiation programme [Williams and Klinken, 1999]. But in general, organ-specific stem cells have been considered to have irretrievably lost the capacity to differentiate into a wide spectrum of tissue types and only perceived to generate cells specific to the tissue in which they reside.

Unexpected developmental potential of progenitor cell populations was observed first in total bone marrow cell preparations. It is well known that these preparations contain both haemopoietic stem cells and marrow stromal fibroblastic or mesenchymal stem cells [Aubin and Triffitt, 2001]. The latter cells are known to

give rise to bone, cartilage, fat, and fibrous tissues and these potentials may be expected as these tissue cell types have long been known to constitute the same cell lineage. Recently more extensive cell phenotypes appear to be generated by these mesenchymal stem cells under specific conditions. So skeletal muscle [Caplan and Bruder, 2001], cardiac muscle [Wang et al., 2000], lung [Pereira et al., 1995], brain [Kopen et al., 1999], and thymic stromal [Liechty et al., 2000] cells are claimed to develop from these stem cells. When genetically-marked total bone marrow cells were transplanted in immunodeficient mice into areas of muscle, damaged one day earlier by using local cardiotoxin injection, it was observed that they participated in muscle regeneration [Ferrari et al., 1998]. Such myotube formation with contractile elements had been documented earlier in marrow stromal fibroblastic cells in vitro but not in vivo and wide mesodermal differentiation seemed evident. However, the specific cellular origin of the myogenic cells present in the marrow was not definitively established in these studies and derivation from the haemopoietic stem cell or the stromal fibroblastic system was theoretically possible. Evidence from previous in vitro studies suggested origins from the latter system was most certain [Wakitani et al., 1995]. Despite the minimal contribution of the marrow cells to the muscle regeneration, an indication that engineered marrow populations could be sytematically delivered to a large number of muscles, as cell-mediated replacement therapy for muscle diseases such as Duchenne's muscular dystrophy, was an exciting possibility from these studies. Subsequent experiments with highly purified haemopoietic stem cells derived from mouse bone marrow showed dramatically that it was these cells, which reconstituted completely lethally-irradiated recipient mice, rather than the "mesenchymal stem cells" of the marrow stroma that could seemingly give rise to muscle cells after systemic injection in vivo. This process was "proven" by the detection of the presence of donor male chromosomes in fused myofibres, [Gussoni et al., 1999] and restored dystrophin expression in the mdx mouse, used as a model of muscular dystrophy. However, in the mdx mouse mutation, there is a high spontaneous reversion of synthesis of dystrophin in the muscle fibres. Furthermore, recent studies in a different mdx mutant, which shows no spontaneous reversion of dystrophin synthesis, indicated that muscle repair by bone marrow cells was less than one percent of the total muscle and was of little benefit [Ferrari et al., 2001].

Further startling evidence that the haemopoietic stem cells are perhaps multipotent was shown, when highly purified haemopoietic stem cells were found have hepatic as well as haemopoietic reconstitution ability [Lagasse et al., 2000]. In other studies, adult cloned neural cell cultures were shown to generate not only neurons and glia but also blood cells and skeletal muscle cells [Galli et al., 2000].

From these reports and an increasing number of others, it appears that adult stem cells derived from a variety of tissue may approach the pluripotential nature of embryonic stem cells. The latter have the capacity to grow indefinitely in vitro but may this property be realised by somatic stem cells under the appropriate conditions? If confirmation of their functional potential is realised practically, new possibilities for therapeutic use of readily accessible adult stem cells will open new vistas in medical practice.

Are There Alternative Explanations for Transdifferentiation Phenomena?

Because of the implications, the observed phenotypic conversions require careful and unambiguous proof. This is doubly so since popular reporting of Science now appears driven by an "accentuating the positive" requirement and all adverse or less dramatic possibilities may not be emphasized. A variety of requirements for this definitive proof have been detailed in excellent reviews by others and will not be repeated here [Anderson et al., 2001; Morrison, 2001; Temple, 2001]. Many of the experimental approaches make use of phenotypic markers for identification with all the pitfalls this entails. In addition, an interesting possibility has emerged that may have some bearing on the detection of donor genes in host tissues, particularly seen in damaged and regenerating tissues. As mentioned previously, the injection of relatively massive amounts of syngeneic or allogeneic fibroblastic cells into the blood stream may be considered by most physiologists as an artificial situation. What is the fate of such cells and particularly their genetic material? Is it completely destroyed by catabolic procedures or could it be integrated into the host genome? The answer to this very important question appears to be that

it is possible that uptake of donor DNA fragments at sites of injury, or activation of regeneration, in vivo could allow for the transduction of host cells [He et al., 2001]. It is interesting that many observations require tissue damage to show donor cell proliferation and transdifferentiation. This could be due to activation of growth factor systems in the regenerating site, which affects donor cell survival, but this could also encourage genomic events, which are mediated by factors such as fibroblast growth factor, for example [He et al., 2001]. No-one to date has performed the crucial control studies in cell fate studies, in which non-proliferative genetically-marked or identifiable cells have been compared with their viable counterparts in tissue distribution studies. At present, the possible affect of such transduction on marked cell distributions and function is not known. Even with the use of viable cells, the fate of genetic material needs careful study and the above possibility assessed. Clearer identification of the actual contribution of the transplanted cells to any regeneration observed should be unambiguously shown to occur.

"Homing" of Stem Cells From the Systemic Circulation

Many reports suggest that systemic delivery indicates homing to preferred sites in the body. But is this really relevant homing or is it mainly determined by blood flow and capillary properties at the tissue site? It is well known that the major venous return carries injected cells first to the lung and non-pulmonary arterial supply is needed for wider systemic distribution of injected moeities. Certainly a number of reports indicate that marked cells appear to target damaged tissues when injected systemically, but is their distribution and accumulation determined mainly by extrusion through damaged blood vessels with increased permeability? Even if homing to a preferred tissue, as seen in the haemopoietic stem cell system, is a possibility, is this a normal physiological event for other tissues? Furthermore, just because a systemically, artificially-introduced cell type appears to contribute to tissue growth and function [Hou et al., 1999] does not necessarily mean that in normal physiology such a process of systemic distribution of stem cells occurs. In the case of marrow stromal fibroblastic cells even the presence of fibroblastic progenitors in the blood, known for many years [Luria et al., 1971], does not prove that a circulatory fibroblastic stem cell theory equates to the known circulation of the haemopoietic stem cell. The fibroblastic cells in blood are known to be inducible by bone morphogenetic agents but now a recent report suggests that even determined osteogenic stem cells are present in the blood stream [Kuznetsov et al., 2001]. If these are functional "stem cells" in vivo, which is questionable, they do not appear to have extensive capacity for tissue production judging by the low levels of bone tissue observed to be formed practically in this and other in vivo implantation sytems. And why would these "circulating" stem cells be of physiological relevance when local osteogenic stem cells apear to exist in appreciable numbers into old age with negligible diminution in numbers during adult life. In addition, many studies have demonstrated the local origins of tissue regeneration in all organs, other than blood, and any appreciable systemic regeneration of tissues has not been reported and may be considered nonexistent in all other cases postnatally. With respect to bone formation, some reports have documented that synthetic hydroxyapatites can induce intrinsic osteogenesis, possibly by concentrating bone morphogens from tissue fluids [Ripamonti, 1996] and the influence of such a process on the implanted cells is difficult to assess. It could, however, contribute significantly to the idea that hydroxyapatite ceramics are of great value in demonstrating osteogenesis in specific cell transplants.

CONCLUDING COMMENTS

Can almost any cell be converted into a self-renewing unit, with resultant developmental plasticity of tissue phenotype as required? This possibility seems increasingly more practicable, especially with application of genetic engineering technology, with the promises of immense potential clinical benefits. Even so, the functional potentials of "stem cells" are probably going to be more restricted than present investigators would wish and many of the observed cell plasticities need further verification. It is, therefore, crucially important that studies should also assess the latent problems in the long term of introducing these highly proliferative cells, modified by culture and molecular biology techniques, locally or systemically into the body. When a living organism or single cell is

introduced into a closed system that is not definitively antagonistic, the cell will attempt to survive in whatever environment it finds itself. If this is a specific autologous cell derived from a distant site and carried to its new location by the blood stream, or cultured in vitro and implanted systemically or into local tissue sites, it is likely to be exposed to different developmental cues and signals to those normally perceived. These epigenetic signals in the environment of the donor cells seem to have profound effects on the subsequent patterns of differentiation. This may not be a surprising conclusion with the knowledge that physiologically normal animals of a number of mammalian species have been born following the transfer of somatic nuclei into recipient enucleated oocytes. The "environmental" signals in the oocyte promote reacquisition of an undifferentiated, totipotent nuclear status, and unravelling the mechanisms of how this process occurs is a significant biological target of the future. When deposited at a particular tissue site, the potential for the required tissue differentiation of the donor cell used in cell based therapy may become apparent, it may be non-beneficial, or even unlimited growth and cancerous development could result. Not enough is known about the long term effects related to the future therapeutic use of the variety of human stem and progenitor cells now being considered for clinical use. Furthermore, comprehensive investigations on the immunogenicity of stem cell transplants will be required to prevent possible host rejection. Embryonic stem cells may be considered more likely to have malignant potential because of their totipotent nature, but all highly proliferative cells and adult stem cells could be transformed into harmful agents. Risk assessment and determining the conditions for optimizing delivery to the required target tissue site for beneficial use of cellular potential for tissue regeneration should be the goal of future research in this area. The methods developed for using stem or progenitor cell-based therapy may or may not be relevant to normal physiological processes, however, the potential for genomic manipulations of these cells indicates that in the future functional and useful cell phenotypes may be attained for clinical benefit. The possibilities seem only limited by the imagination and wizardry of the experimenter, but these are likely to require many years of rigorous investigation before extensive clinical benefit is realised.

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