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Cardiovascular Research 58 (2003) 324–335

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

Origin and use of embryonic and adult stem cells in differentiation and tissue repair

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Received 6 September 2002; accepted 7 November 2002

Abstract

Stem cells are self-renewing, unspecialised cells that can give rise to multiple cell types of all tissues of the body. They can be derived from the embryo, foetus and adult. The ability of stem cells to divide but also to differentiate to specialised cell types like nerve and muscle, have made them candidates on which to base therapies for diseases and disorders for which no, or only partially effective, therapies are available. Replacement of defective or absent cells in defective tissues and organs could represent a cure. Here, we introduce the background to stem cell research and review the present state-of-the-art in stem cell biology, directed differentiation and tissue repair. In particular, we distinguish embryonic versus adult sources of stem cells and data derived from animal versus human experiments in order to place current research and perspectives for clinical application in their correct context.

Keywords: Developmental biology; Stem cells

1. Introduction

Stem cells are primitive cells present in all organisms that can divide and give rise to more stem cells, or switch to become more specialised cells, such as those of the brain, heart, muscle and kidney. Stem cells in early embryos give rise to cells of all tissues of the adult body and are therefore termed 'pluripotent'. Stem cells in some adult tissues, involved in tissue replacement and repair, usually give rise only to cell types already present in the surrounding tissue from which they are derived. Stem cells of the bone marrow, for example, give rise to hematopoietic cells. These adult stem cells are generally regarded as 'multipotent'.

Since the first report of the isolation human stem cells from surplus IVF embryos in 1998, their derivation and use has been hotly debated by governments, press and society in many parts of the world. Driving the debate has been the shortage of donor organs and tissues for regenerative medicine; diseases such as diabetes, Parkinson, rheumatoid arthritis and myocardial infarction caused by the loss or loss-of-function of specific cell types, could be cured if healthy cells replaced defective cells. However, ethical considerations question the instrumental use of embryos for the isolation of stem cells, even if those embryos are surplus to requirements for assisted reproduction and destined for destruction. The debate has been further fuelled by recent evidence that adult stem cells have a greater capacity for differentiation than had previously been thought; they have entered the discussion as alternatives. Although with obvious ethical advantages, the scientific question concerns whether embryonic and adult stem cells are equivalent in their capacity to produce large numbers of specific cell types for transplantation, which retain their function over long periods. Here, we first introduce embryonic stem cell biology in its historical context then consider current problems in controlling their growth and differentiation. Their potential in regenerative medicine is considered in the light of state-of-the-art advances in adult stem cell biology.

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Time for primary review 31 days.

0008-6363/03/$ – see front matter
doi:10.1016/S0008-6363(02)00770-8

2. Embryonic stem cells and teratocarcinomas

Exactly 20 years ago, the first embryonic stem cells were isolated from mouse preimplantation, blastocyst-stage embryos. The background to the discovery lay in the study of teratocarcinoma, a spontaneous tumour of the testis in mice and humans, consisting of tissues as diverse as hair, muscle, bone and even complete teeth. They resemble a disorganised foetus and have fascinated pathologists for a century or more (reviewed in Refs. [1,2]). In the mid-1970s, developmental biologists discovered that teratocarcinomas could be induced in mice by transferring embryos to extra-uterine sites and that they contained undifferentiated stem cells. These embryonal carcinoma (or EC) stem cells could be isolated and grown in culture without losing the capacity to differentiate. This was most strikingly demonstrated by introducing them into embryos; if derived from a brown mouse and placed in a blastocyst from an albino, the pups delivered by the foster mother were brown and white. The stem cells had formed 'chimeras' and had contributed to all somatic tissues, most obviously in the melanocytes of the skin. After differentiation, EC cells are no longer malignant; they therefore became not only a useful model for the study of development, but were also of interest to oncologists testing differentiation-induction as therapy for teratocarcinoma. Although this ultimately failed, pathologists gathered several diagnostic markers for the undifferentiated cells, useful in determining therapy and prognosis. Meanwhile, developmental biologists addressed the question of whether it would be possible to isolate stem cells directly from mouse embryos, without an intermediate teratocarcinoma stage. In 1981, two groups succeeded in establishing mouse embryonic stem (or ES) cell lines.

In view of the similarities between mice and human teratocarcinomas, it was predicted that ES cells could be isolated from humans. The motivation initially was for studying early human development but later, the perspectives for cell transplantation therapies became evident. First attempts were made in the mid-1980s, when embryos could not be cryopreserved and excess was discarded after IVF. The attempts were unsuccessful and were mostly discontinued when freezing of embryos became common practice. Exceptionally, Thomson [3] in the US continued, first in primates then in humans, and in 1998, his group published their breakthrough. In humans, verification of an embryonic stem cell phenotype by generation of a chimeric individual is of course not possible. The markers developed by pathologists to diagnose EC stem cells in tumours, thus provided the first evidence of their undifferentiated phenotype; their capacity to form teratocarcinomas containing many tissue types in immunodeficient mice confirmed their pluripotency.

An Australian–Singaporean group lead by Bongso and Trounson [4] later described the isolation of two human ES cell lines independently. As experts in human reproductive biology, their ability to assess the quality and stage of human IVF embryos has lead to exceptionally high efficiencies for hES cell line isolation, with 50% of blastocysts now yielding cell lines. Since then, a handful of publications have described their differentiation to various cell types in culture in response to cytokines, hormones and growth conditions. These include neural cells (neurons, glia, and oligodendrocytes) and, most recently, insulin-producing pancreas cells, cartilage and bone, cardiomyocytes, hematopoietic cells, endothelial cells and hepatocytes. Some of these differentiated hES cells have been transplanted into mice but the majority of the studies so far only differentiated phenotypes in vitro. The hype around embryonic stem cells derives largely from studies using mouse ES cells to derive differentiated derivatives which when transplanted to ailing mice have cured or relieved their disease. These results have often been extrapolated directly to the human equivalent, which cannot be justified without further validation using hES cells. In the following sections, we will provide an overview distinguishing results derived from human versus mouse ES cells both in terms of their ability to grow and differentiate in culture as well as in their ability to contribute to tissue repair. We provide a similar overview of the results in adult stem cells, largely concentrating on those derived from bone marrow as hematopoietic and mesenchymal stem cells are probably among the most accessible sources in humans and research on their potential usefulness is more advanced than that using stem cells from other adult tissues.

3. Growth and differentiation of embryonic stem cells in culture

3.1. Growth

The first mouse and human embryonic stem cell lines derived from blastocyst stage embryos were dependent on mouse foetal fibroblasts for maintenance of growth in an undifferentiated state [3–6]. In 1988, three groups identified the activity secreted by the fibroblast 'feeder' cells (MEFs) as leukemia inhibitory factor (LIF) and showed that this was sufficient to replace the feeder requirement for continuous undifferentiated growth [7–9] (see Smith [10] for review of signal transduction pathways involved in stem cell self-renewal). Unfortunately, this is not the case for hES cells and LIF cannot supplant MEFs [3,4], although semi-defined, feeder free conditions have recently been described by Xu et al. [11] where 100% MEF-conditioned medium is used in combination with basic fibroblast growth factor (bFGF) and a Matrigel substrate. A possible clue to this LIF insensitivity was provided by a study in human EC cells, which express both the LIF binding receptor and it, co-receptor, gp130 [12]. Human EC cells express elevated levels of the negative feedback protein suppressor of cytokine signalling 1 (SOCS-1) compared with mouse stem cells; this constitutive expres-

sion inhibits LIF signalling via STAT3 and may be applicable to hES given their similarities in other respects. Amit et al. [13] also described a refinement to culture conditions, which promoted clonal growth. Noting that foetal calf was often inhibitory to colony formation, they used a commercial serum replacement in combination with bFGF to show a 3.5-fold increase in colony forming efficiency above that under serum conditions. Apart from being important in demonstrating the pluripotentiality of single cells and possibly yielding more phenotypically stable cells lines [14], the ability to support clonal growth is essential for selecting transgenic hES cell lines on the basis of antibiotic resistance. Efficient stable transfection methods may be needed for creating or selecting pure populations of cells [15–17], directed differentiation [18], marking cells so that they can be recognised histologically and examining the role of specific genes in early human development.

Despite these advances, the culture and frozen storage of hES cells remains difficult, slow and labour intensive; some cell lines require manual dissection and transfer for passage [4,19] while bulk culture often results in non-specific differentiation. The presence of differentiated cells in mES cell cultures can in itself promote differentiation of any remaining stem cells; their systematic removal as new lines are established in culture increases the efficiency of their isolation [20,21]. Similar approaches may substantially improve hES culture methods. Likewise, identification of the MEF-derived hES differentiation inhibiting factor(s) will represent an important breakthrough once available as a culture medium supplement. Culture quality remains inexplicably variable for many researchers and may be the reason that the distribution of the cell lines on the National Institutes of Health's registry of lines accepted by President Bush as eligible for NIH funding has been slow [22]. An important step forward has recently been described where MEFs have been replaced by human foetal fibroblasts [23]. Not only has this eliminated the 'roller-coaster' variability in cultures of existing hES cell lines, the researchers have been able to derive a new cell line under these xenofree conditions. Although raising its own ethical questions, this new method represents a possible solution to the risks of cross-transfer of animal pathogens in xenosupport systems, which would have compromised future clinical applications. The cell line derived has a normal karyotype, tested positive for alkaline phosphatase activity, oct-4 expression and cell surface antigens SSEA-3, SSEA-4, Tra-1-60 and GCTM-2, the same panel of markers used to validate hES cells in the first publications of Thomson et al. [3] and Reubinoff et al. [4] and those on the NIH list. In addition the new cell line formed teratomas in SCID mice containing multiple differentiated tissues.

3.2. Differentiation

The ability to direct pluripotent stem cells into specific differentiation pathways and support the viability and maturation of differentiated phenotypes is still limited and essentially based on techniques developed with mouse EC cells [24,25]. Cell aggregation in suspension culture triggers differentiation in multilayered structures called embryoid bodies. Despite the absence of a body axis, differentiation proceeds in a manner reminiscent of the early mouse embryo and results in a range of differentiated cell types, including yolk sac endoderm, cardiomyocytes, embryonic and definitive hematopoietic cells, endothelial cells, skeletal myocytes, neurons and glia [26]. It is possible to bias the differentiation of mES cells using growth factors and/or retinoic acid [27–31] or blocking certain inhibitory pathways [32,33] or growing cells in co-culture with various differentiated cell types [34–37] but the final cultures are always a heterogeneous mixture of various cell types. Induction and selection [15,16,35,38,39] is probably the only way to obtain pure populations of cells suitable for transplantation. In monolayer culture in the absence of LIF or MEFs, mES cells also differentiate to cell types with various morphologies expressing a mixture of endoderm and mesodermal markers although no obvious mature phenotype [40]. However, clonal progenitors of the endothelial and hematopoietic lineages have been isolated more recently by FACS from these mixed monolayer cultures [41] and under appropriate conditions, these will go on to form an organised vasculature [42]. This demonstrates that somatic differentiation can take place in the absence of complex multicellular interactions and has practical implications since monolayer cultures are much more amenable to experimental manipulation and analysis than aggregation. An alternative approach would be forced expression of transcription factors known to be essential for specific differentiation events. A recent example is the ectopic expression of the transcription factors GATA-4 or GATA-6 in mES cells, which resulted in differentiation towards visceral endoderm [18].

Whilst mES cells and many mEC cell lines undergo substantial differentiation in embryoid bodies, most hEC cell lines require aggregation and/or high concentrations of retinoic acid [43]. They will then form extraembryonic endoderm or neural cells but few, if any, other somatic derivatives. By contrast, hES cells will undergo extensive (somatic) differentiation (Fig. 1) not only in vivo in teratomas in SCID mice [3,4,44] but also in culture where the formation of neurons, pancreas cells, cardiomyocytes, hematopoietic cells and endothelial cells has been described [4,13,45–53]. It is clear that high local cell densities are required for differentiation to take place and, just as for mES cells, the additional presence of growth factors can bias the differentiation program, but whether classical embryoid bodies actually form has been a point of discussion between different research groups (see Ref. [54]). The groups working with the H9 hES cell line derived by Thomson et al. [3] and its subclones, grow the cells as aggregates in suspension to trigger differentiation (e.g. Ref. [49]) but it is not clear whether an outer

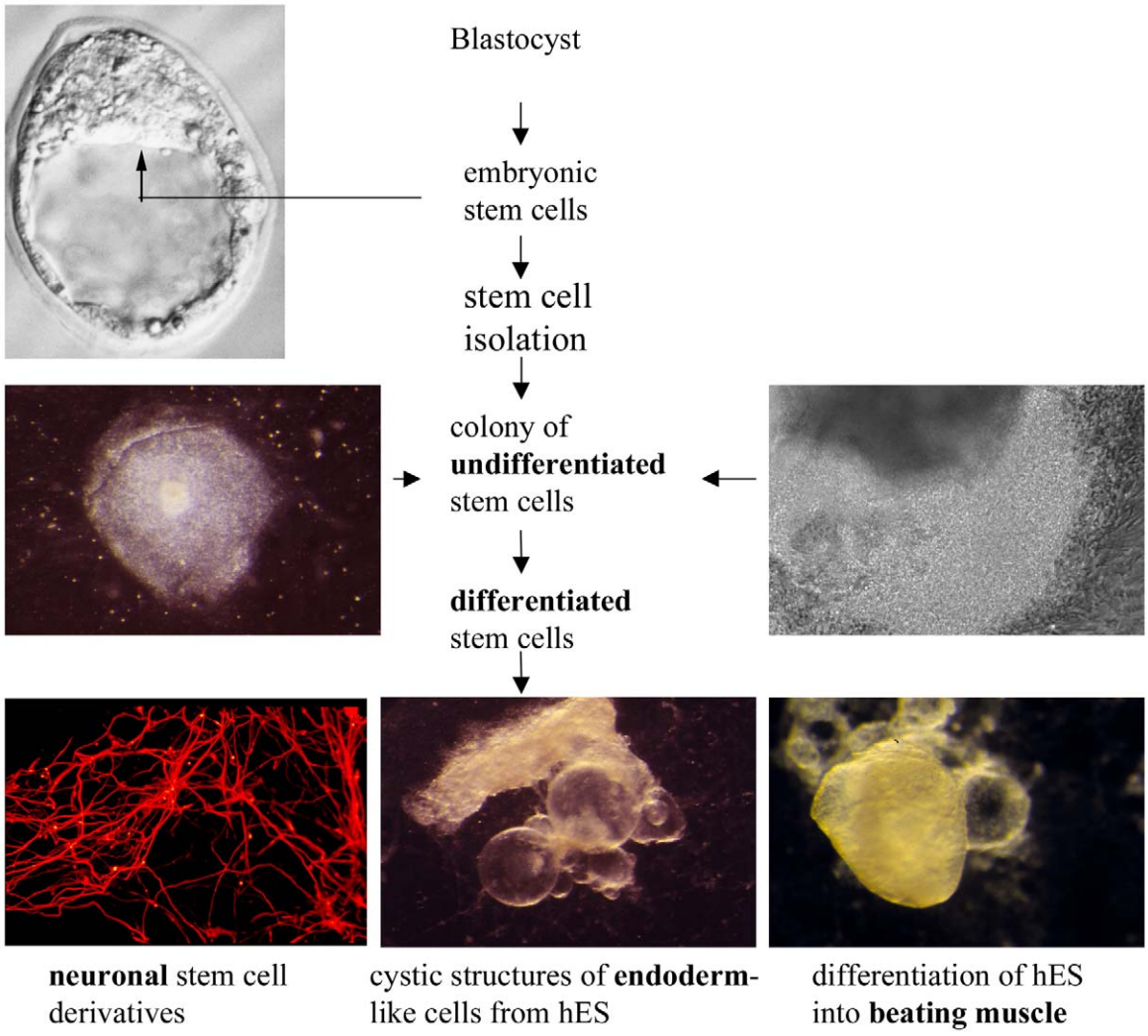

Fig. 1. From blastocyst, to embryonic stem cell line, to somatic cells. hES: human embryonic stem cell. Photographs D. Ward and L. Tertoolen, Hubrecht Laboratory.

endoderm layer forms as in mES-derived embryoid bodies; the group working with the cells of Reubinoff et al. [4], however, grow the cells to high densities attached to a substrate. Overall, however, the extent to which the hES cell lines differentiate in vitro does not match their more extensive differentiation capability in teratomas [44].

4. Embryonic stem cells and tissue repair

The ability of human ES cells to differentiate to cells from all three germ layers (ectoderm, endoderm and mesoderm) in culture is still a long way from contributing to regenerative medicine. Speculation here is being driven primarily by experiments in rodents with a variety of experimentally induced or genetic lesions. Mouse ES-derived cardiomyocytes have been transplanted to the heart and survived [15], rats with spinal cord lesions receiving mES cell-derived embryoid bodies were again able to bear their own weight [29,55] while more directed differentiation of mES cells into motor neurons showed that they were able to repopulate the spinal cord, extend axons and form synapses with target muscles [35]. Transplantation of ES-derived neural cells resulted in functional improvement in mice with Parkinson-like lesions [28,56,57] and ES-derived pancreas-like cells secreted insulin after transplantation, although in insufficient amounts to cure their diabetes [31]. Myelin became detectable around axons in mutant rats lacking myelin, after transfer of mouse ES-derived glial cells [58] suggesting a potential treatment for multiple sclerosis. Together, these results are highly encouraging of the feasibility of cell transplantation studies but are still far from clinical application. The major question is the extent to which these studies in rodents can be extended to humans. The differences in culture requirements between mES and hES have been described above but obstacles related to directed differentiation of hES and appropriate scale-up of transplantable cells to account for

the differences in size of humans and rodents only multiply the problems to be overcome before clinical application is a reality. So far there are three reports of transfer of differentiated hES derivatives to rodent models: two describe the derivation of neural progenitors from hES using a combination of high cell density and FGF and their transfer to the brains of neonatal mice [46,50] while a third describes the seeding of human embryonic endothelial cells, selected from hES cell aggregates using antibodies against PE-CAM, on biodegradable polymer scaffolds [51]. Upon subcutaneous insertion of these scaffolds in SCID mice, the (human) endothelial cells organise into vessel-like structures with mouse blood in their lumen, suggesting that microvessels had formed and anastomosed with the mouse vasculature, becoming functional blood-carrying microvessels. FGF and vascular endothelial growth factor (VEGF) known to promote vasculogenesis in mES embryoid bodies had apparently no effect on the hES aggregates, suggesting different mechanisms might be involved. In no case was there evidence of teratoma formation. There is one report of colonies of hES being grafted to somites in chick embryos where they were shown to divide and differentiate in the host tissue [59]. The host embryonic environment appeared to modulate their differentiation, suggesting that the chick may be a useful model system in which to study the integration of hES cells and their derivative into tissues in vivo.

There has been no transfer of hES derivatives to humans to date although there has been a report of transfer of neural derivatives of hEC cells to the brains of 11 stroke victims in a phase I (safety) trial [60]. The efficiency with which EC cells form teratocarcinomas is much higher than that of ES cells so this was as such a high-risk trial. In addition, and given the anxieties expressed over potential risks of the present xenosupport systems used for hES cells, the hEC cell lines used had been derived through cloning via transfer to mice. Fortunately after two years there was no evidence of tumour formation or other pathologies and the transplanted cells were still detectable; for two patients, a slight improvement in their symptoms was reported and a phase II trial has been initiated.

For human clinical use, graft rejection is likely to occur after transfer of differentiated hES derivatives to most organs except the (immune privileged) central nervous system. Evidence from human embryos had raised the possibility that hES cell themselves might be immune privileged since they did not appear to express MHC proteins. However, a recent study has shown that MHC class I molecules are expressed on the undifferentiated hES and that these increase after differentiation [61]. Among possible solutions to graft rejection would be building up a hES cell bank with a range of MHC profiles for matching to individual patients in combination with immunosuppressive drugs; less feasible alternatives would be genetic alteration of hES cells to develop a ‘universal donor’ that would not express MHC proteins or using nuclear transfer techniques to derive genetically matched stem cells for individual patients (therapeutic cloning). Both of these have their own particular disadvantages although a recent experiment carried out in mice demonstrated a particularly elegant combination of nuclear transfer, stem cell isolation and tissue transplantation [62]. Here, immune-deficient Rag2−/− mice were used as nuclear donors for transfer into enucleated oocytes, the resulting blastocysts were cultured to isolate an isogenic embryonic stem cell line and one of the mutant alleles in the Rag2−/− ES cells was repaired by homologous recombination. Mutant mice were then treated with repaired ES cells induced to differentiate in vitro to hematopoietic precursors; 3–4 weeks after engraftment, mature myeloid and lymphoid cells as well as immunoglobulins became detectable, establishing a paradigm for treatment of a genetic disorder by combining therapeutic cloning with gene therapy. For the present though, tissue matching is the major advantage that stem cells from adult human tissues represent above hES cells, as will be described in the following sections.

5. Transdifferentiation of adult stem cells

Success stories reported for embryonic stem cells have recently extended to adult stem cells. Adult stem cell function was assumed previously to be restricted to cell lineages present in the organ from which they were derived and to be involved solely in their repair. However, in recent years several lines of evidence have suggested that these adult stem cells are multipotent and can transdifferentiate into different cell lineages (Table 1). Adult bone marrow, brain, skeletal muscle, liver, pancreas, fat, skin and skeletal muscle, have all been shown to possess stem or progenitor cells with the capacity to differentiate or transdifferentiate into cell types other than their tissue of origin. Among all presently known adult stem or progenitor cells, cell populations from bone marrow have shown the highest potential with respect to multilineage differentiation and functional engraftment into host animals.

5.1. Bone marrow stem cells

Studies with bone marrow stromal or mesenchymal stem cells, a subset of cells that can be separated by plastic adherence, have shown differentiation into various cell types, including bone [63,64], tendon, cartilage [65] and fat [66]. A cardiomyogenic cell line was established by culturing mesenchymal stem cells from mice in the presence of the DNA demethylation agent 5-azacytidine. Upon induction, the mesenchymal stem cells showed a cardiomyocyte phenotype, expressed cardiac differentiation markers and began spontaneous beating [67]. Furthermore, these cells displayed functional adrenergic and muscarinic receptors [68]. The cardiogenic potential of

Table 1
Transdifferentiation of adult stem cells

Tissue of origin	Newly formed tissue	References
Bone marrow		
Unfractionated	Brain	[72,73]
	Kidney	[79,80]
	Skeletal muscle	[113]
Mesenchymal/	Brain	[71]
stromal	Bone	[63,64]
	Fat	[66]
	Heart	[67–69]
HSC	Liver, lung, skin, gastro-intestinal tract	[77]
MAPC	Brain, retina, lung, heart, skeletal muscle, liver, intestine, kidney, spleen, bone marrow, blood and skin	[84]
Brain	Heart, skeletal muscle, kidney, stomach, intestine, liver	[87]
	Blood	[85,86]
	Blood	[94,95]
Skeletal muscle	Heart	[99]
Liver	Bile duct	[97]
Fat	Pancreas	[98]
Skin	Bone, skeletal muscle	[101,102]
Pancreas	Fat, brain, muscle	[103]
	Liver	[100]

HSC, hematopoietic stem cells; MAPC, multipotent adult progenitor cell.

mesenchymal stem cells was further demonstrated in vivo by injecting early-passage human mesenchymal stem cells, expressing β-galactosidase (β-gal), into the left ventricle of SCID mice. Although only a small percentage β-gal$^+$ donor cells could be detected in the heart (at the most 0.44% of injected cells) 60 days after injection, engrafted β-gal$^+$ cells did express several cardiac markers, such as cardiac troponin T, alpha-actinin and phospholamban [69].

Injection of human marrow stromal cells into rat brains showed that cells migrated from the injection site to successive layers of the brain [70]. These findings were confirmed and extended by Kopen et al. [71]. Injection of murine marrow stromal cells into the lateral ventricle of neonatal mice, resulted in their migration throughout the forebrain and cerebellum and their differentiation into astrocytes and presumably neurones. Transplantation of unfractionated mouse bone marrow cells into mutant mice, lacking the ability to develop cells of the myeloid and lymphoid lineages [72], or in lethally irradiated mice [73], resulted in their migration into the brain and, most importantly, the donor-derived bone marrow cells differentiated into cells that expressed neuronal markers, such as NeuN. However, in a recent study, Castro et al. [74] did not observe transdifferentiation in a transplantation experiment where they used a similar unfractionated or subpopulation of mouse bone marrow cells. They came to the same negative conclusion following bone marrow transplantation in a model of neural injury, raising the question whether the transformation from bone to brain is a general phenomenon or dependent on the experimental system.

Hematopoietic stem cells (HSC) are present in the bone marrow at very low frequency and are able to repopulate the hematopoietic system. Recent studies showed that transplanted bone marrow cells, enriched for HSC are able to differentiate into hepatocytes in liver of rodents [75,76]. Krause et al. [77] demonstrated that a single HSC was not only able to repopulate the hematopoietic system in irradiated mice, but also differentiate into epithelium of lung, skin, liver and the gastro-intestinal tract. These HSCs showed increased expression of CD34 and SCA-1 during homing to the bone marrow. Although HSC may have the capacity to transdifferentiate, it was recently demonstrated that this is an extremely rare event. Injection of single mouse green fluorescent protein (GFP) positive c-kit$^+$Thy1.1^{lo}Lin$^-$Sca-1$^+$ HSC into lethally irradiated mice showed reconstitution of peripheral blood leukocytes, but no (kidney, gut, muscle and lung) or only a few (liver and brain) GFP$^+$ non-hematopoietic cells were detected. These data were confirmed in GFP$^+$:GFP$^-$ parabiotic (surgically joined) mice, which share a common anastomosed circulatory system [78].

Transplantation of gender mismatched bone marrow cells into mouse and human resulted in engraftment of donor-derived cells into kidney. Using epithelial markers it was demonstrated that donor-derived CD45$^-$ engrafted cells had differentiated into renal parenchymal and tubular epithelial cells in mouse and humans, respectively [79]. In accordance, transplantation of mouse bone marrow cells expressing GFP into irradiated mice demonstrated the presence of donor-derived mesangial cells in the glomeruli [80].

The group of Verfaillie [81,82] has described another

sub-population of bone marrow cells. These cells, that copurify with mesenchymal cells, are found in human, mouse and rat and have been named multipotent adult progenitor cells or MAPCs. Human and rodent MAPCs are $CD44^-$, $CD45^-$, HLA class I^- and II^- and $cKit^-$. Single MAPCs from human and rodents have been shown to differentiate in vitro, not only into mesodermal and neuroectodermal cells, but also into endodermal cell types with hepatocyte phenotype and function. Optimal differentiation was obtained by culturing MAPCs in the presence of FGF-4 and HGF on fibronectin, or a mixture of extracellular matrix components (Matrigel) [83]. In addition to in vitro differentiation into endoderm, mesoderm and ectoderm derivatives, injection of a single mouse MAPC into mice blastocysts and transfer to foster mothers, resulted in chimeric mice. When these chimeric mice were sacrificed MAPC-derived $\beta\text{-gal}^+$ cells were found in many tissues, including brain, retina, lung, myocardium, skeletal muscle, liver, intestine, kidney, spleen, bone marrow, blood and skin. The same study also demonstrated that mouse MAPCs engrafted and differentiated into tissue-specific cells following injection into irradiated SCID mice. Between 1 and 10% $\beta\text{-gal}^+$ cells were detected in blood, bone marrow, spleen and epithelium of lung liver and intestine, whereas no contribution to skeletal or cardiac muscle was observed in mice analysed 4–24 weeks after transplantation [84]. The single cell experiments have provided the most rigorous proof to date of the capacity of MAPCs to transdifferentiate into several lineages. Without these experiments it remained possible that the bone marrow cell populations used in fact contained a mixture of precursor by various lineages. It is unclear why several months are necessary before MAPC colonies start to grow in bone marrow cultures and it has been suggested that the cells may represent a tissue culture specific cell with no in vivo counterpart. Nevertheless, if a true source of transplantable autologous cells then their exact identity may not be important.

5.2. Brain stem cells

In addition to the observed plasticity of cell populations in bone marrow, it has also been shown that adult neural stem cells have a broader differentiation potential than previously thought. Embryonic and adult stem cells, clonally derived from the forebrain of mice and expressing β-gal ubiquitously, were systemically injected into sublethally irradiated mice. Although normally giving rise to neurons, astrocytes and oligodendrocytes, neural stem cells were able to engraft into the hematopoietic system of irradiated hosts to produce a range of blood cell types. First $\beta\text{-gal}^+$ blood cells appeared 20–22 weeks after injection, which was approximately 3 weeks later than following bone marrow transplantation [85]. The contribution of neural stem cells to the hematopoietic system was confirmed by transplantation of human brain derived cells into SCID mice [86]. Plasticity of neural stem cells was also shown by injecting neurospheres derived from single-cell cultures, into the amniotic cavity of chick embryos or aggregating them with mouse morulae. These chimeras were allowed to develop until different stages of development [87]. Although neural stem cell progeny were found, not only in ectodermal tissues, but also in mesodermal and endodermal tissues, such as heart, kidney, skeletal muscle, lung stomach, intestine and liver, no neural stem cell progeny were detected in the hematopoietic system, in contrast with the studies described above. The contribution of neural stem cells to repopulation of the hematopoietic system was further questioned by Morshead et al. [88], who used short-term and long-term passaged neurosphere cells for transplantation in irradiated mice. Although they found neural stem cells continuing to generate neural progeny, no neural stem cell progeny could be detected in the hematopoietic system; they concluded that the findings of Bjornson et al. [85] represented a rare event, and possibly caused by an accompanying genetic or epigenetic change.

5.3. Skeletal muscle stem cells

Adult skeletal muscle contains a population of myogenic precursors, the so-called satellite cells, which are capable of self-renewal and myogenic differentiation in response to physiological and pathophysiological stimuli. Furthermore, satellite cells or myoblasts have been shown to differentiate into slow-twitch muscle fibers when injected into the heart and improve cardiac function [89–91] (for reviews, see Refs. [92,93]). To investigate their possible plasticity, cells were isolated from skeletal muscle from adult mice, cultured and injected retroorbitally in irradiated mice [94]. Six weeks after transplantation high percentages (>50%) of donor-derived B-, T-cells, granulocytes and macrophages were found by fluorescence activated cell sorting (FACS). The adult skeletal muscle was demonstrated to contain a population of cells (approximately 1% of total) with a high efflux of the fluorescent dye Hoechst 33342 and expressing Sca-1 and c-Kit, characteristics of HSCs, but lacking the hematopoietic marker CD45. The authors suggested that these putative stem cells may be identical to satellite cells. In another study, a sub-population of skeletal muscle cells, $Sca\text{-}1^+$, lin^-, $c\text{-}Kit^-$ and $CD45^-$ were injected into the tail veins of lethally irradiated. Four to eight weeks after transplantation regeneration of muscle and blood cells, derived from donor cells, was detected [95]. However, recently, the group lead by Goodell [96] came to different conclusions. They used freshly isolated adult skeletal muscle cells from mice and separated these cells by FACS, based on the expression of CD45 and Sca-1. $CD45^+$ and $CD45^-$ cell populations were injected into irradiated mice and their contribution to regeneration of muscle cells and cells of the hematopoietic system was studied. Only the $CD45^+$ fraction generated hematopoietic

colonies in vitro and displayed a limited myogenic potential, whereas the $CD45^-$ fraction showed high myogenic potential, but lacked hematopoietic reconstitution. These findings made the authors conclude that muscle-derived HSCs are derived from the hematopoietic system rather than the adult muscle progenitor cell population as originally proposed [94,96].

5.4. Liver stem cells

A population of small oval shaped cells in the liver termed oval cells, is capable of proliferation and is thought to be the stem-cell compartment in the liver. In addition to the regeneration of hepatocytes, hepatic stem cells from rat have also been shown to give rise to bile duct cells [97] and pancreatic cells, producing insulin and glucagon [98]. Furthermore, cells from a clonal stem cell line (WB-F344), derived from an adult rat liver, were injected into the left ventricle of mice. Before injection cells were infected with a retrovirus expressing β-gal. $\beta\text{-gal}^+$ cells were detected in the mouse heart and a cardiomyocyte phenotype was observed as determined by the striated patterns and the co-localisation of cardiac troponin T and β-gal. Furthermore, electron microscopy showed the presence of well-organised sarcomeres, intercalated discs and gap junctions, co-localised with β-gal, suggesting but not proving their multipotency [99].

5.5. Pancreatic stem cells

Besides the ability of hepatic stem cells to differentiate into pancreatic cells, cell fractions from the rat pancreas were shown to differentiate into hepatocytes after transplantation to the liver. Following transplantation donor-derived cells expressed liver-specific proteins and showed phenotypical resemblance with hepatocytes [100]

5.6. Fat stem cells

Human adipose tissue obtained from liposuction procedures has also been used to isolate a fibroblast-like cell population, called processed lipoaspirate (LPA) cells. In vitro studies with LPA cells demonstrated differentiation into adipogenic, chrondogenic, myogenic and osteogenic cells [101,102]. However, contamination with stem cells derived from peripheral blood can not be excluded in these studies.

5.7. Skin stem cells

Cells isolated from the skin of juvenile or adult mice were cultured and studied for their ability to differentiate into cell-types of different lineages. In vitro experiments demonstrated differentiation of skin-derived cells and clones of individual cells into neurones adipocytes, glial and smooth muscle cells, based on phenotype and cell-specific markers. Furthermore, cultured skin cells from human scalp were also able to produce neural proteins, when induced to differentiate. These skin stem cells were different from mesenchymal or neural stem cells, as determined by their phenotype and the production of nestin and vimentin [103].

6. Adult stem cells and tissue repair

A sub-population of Lin^- and $c\text{-}Kit^+$ bone marrow cells was obtained from transgenic mice, expressing GFP ubiquitously. Following intra-ventricular injection of these cells in mice that had undergone experimental myocardial infarction, regeneration of cardiomyocytes was found in 40% of the animals [104]. Besides regeneration of cardiomyocytes, newly formed endothelial and smooth muscle cells, organised in coronary vessels, were also demonstrated. Nine days after transplantation, the percentage of newly formed tissue, derived from the injected bone marrow cells, was 68% of the infarcted region of the ventricle. Most importantly, cardiac function improved following transplantation with the Lin^-, $c\text{-}Kit^+$ sub-population, as opposed to the effects of transplantation of the Lin^-, $c\text{-}Kit^-$ sub-populations. In a subsequent study, the same group used a non-invasive method to increase the number of circulating stem cells in mice by treatment with stem cell factor (SCF) and granulocyte-colony-stimulating factor (G-CSF). Cytokine-treated mice displayed newly formed cardiac tissue in the infarcted region, and as a result decreased mortality and increased ejection fraction, parameters reflecting cardiac function. This experimental approach circumvented the high mortality of their previous study [105] and demonstrated the successful homing of donor-derived bone marrow cells into the infarcted region of recipient mice. However, it is not clear which sub-population of bone marrow cells is responsible for regeneration of cardiac tissue.

Another sub-population ($CD34^-$/low, $c\text{-}Kit^+$, $Sca\text{-}1^+$) of mouse bone marrow cells, also called side-population or SP, expressing β-gal were selected by FACS and injected into irradiated mice. SP cells also expressed the adhesion molecule PECAM-1 (CD31) and mRNA of Tie-2, Tal-2, VEGF-A and angiopoietin, all features of endothelial precursors. After 10–12 weeks animals with high contributions of engrafted cells were subjected to an experimental ischemia–reperfusion regime to mimic cardiac infarct. Homing and engraftment of donor-derived SP cells were found in the infarcted region of mice. These engrafted cells had differentiated into both cardiac muscle and had formed vessel-like structures [106]. Cardiac cells positive for β-gal were predominantly located at the border zone of the infarct region. A higher percentage was seen for endothelial engraftment (3.3%) than for cardiomyocyte engraftment (0.02%). In all cases however the contribution of donor

cells was low so that restoration of tissue function would remain questionable

Endothelial progenitor cells (EPC) have been identified in adult peripheral blood, bone marrow and human umbilical cord blood [107,108]. HSCs, which can also be isolated from human umbilical cord blood and EPCs have common cell markers, such as Flk-1, Tie-2 and CD34. Intravenous injection of G-CSF-mobilised human $CD34^+$ bone marrow cells into rats, which were subjected to myocardial infarction, resulted in homing of these cells in the infarct zone within 48 h after the ligation of the left descending coronary artery. No infiltration was observed in non-infarcted myocardium or in control hearts. The increased infiltration of $CD34^+$ cells in the infarct zone, lead to formation of capillaries of human origin, determined by DiI fluorescence labelling, within the central infarct zone. In addition, injection with $CD34^+$ cells also improved cardiac function in infarcted rats as opposed to injection of $CD34^-$ cells or saphenous vein endothelial cells [109]. In accordance, rat EPCs have also been shown to augment angiogenesis in ischemic tissues [110,111]. Furthermore, Condorelli et al. [112] showed that differentiated endothelial cells from the human umbilical vein can transdifferentiate into beating cardiomyocytes when cocultured with neonatal rat cardiomyocytes or when injected near the border zone in infarcted rat hearts.

Unfractionated bone marrow cells obtained from transgenic mice (under control of the muscle specific MLC3F promoter) were injected into injured muscle of SCID mice. In four of six $\beta\text{-gal}^+$ positive cells were found in injured muscle, 2 weeks after injection. Furthermore, bone marrow transplantation followed by induction of muscle injury was studied. After 5 weeks bone marrow-derived myogenic progenitors had migrated into regenerating muscle and participated in the regeneration process, giving rise to fully differentiated muscle fibers [113].

Bone marrow transplantation experiments in a model for hepatic injury, showed that new liver cells, partly derived from donor-bone marrow cells, were generated [75]. Bone marrow transplantation in a mouse model (FAH mutants) of a lethal hereditary liver disease resulted in improved survival of mice. Seven months after transplantation, mice were sacrificed and in all four mice $\beta\text{-gal}^+$ staining was observed in 30–50% of the liver cells. They also observed increased biochemical liver function (assessed by measuring levels of serum transaminases, bilirubin and amino acids) [114].

Recently, two research groups performed co-culture of mouse adult neural stem cells [115] or bone marrow cells [116] with mouse embryonic stem cells. Both groups came to the same surprising conclusion: adult stem cells can fuse with embryonic stem cells in culture. Co-culture of bone marrow cells with embryonic stem cells using selection markers that would eliminate the growth of embryonic stem cells, lead to colonies with an embryonic stem cell morphology, which they called bone marrow-derived embryonic stem-like (BMESL) cells. In vitro differentiation of these BMESL cells into endoderm, ectoderm and mesoderm was observed. However, unexpectedly BMESL cells showed tetraploid and hexaploid DNA content. The karyotype of two tetraploid cells was XXXY and these expressed genetic markers for both bone marrow cells and embryonic stem cells, indicating that fusion had occurred. Blastocyst injection of BMESL failed to generate chimeric mice [116]. Similar findings were obtained with the co-culture of neural stem cells and embryonic stem cells. Pluripotency of hybrid cells, expressing β-gal, was shown in vitro and by generating chimeric mice contributions to multilineage tissue in vivo was observed [115].

7. Conclusions

The results from the studies described in this overview suggest that undifferentiated stem cells derived from adult tissues are not determined progenitor cells with limited differentiation potential. Rather, these cells seem to possess a much broader capacity for cellular differentiation that is dependent on and responsive to specific cues present in the environment of the engrafted site. However, with the exception of three studies with bone marrow cells [77,78,84], this has not been rigorously demonstrated for any other stem cell of adult origin. Questions of scale-up and directed differentiation for transplantation and tissue repair remain to be answered, just as for embryonic stem cells, but their potentially autologous origin give them extremely promising potential in the development of cell replacement therapies.

Although considerable progress has been made towards understanding the control of differentiation of adult and embryonic stem cells, both have potential but a long way to go before clinical application. It is incorrect to say that adult stem cells are equivalent to embryonic stem cells for treating diabetes, Parkinson and probably other diseases. Adult stem cells have tissue compatibility advantages in transplantation but in certain genetic or autoimmune diseases, (matched) tissue from ES cells and immunosuppressive drugs may be preferable. Existing human ES cell lines are probably sufficient for current research if made widely available; only when transplantation becomes an issue may isolation of new lines for histocompatibility matching become necessary.

Acknowledgements

We thank colleagues at the Hubrecht Laboratory, University Hospital Medical School Utrecht, Interuniversity Cardiology Institute of the Netherlands, Monash University, Australia and Embryonic Stem Cell International for their input in our stem cell projects. Leon Tertoolen and Dorien Ward are thanked for contributions to Fig. 1.

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