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Editorial: Our Top 10 Developments in Stem Cell Biology over the Last 30 Years

LYLE ARMSTRONG,[a] MAJLINDA LAKO,[a] NOEL BUCKLEY,[b] TERRY R.J. LAPPIN,[c] MARTIN J. MURPHY,[d] JAN A. NOLTA,[e] MARK PITTENGER,[f] MIODRAG STOJKOVIC[g]

[a]The Institute of Genetic Medicine, Newcastle University, The International Centre for Life, Central Parkway, Newcastle upon Tyne, United Kingdom; [b]Department of Neuroscience, Institute of Psychiatry, King's College London, London, United Kingdom; [c]Centre for Cancer Research and Cell Biology, Queen's University, Belfast, Northern Ireland, United Kingdom; [d]AlphaMed Consulting, Inc., North Carolina Division, Durham, North Carolina, USA; [e]University of California, Davis School of Medicine, Davis, California, USA; [f]Johns Hopkins University School of Medicine, Severna Park, Maryland, USA; [g]SPEBO Medical, Leskovac, Serbia

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ABSTRACT

To celebrate 30 years of peer-reviewed publication of cutting edge stem cell research in *Stem Cells*, the first journal devoted to this promising field, we pause to review how far we have come in the three-decade lifetime of the Journal. To do this, we will present our views of the 10 most significant developments that have advanced stem cell biology where it is today. With the increasing rate of new data, it is natural that the bulk of these developments would have occurred in recent years, but we must not think that stem cell biology is a young science. The idea of a stem cell has actually been around for quite a long time having appeared in the scientific literature as early as 1868 with Haeckels' concept of a *stamzelle* as an uncommitted or undifferentiated cell responsible for producing many types of new cells to repair the body [Naturliche Schopfungsgeschichte, 1868; Berlin: Georg Reimer] but it took many years to obtain hard evidence in support of this theory. Not until the work of James Till and Ernest McCulloch in the 1960s did we have proof of the existence of stem cells and until the derivation of embryonal carcinoma cells in the 1960s–1970s and the first embryonic stem cell in 1981, such adult or tissue-specific stem cells were the only known class. The first issue of *Stem Cells* was published in 1981; no small wonder that most of its papers were devoted to hematopoietic progenitors. More recently, induced pluripotent stem cells (iPSCs) have been developed, and this is proving to be a fertile area of investigation as shown by the volume of publications appearing not only in *Stem Cells* but also in other journals over the last 5 years. The reader will note that many of the articles in this special issue are concerned with iPSC; however, this reflects the current surge of interest in the topic rather than any deliberate attempt to ignore other areas of stem cell investigation. STEM CELLS *2012;30:2–9*

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GOING WITH THE FLOW—FINDING AND ISOLATING ADULT STEM CELLS

July 16, 1945 was not an auspicious day. At the White Sand Proving Grounds, New Mexico at 5:29 a.m. local time, the world entered the nuclear age with the detonation of a 20-kiloton plutonium implosion device that although puny by the standards of later weapons changed our atmosphere forever by adding the first of many increments of radioactive nuclides. After 3 weeks, broadly similar devices were detonated over Japan, bringing an end to World War II and starting an enormous program of research into the effects of radiation on the human body. One of these effects turned out to be destruction of the cells in the bone marrow (BM) leading to catastrophic anemia in individuals exposed to high doses of ionizing radiation; a logical inference from this was that transplantation of unaffected BM might be a treatment for radiation sickness. The next logical step was treatment of hematological malignancies by radiation-induced ablation followed by transplantation of BM taken from donating individuals. It should come as no surprise, with the benefit of hindsight that these early attempts were

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Correspondence: Lyle Armstrong, PhD., The Institute of Genetic Medicine, Newcastle University, The International Centre for Life, Central Parkway, Newcastle upon Tyne NE1 3BZ, United Kingdom. Telephone: 00+44 191 241 8695; Fax: 00+44 191 241 8666; e-mail: Lyle.Armstrong@ncl.ac.uk or Majlinda Lako, PhD, The Institute of Genetic Medicine, Newcastle University, The International Centre for Life, Central Parkway, Newcastle upon Tyne NE1 3BZ, United Kingdom. Telephone: 00+44 191 241 8688; Fax: 00+44 191 241 8666; e-mail: Majlinda.Lako@ncl.ac.uk Received November 28, 2011; accepted for publication November 28, 2011; first published online in STEM CELLS *EXPRESS* December 7, 2011. available online without subscription through the open access option. 1066-5099/2011/$30.00/0 doi: 10.1002/stem.1007

unsuccessful due to immune rejection both from the recipient and the incoming donated BM (graft versus host disease); however, a detailed discussion of the development of the entire field of BM transplantation is beyond the scope of this article. For our present discussion, it is sufficient to note that the hematopoietic tissues of the BM produce a continuous supply of differentiated blood cells whose functions are essential for life. The resulting cells have short lifetimes and do not self-renew; therefore, replacing these cells relies upon the existence of cells that can not only produce new copies of themselves but also give rise to new differentiated cells to maintain blood function. The basis of this system is the hematopoietic stem cell (HSC) whose existence was first demonstrated by James Till and the late Ernest McCulloch in 1963 [1] in culmination of their earlier work on the radiation sensitivity of mouse BM cells by showing that limiting numbers of BM cells could give rise to clonal colonies of erythroid and myeloid cells in the spleens of the irradiated hosts. At this stage all we knew was that each spleen colony was genetically unique and had thus arisen from a single cell but we did not know much about the phenotype of the cells in question. Moreover, we had no protocols to isolate them; however, the development of flow cytometry by the 1980s permitted enrichment of the HSC from BM and so qualifies as the first major breakthrough in the history of our journal.

Flow cytometry was a well-established technique by the late 1980s by which time the original principles used to build early coulter cell counters had been advanced considerably. Fluorometry of a single cell as it traversed a beam of exciting light could be used to give quantitative information about the amounts of fluorescent dyes bound to the cell surface, and since a number of excitation wavelengths could be used, the number of possible combinations of dyes was large. Conjugation of such dyes to monoclonal antibodies directed against known terminally differentiated hematopoietic cell markers (such as those on B or T cells) meant that fluorescence-activated cell sorting could be used to separate these cells (lineage depletion) from less-committed progenitor cells. Development of antibodies against antigens present on the progenitor populations allowed us to fractionate this population still further. The investigations of Irving Weissman [2] showed that a population of mouse BM cells expressing the surface antigens Thy1 (low) and Sca1 from which lineage differentiation markers were absent (Lin negative) was able to rescue irradiated BM far more effectively than unsorted BM cells. Injection of as little as 30 cells into a lethally irradiated mouse rescued half of the recipients from BM failure whereas 13,000–33,000 unsorted BM cells were needed to achieve the same effect, indicating that the proposed stem cells were greatly enriched by this protocol. Many studies followed, defining flow-based isolation methods for human stem cells, assessed by xenotransplantation into mice that lacked the capacity for immune rejection. Flow cytometry is now used to enrich many types of adult stem cells and the differentiated progeny of pluripotent cells; without this technique, stem cell biology would be enormously difficult, and so even though its uses are not restricted to the stem cell arena, we have no hesitation in placing it high on our list of major stem cell breakthroughs.

The First Embryonic Stem Cells From MICE

Winning a Nobel Prize does not guarantee inclusion in our list but it does help! Quite apart from that, the work of Martin Evans, Matthew Kaufmann, and Gail Martin [3] was a tremendous boost to the study of pluripotent cells because it provided a seemingly stable and immortal cell line that was not transformed like the immortal lines derived from cancers. By the time mouse embryonic stem cells (mESCs) were derived, the concept of pluripotency, the ability of a single cell type to differentiate into multiple other types of cells, was far from new. The concept arose hand-in-hand with the theoretical proposition that stem cells were responsible for tissue repair in the adult from detailed histological studies of teratocarcinomas which are neoplasms composed of seemingly undifferentiated cells randomly interspersed with somatic tissues. The nature of these somatic tissues was fascinating because they appeared to represent various stages of differentiation. However, the fact that a single cell suspension produced from teratocarcinomas could give rise to completely new neoplasms in experimental animals suggested the presence of stem cells in their tissue mass. Lewis Kleinsmith and Barry Pierce [4] were the first to develop conditions to isolate and culture these cells and they coined the term “embryonal carcinoma cells” (EC cells) in 1964. What did Evans and Kaufmann do that was so special? They showed for the first time that it was possible to isolate the inner cell mass (ICM) of a mouse embryo at the blastocyst stage and coax those cells to survive and develop into colonies of cells that could be cultured as cell lines. This was a lot more difficult than it seems since several groups had attempted unsuccessfully to grow cells from the ICM, although much of this work was done to gain more understanding of embryonic development [5].

By the mid-1970s, we had learned that mouse embryos could complete at least some of their development outside the uterus and that the cells of the trophectoderm could proliferate in the absence of the ICM, but all attempts at independent culture of the ICM had failed. Michael Sherman observed that an enriched culture medium containing 10% heat-inactivated fetal calf serum promoted 90% of mouse blastocysts to hatch from their zona pellucidae and attach to the culture dish but he was unable to prevent differentiation of the ICM. Although the ICM cells expanded in culture to overgrow the trophoblast cells, they seemed to differentiate primarily to epithelial cells. Evans and Kaufmann solved this problem by removing the ICMs as they began to develop into egg cylinder-like structures and disaggregating them into single cell suspensions. These cells were passaged onto irradiated fibroblasts, whereupon colonies of cells that were morphologically similar to EC cells appeared [6]. Unlike EC cells, the mESCs (as they were called by Evans) had normal karyotypes and were able to form embryoid bodies when cultured away from their feeder fibroblasts. Prior to this development, EC cells were widely used to model developmental processes and early embryonic cell differentiation but within a few years, these were supplanted by mESCs principally because the latter are thought to be more representative of those cells present in the ICM (a snapshot of early development, if you will). A major contributing factor to the success of mESCs was the introduction of leukemia inhibitory factor (LIF) into mESC culture media to prevent differentiation and allow them to grow under feeder cell-free conditions [7]. This works because of the diapause phenomenon which permits several rodent species to generate a new batch of fertilized embryos while still pregnant. The new embryos arrest at the blastocyst stage of development until the uterus is vacated, whereupon they implant and resume their development.

Most of the last 10 years has seen the notion of pluripotency evolve as one of a “ground state,” a view protagonized by many notably Austin Smith and Rudi Jaenisch. This perspective sees the job of pluripotency factors as maintaining this ground state (at least in part) by inhibiting differentiation. In an elegant recent review [8] in which Loh and Lim challenge this view and present an alternative view of

pluripotency factors maintaining pluripotency (at least in part) by acting as mutually antagonistic lineage specifiers. As long as all factors are present and correct then this results in a metastable state that is pluripotency. Which view prevails, and whether stem cell biology is broad enough to accommodate both views, only time will tell.

Copying Genomes—The Development of Mammalian Cloning

One might not immediately think of mammalian cloning as a stem cell-related topic but enforcing epigenetic plasticity on a somatic genome, the essential basis of the cloning technique, told us a great deal lot about the molecular basis of pluripotency. Also, the technique was hailed as a possible means of producing ESCs for individual patients and since a wealth of data was derived from cloning experiments that led, albeit indirectly, to improved techniques for reprogramming somatic cells, cloning has its place in our list. Briggs and King [9–12] demonstrated that somatic cell nuclear transfer (SCNT) could be used to clone frogs. Using oocytes and donor nuclei from *Rana pipiens*, they found that the "reconstructed" embryos were capable of developing to at least the early cleavage stages and in some experiments as far along as the tadpole stage. The use of nuclei from blastomere cells was instrumental in this process because such cells are relatively unspecialized [13, 14]. In retrospect, it was not surprising that early attempts to use SCNT to clone frogs from adult somatic cells met with failure. However, later work by Gurdon [15] using intestinal cells from tadpoles demonstrated that differentiated somatic cells were capable of producing viable embryos. In contrast, SCNT in mammals was more difficult and for many years it was believed that the cells of adult vertebrates were simply too specialized to revert to a totipotent state. This opinion was decisively contradicted with the cloning of "Dolly" in 1996 [16] by fusion of a mammary gland epithelial cell from a Finn Dorset ewe with the enucleated oocyte from a separate donor. Many studies have now shown the possibility of SCNT in various mammalian species [17–19] but for a while the interest lay with mouse SCNT because it proved possible to derive ESC from blastocysts obtained from SCNT of murine fibroblasts into early-stage oocytes [20]. For a while, such nuclear transfer (nt) ESCs were hailed as the solution to the problem of immune rejection of differentiated cells generated from human ESC but so far derivation of nt-ESC from human SCNT blastocysts has been elusive. Even if such derivation is achieved, supply problems with human oocytes may render the technique only marginally useful.

Human Embryonic Stem Cells

Derivation of human ESC (hESC) was not rapid following mESC since it took 17 years before James Thomson at the University of Wisconsin reported his first five hESC lines. Fresh or frozen early cleavage stage human embryos, produced by in vitro fertilization (IVF) for clinical purposes, were donated by individuals after informed consent. Embryos were cultured to the blastocyst stage, 14 ICMs were isolated, and five hESC lines originating from five separate embryos were derived, essentially as described for nonhuman primate ESC (which was published 3 years earlier [21]). These cells were more difficult to grow in culture than mESCs but behaved in a broadly similar fashion in terms of their apparent immortality, expression of key surface antigens, and their ability to generate teratomas in immune compromised mice. Naturally, it was impossible to examine germline transmission following injection into blastocysts since manipulation of human embryos in this manner is illegal.

The method used by Thomson et al. was actually very similar to that of Evans in his mESC derivation work; however, the delay in obtaining hESC may be attributed to problems with the ethical issues and the availability of the necessary human embryos. The original article describing the first five hESC lines was published in *Science* in November 1998 [22] and although it is a fairly brief report in its own right, the significance of this development cannot be underestimated since it is the forerunner of hundreds of new hESC lines derived in the 13 following years. Subsequent research has shown that hESC while similar to mESC have many unique characteristics for modeling human development.

When Good Cells go Bad—The Concept of Cancer Stem Cells

We have come a long way in developing methods to kill cancer cells that form a variety of malignancies but relapse is an ongoing problem, along with the development of metastatic tumors at sites remote from that of the original tumor. One suggestion to account for these phenomena is the existence of a tumorigenic stem cell that is capable of regenerating all the differentiated cell types present in the original tumor. Most chemotherapeutic treatment strategies kill the replicating differentiated cells that form the bulk of the tumor mass and these may not be able to destroy all of the rare quiescent putative cancer stem cells (CSCs). If these CSC have self-renewal and expansion characteristics similar to nontumorigenic stem cells, it would only require a few survivors to generate a whole new tumor. The key paper supporting the CSC hypothesis from the laboratory of John Dick appeared in 1997, in which he and Dominique Bonnet demonstrated that an isolated cell type was capable of initiating acute myeloid leukemia [23]. These cells were exclusively $CD34^{+}CD38^{-}$ similar to normal hematopoietic progenitors suggesting that normal primitive cells rather than the more committed hematopoietic cell types are responsible for leukemic transformation.

Induced Pluripotent Stem Cells

The pluripotency of ESC makes them a potentially attractive resource for generating clinically useful somatic cells but for the problem of immune rejection. Transplanting differentiated cells obtained from ESC lines is the same as transplanting those cells from the individual from whom they were derived. There have been several attempts to avoid this problem, not the least of which was the therapeutic cloning approach described above, but the field was excited in 2006 by the generation of induced pluripotent stem cells (iPSCs) from the laboratory of Shinya Yamanaka which demonstrated the reprogramming of mouse somatic cells to pluripotency. Retroviral transduction of just four genes (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*) reprogrammed mouse embryonic fibroblasts and adult tail fibroblasts to show characteristics reminiscent of mESC. The resulting cells were named iPSCs and they were capable of contributing to chimeric animals with germline transmission and contribution to all tissues of the resulting offspring indicative of their pluripotency [24, 25].

Yamanaka's group extended their earlier work and showed that human adult dermal fibroblasts could be

reprogrammed by retroviral transduction of *Oct4*, *Sox2*, *Klf4*, and *c-MycOCT4*, *SOX2*, *KLF4*, and *C-MYC* with good efficiency [26]. James Thomson's laboratory found that a slightly different set of factors, *OCT4*, *SOX2*, *NANOG*, and *LIN28*, were sufficient to allow iPSC generation from fetal and adult fibroblasts [27] via lentiviral, rather than retroviral transduction, allowing the transduction of nondividing cells, which was not previously possible using retroviruses. iPSCs are a truly remarkable development which could open the way to patient-specific regenerative medicine and so they qualify as one of the most significant events in our list of the top 10 developments in stem cells but they are not without problems. Initial iPSC studies used retroviruses for gene transfer into target cells, since it was believed these genes would be silenced [28, 29], but alongside their inability to infect nondividing cells [30], it was noted that gene silencing was not maintained in iPSC raising the risk of tumorigenesis [31, 32]. Constitutive lentiviral use, in which transgene silencing is poor [29, 33, 34] was superseded by inducible lentiviral methods which hoped to attain full silencing of transgene expression upon attainment of the pluripotent state. However, the common problem with these vector types is the possibility of mutations upon integration or reactivation of the transgenes, which has been shown to lead to tumorigenesis [25]. New vectors have been developed that can be removed from the reprogrammed genome and further developments of RNA-based reprogramming systems such as those using microRNAs, isolated proteins, and small molecules show some promise for deriving integration free iPSC lines but there are still concerns that the somatic genome may not have been fully reprogrammed to pluripotency [35–38]. Some of the concerns are manifested as an epigenetic trace left over from the cell of origin. Even though this trace may be erased over extended passage, the observation raises the concern over the impact and importance of cell-of-origin and its subsequent translational value. Another comfortably forgotten fact in the iPSC field is the representative nature of a single iPSC line—this again points to "not all iPSCs are equal" and again should act as a caution in considering patient-specific iPSCs (see below). These problems could restrict the clinical utility of iPSCs and are under intense investigation by many groups. However, generation of in vitro models of human disease using patient-specific iPSCs is allowing investigation and the generation of a wealth of data that promises to make a major impact on science and medicine.

As an illustration of the use of iPSCs for modeling disease in a dish we can turn to the work of Rusty Gage. At a time when Pharma is retreating from neurodegeneration/neuropsychiatric research because of cost and paucity and inadequacy of animal models, the use of iPSCs as "disease in a dish" assume massive potential. Gage derived iPSC from fibroblasts of schizophrenic patients and subsequently differentiated them into neurons and showed reduced connectivity and synapse formation. Most dramatic was the rescue of this "disease phenotype" by application of the antipsychotic, loxapine [39]. There is a long road between disease in a dish and cures for psychiatric disorders, but at least iPSCs offer a tractable system to interrogate cellular and molecular mechanism.

Mesenchymal Stem Cells

Number 7 in our top 10 is devoted to the study of mesenchymal stem cells (MSCs). The reasons behind the inclusion of MSCs are simply that they are currently the most prolific source of potential therapeutic strategies for human disease and numerous clinical trials are underway using this versatile source of stem cells.

MSCs may be isolated from human BM and the first experimental evidence for the existence of a stem cell population in this tissue compartment other than the HSCs arose in the 1960s. This predates the focus of our review but it is worth mentioning to set the later studies in context. Transplantation of decellularized bone to ectopic sites demonstrated that cells from other tissues could generate bone. Pursuing the source of this potential, Friedenstein identified adherent fibroblast-like cells from BM capable of osteogenesis in vivo [40]. Such cells were believed to be a component of the BM stroma needed to support and nurture the hematopoietic functions of the BM but their ability to differentiate into other cells types, such as chondrocytes and adipocytes demonstrated additional lineage potentials. At this point, the multipotency of such adherent fibroblastic cells was recognized and they became known as MSCs rather than simply marrow stromal cells [41]

The name change proved to be quite fortuitous because it soon became apparent that MSCs could be derived from sources other than BM. By the late 1990s, it seemed that umbilical cord blood, in addition to being a valuable source of HSCs, also contained multipotential cells similar to those found in the BM [42]. While it should have been no surprise that the umbilicus would require the presence of stromal cells to generate a niche capable of supporting its HSCs, some sources of MSC were less obvious. For example, cells very similar to MSCs have been isolated from adipose tissues, amnion, placenta, and even the deciduous teeth of younger individuals [43–45]. An entire field of medicine now centers on the use of adipose-derived MSCs for tissue repair. The degree of similarity between MSCs from such seemingly diverse sources is still a matter of some debate despite early suggestions that they are present in the connective tissues of many organs and surround blood vessels as pericytes and may contribute to maintenance of organ integrity.

We may find that other adult or tissue-specific stem cells are capable of similar feats but to date the evidence for this is not so strong and the ubiquitous nature of MSCs suggests they may have a degree of plasticity not enjoyed by other stem cell types. The ease of obtaining MSCs means they have been the subject of more intensive investigation which has brought them closer to the point of medical or commercial application than many other types of stem cell. A recent example in which MSCs were applied to tissue engineering is given in our next top 10 topics.

Tissue Engineering With Stem Cells

Growing stem cells in the laboratory is fine for investigating their molecular characteristics and differentiation ability; however, long-term goals of making whole human organs for transplant into patients requires some different approaches. One of the major problems of growing stem cells enriched from the body is that they are no longer in the three-dimensional (3D) microenvironment that supports and nurtures the cells and encourages their efficient function. For these reasons, it is difficult to get stem cells to re-create the complex 3D structures of organs, especially since the patterns of most organs were laid down during embryonic development and the stem cells were only required to replace cells lost from the existing structure. One way around this problem is to build a scaffold onto which the stem cells (and other types of cell) can be engrafted. Artificial scaffolds have been created

from collagen, hydroxyapatite, and various biodegradable polymers most of which have been used for building artificial bone.

The technique of tissue or organ decellularization strips away cells and antigens, to leave a scaffold composed mostly of the extracellular matrix deposited by the cells in the original structure. This is an interesting concept since it suggests that all the positional information required to build an organ is present in its extracellular matrix "skeleton." This implies the presence of signals to tell specific cell types where they need to attach and this was the basis of attempts to construct animal hearts from cadaveric examples from which the cellular material had been removed by perfusion with detergent solutions. A specific example of this type of experiment was performed by the group of Doris Taylor at the University of Minnesota in which beating rat hearts were generated by recellularization of the extracellular matrix scaffolds using suspensions of neonatal cardiac cells [46]. Taylor's group thus introduced the concept of tissue decellularization as "nature's platform" for rebuilding organs. Decellularized tissues were prominent in the 1980s when bioengineers were comparing their polymer materials to natural products. Small intestinal submucosa has been used since 1960s, and decellularized pig valves were used clinically for many years. An exciting extension of this technique is Anthony Atala's use of collagen–polyglycolate polymers as scaffolds on which smooth muscle cells obtained from a biopsy taken from a diseased bladder could be seeded followed 48 hours later by urothelial cells from the same biopsy. After 3–4 days, these cells had colonized the scaffold sufficiently well to allow the whole structure to be transplanted into the patient [47]. The patients' cells remodeled and replaced the foreign materials and the resulting bladders functioned well in the recipient patients.

The most successful and well noted clinically used decellularized tissue engineering product was the artificial trachea transplanted into a patient in Barcelona in 2008 (to replace the patient's left bronchus) [48]. This procedure was exciting because the research group used a cadaveric trachea that had been decellularized. This scaffold was readily colonized by the patient's own epithelial cells and chondrocytes derived from MSCs and provided a functional section of airway that was an immediate replacement for the patients damaged bronchus. The patient is healthy 3 years after surgery and several other attempts to transplant sections of trachea made outside the body have been recorded worldwide and are now extending to pediatric patients.

Some organ structures are more complex than others so it remains to be seen how effective these techniques will be in future studies especially since many cell types are needed to build the various parts of the organs. These problems are being addressed in various investigations worldwide, such as in lung and liver bioengineering, where reconstructed organs based on the innate decellularized tissue can survive for at least 2 months in vivo [49, 50] and if they can be overcome, this could be a most interesting method of generating new organs for transplant, and hence this development has been included in our top 10 list.

Improving Genetic Manipulation

Until a few years ago, what you saw was largely what you got with ESC, at least as far as their genomes were concerned. It was possible to insert constructs with reporter genes and as long as you did not break up the colonies of cells too much, you could put them under antibiotic selection and probably obtain a cell line with a stable integrated reporter. The trick was not to be too liberal with the trypsin because pluripotent cells did not like being on their own very much. Single hESC will adhere to feeder cells or extracellular matrix monolayers but their survival rates are low. Another significant problem was that one could never be sure quite where the transfected construct was going to integrate into the genome meaning that expression of the transgene could be unpredictable over time due to atypical epigenetic changes occurring near its site of integration. There was also the complication of multiple integrations of the transgene per cell and disruption or activation of other genes, creating a nonisogenic experimental setting.

Targeting gene constructs to specific genomic loci offers the possibility of specific permanent editing of the genome providing a truer representation of genetic behavior in its native environment. The best way to achieve this uses homologous recombination by delivering a DNA template with long regions of homology to the target locus. This technique has been routinely used for successful and efficient gene targeting in mouse ESC with a homologous recombination rate of 1 in 10^3 cells, to knock genes in and out, and generate transgenic lines, which have been important for elucidating gene function. Applying the same procedure to hESC only achieves a recombination rate of 1 in 10^6 cells because successful transfection with the gene targeting construct relies on a single cell suspension. This is not a problem for mESC because we can prevent their differentiation with LIF but since this does not apply to hESC, the procedure does not work well and following the first report of human gene targeting by Thomas Zwaka [51] only a few publications reported the use of this technique.

The recent development of zinc finger nucleases (ZFNs) promises to change this state of affairs and so are worthy of inclusion into our top 10 list. ZFNs [52, 53] are designed to recognize specific DNA sequences by combining C2H2 zinc finger proteins [54] into a customized array [55]. This zinc finger domain is linked to the nonspecific FokI nuclease which cleaves the DNA into a double-strand break (DSB) [56–59]. For its activity, the FokI domain needs to dimerize [60]; thus ZFN pairs are required and hence designed to bind to the region of interest in the opposite orientation. DSBs are bad for genome stability so the cell proceeds to repair them as quickly as possible using either one or both of two available methods. Homologous recombination is more accurate but relies on the presence of the homologous sequence from the undamaged sister chromatid as a template. It has been shown that the HDR apparatus can use a supplied donor DNA plasmid which contains homology arms as a surrogate template. This approach allows for gene correction of single nucleotide changes from an exogenous episomal donor to the endogenous locus. Larger sections of DNA can also be inserted into the genome at a desired location using this technique making this one of the most powerful methodologies available for manipulation of pluripotent stem cell genomes (for a comprehensive review of ZFN-based gene targeting see [61]).

Getting Closer to Cures

The ultimate objective of all stem cell research is to understand human biology and use this knowledge to cure human diseases but how close are we to this goal? In truth, stem cell transplants have been used for many years in the treatment of leukemias (BM transplant) but given the amount of media

attention devoted to the field since the first derivation of hESC, it is important to demonstrate that clinical application of stem cell biology is possible. For the final entry in our top 10 list, we have included a number of examples of ongoing clinical trials to demonstrate that stem cell-based cures are not merely some hypothetical concept that are always, "just a few years down the line."

Our first example illustrates an application of adult tissue-specific stem cells to restore sight and results from the efforts of several groups worldwide. The human cornea is maintained by a population of stem cells residing in the limbus which is the border between the transparent region of the cornea and the opaque conjunctiva. In cases of chemical, mechanical, or thermal injury to the eye, the stem cells can be destroyed leading to reduced corneal maintenance which in turn permits the conjunctiva to grow over the spaces they leave behind. The excessive growth of opaque tissue naturally occludes vision but is also very painful. The global term for this condition is limbal stem cell deficiency (LSCD) and it often requires long-term, costly treatment with frequent clinic visits and intensive hospital admissions. The vision loss due to LSCD makes this disease not only costly but also often requires social support due to the enormous impact on the patient's quality of life. This is further magnified by the fact that LSCD mostly affects young patients. If the damage is unilateral, it is possible to excise a small amount of tissue from the limbus of the healthy eye and after appropriate culture, colonies enriched in limbal stem cells can be derived. Attachment of these cells to small pieces of human amniotic membrane or culture of single cell suspension on mitotically inactivated feeder cells allows them to be engrafted into the surgically exposed limbus of the damaged eye whereupon the stem cells can recolonize their intended niche [62, 63] eventually restoring sight. The technique also avoids the need for drugs to suppress immunity and means there is no chance of the implanted cells being rejected.

Our next example of a stem cell-related therapy takes us back to the MSC arena. Cellular therapies for myocardial infarction (MI) are currently emerging that include i.v. delivery of culture-expanded BM-derived MSC. It was initially hoped that MSCs would differentiate to the tissue of interest, but their potent secretion of factors to help heal and revascularize tissues is emerging as the more important mechanism. A great advantage of MSCs is their seemingly low immunogenicity which permits the use of allogeneic cells. This is a significant benefit since expansion and application of limited numbers of batches, or "lots" of MSC would be more cost-effective and well-controlled in a Good Manufacturing Practice setting than isolation and expansion of MSC from every patient requiring treatment of this type. Clinical trials have taken place which established the safety and efficacy of allogeneic MSC transplantation, first for graft versus host disease [64] and then for MI treatment [65]. In terms of pulmonary function and cardiac performance, MSC-treated MI patients showed significant improvement relative to those treated with placebo, and there was no evidence that i.v. administration of MSC resulted in formation of tumors or ectopic tissues. In addition, there was no evidence of organ damage due to MSC lodging in the microvasculature. Thousands of patients have been safely treated with expanded MSCs worldwide, and phase II and III clinical trials for many indications are ongoing. However safe, there remains significant room for improvement in the engraftment of MSCs, as only 1%–2% are detectable in the recipients after a short period (1–2 weeks).

The progress toward stem cell cures is not always straightforward as shown by the recent controversy surrounding the Geron Corporation's phase I clinical trial to examine the safety and efficacy of hESC-derived oligodendrocyte progenitor cells in treating spinal cord injuries (SCIs). Approximately 12,000 people in the U.S. sustain SCIs every year caused by trauma to the spinal cord that results in a loss of such functions as locomotion, sensation, or bowel/bladder control. A traumatic blow to the spine can fracture or dislocate vertebrae that may injure the nerve fibers and the glial cells that insulate the nerve fibers in the spinal cord. SCIs do not repair spontaneously but oligodendrocyte progenitor cells have demonstrated remyelinating and nerve growth stimulating properties leading to restoration of function in animal models of SCI. This works because oligodendrocytes naturally synthesize the myelin that wraps around the axons of neurons to enable them to conduct impulses in a manner analogous to the insulation surrounding electrical wires. Oligodendrocytes also produce several neurotrophic factors that promote the survival of neurons and preclinical studies have shown that injecting oligodendrocyte precursor cells (made from ESC) into rats with SCI allows new oligodendrocytes to colonize the injury site where they proceed to generate new myelin and promote neuronal growth [66]. These data encouraged the idea that SCI might be treatable using ESC-derived oligodendrocytes and Geron has pressed hard in recent years to push the concept to a clinical trial so it was surprising that the company called a halt to the study in mid-November 2011 citing economic reasons. The company claimed that further development would costs $25 million per year and this was too large a drain on its resources to justify supporting a research program from which no products have yet arisen. We understand that Geron is seeking alternative business partners to continue this project so we can only hope that this would be resolved quickly and that this important pioneering translational work resumes.

Whereas Geron's approach in repairing the damaged nervous system is based on transplantation, as is much of the work on "simple" neurodegenerative diseases such as Parkinson's Disease, it is much less clear what transplantation has to offer more complex neurodegenerative disorders such as Huntington's or Alzheimer's disease. An alternative strategy is to recruit the endogenous neural stem cell machinery that lies within the neurogenic niches of the forebrain; no transplantation, no rejection of heterologous transplants, no trauma. An even more radical idea is to recognize the latent neurogenic capacity of parenchymal "non-niche" reactive astrocytes—reactive astrocytes are by definition at the site of injury/degeneration—right where you need to initiate repair. Magdalena Gotz has led the way on this over the last decade [67] and has clearly shown that reactive astrocytes possess a latent neurogenic capacity that is clear in a dish—the challenge is overcoming the inhibitory signals from the brain that block this capacity in vivo.

Conclusion

There is not enough space in this review to include all the exciting developments that are currently taking place in the use of stem cells to improve the lives of patients suffering from a broad spectrum of diseases throughout the world. We hope that this brief listing of our top 10 developments over the last 30 years of *Stem Cells*' history has convinced the reader that far from being an obscure academic discipline, the study of stem cell biology is making significant contributions to the quality of human life. While we are pardonably proud of the prominent role that *Stem Cells* has played in helping to prosecute the peer-reviewed progress of the past three decades, we are all the more mindful of the abiding responsibilities we shoulder as the first and oldest journal devoted to discovering the universe of secrets still enwrapped within stem cells. We accept it as a sacred trust and, as we did at the

outset 30 years ago, we ask you, our readers, and our authors, for your collegial collaboration.

We are grateful to our four Founding Editors, Donald Metcalf, Fumimaro Takaku, and the late Laszlo Lajtha who, with Martin Murphy serving as Editor-in-Chief, founded the Journal in 1981, "by scientists in the service of science" at the dawn of what may be truly called the Stem Cell Era. *Stem Cells*, first published by Karger (1981–1983), was published by AlphaMed Press as *The International Journal of Cell Cloning* from 1983 to 1994 when AlphaMed Press reclaimed its founding name, *STEM CELLS*®, which was then trademarked on the Primary Register. We are grateful to CurtCivin, who led *Stem Cells* from 2000 to 2007, to Donald Phinney and Miodrag Stojkovic, who were its coeditors from 2007 to 2009, and to Miodrag Stojkovic, who served the Journal as editor from 2009 to 2011. Now, in January of 2012 at the dawn of its third decade, we warmly welcome Jan Nolta as she takes up her role as the Journal's Editor … with the same mission and renewed commitment to publishing excellence by scientists in the service of science.

Finally, we pause to acknowledge our gratitude and our respect for our publisher and managing editor, Ann Murphy, who has been the guiding hand behind every issue of the Journal for three decades. She is the glial element that has bound us together and, by her example, reminds us to devote our very best to this very special journal.

We would be delighted to hear from you, our readers. What is the one scientific advance that you believe—evidence-based—should have been included in your top 10? The best will be published as Letters to the Editor, as space allows.

Working together, the best is yet to come!

Disclosure of Potential Conflicts of Interest

The authors declare no conflicts of interest.

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