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Adult Bone Marrow Stem Cells for Cell and Gene Therapies: Implications for Greater Use

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Abstract There is excitement generated almost daily about the possible uses of stem cells to treat human disease. Much of the interest of late is generated by embryonic stem cells (ESCs). As exciting as ESCs may be, they are quite controversial for moral reasons, given their source. They are also scientifically controversial since they are much less well understood than the original, long-standing, and clinically successful hematopoietic stem cell (HSC). HSCs have the distinct advantage of being reasonably well characterized and have been proven in the clinic. They can be isolated by simple procedures directly from the bone marrow or from peripheral blood after being stimulated (mobilized). They can then be manipulated and delivered to a patient, often producing a cure. Their biology provides the paradigm by which all other stem cells are judged, and they have little in the way of moral controversy surrounding them given they are isolated from adults who have consented to the procedure. Another putative stem cell has gained momentum in the last few years; the mesenchymal stem cell (MSC). MSCs appear to have much in common with HSCs. They were originally characterized from bone marrow, are capable of differentiating along multiple lineages and, at least in vitro, have significant expansion capability. Unlike HSCs, they have not yet been definitively shown to function as stem cells, despite their ability to differentiate into various mesenchymal cell types under the right culture conditions. Still, there is mounting evidence these cells may be useful, if not as true stem cells then at least as vehicles for emerging cell and gene therapies, especially in the field of tissue engineering. While this is an important endpoint, it is more important to thoroughly understand stem cell biology. That understanding can then be applied toward the ultimate goal of using these cells not just for various forms of therapy, but rather as a tool to discover the mechanisms and means to bring about directed repair and regeneration of damaged or diseased tissues and organs. The excitement of HSCs and MSCs has been muted somewhat by the excitement surrounding ESCs, primarily due to the fact HSCs and MSCs are viewed as limited to specific cell types while ESCs could potentially be applied to any cell type. Recent information indicates HSCs, MSCs, and other cells in general may have more universal differentiation abilities than previously thought. J. Cell. Biochem. Suppl. 38: 20–28, 2002.

Key words: stem cells; bone marrow; gene therapy; cell therapy

STEM CELL BIOLOGY

Stem cells, by definition, are a population of cells capable of providing replacement cells for a given differentiated cell type (Fig. 1, panel A) [Blau et al., 2001]. Stem cells can be restricted to a particular cell type, such as colonic epithelial crypt cells, which may be considered monopotent stem cells. At the other end of the stem cell, spectrum is the only known totipotent stem cell, the oocyte, which includes what is essentially a variant of the oocyte, the embryonic stem cell. ESCs are not restricted to any cell type and are capable of giving rise to every cell of every tissue in an organism (Fig. 1, panel B). Somewhere in between are the pluripotent stem cells capable of giving rise to multiple, but restricted, cell lineages, the prime example being hematopoietic stem cells (HSCs) (Fig. 1, panel C).

Stem cells, also by definition, are capable of self-renewal; they can make more of themselves in addition to providing daughter cells that go on to differentiate towards specific lineages. This is an important factor in their normal biology but is especially critical in developing possible therapeutic uses for these cells because a stem cell phenotype is necessary to ensure the longevity of the therapeutic effect. If HSCs, e.g.,

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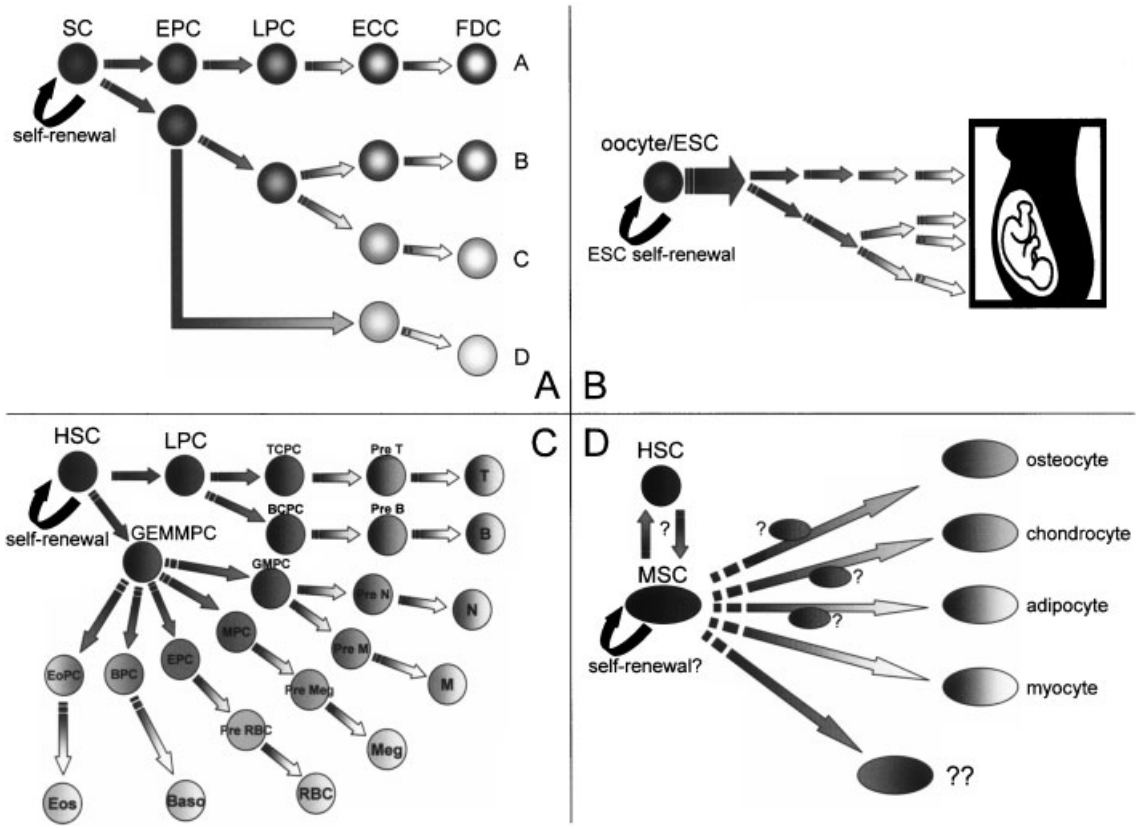

Fig. 1. Diagramatic depictions of various stem cell differentiation paradigms. **Panel A**: Generic depiction of a self-renewing stem cell (SC) differentiating along multiple pathways (**A–D**) sequentially through early progenitor cells (EPC), late progenitor cells (LPC), early committed cell (ECC) and culminating in a fully differentiated cell (FDC). **Panel B**: Generalized representation of a totipotent oocyte/ESC producing an entire organism. While our knowledge of how this occurs through developmental biology has expanded greatly over the years, the exact nature and steps involved in self-renewal, cell fate determination and differentiation remain elusive. **Panel C**: A low detail diagram outlining the main differentiation steps of a HSC down the spectrum of hematopoietic lineages. Corresponding to the stages outlined in Panel A, in order: hematopoietic stem cell (HSC); lymphoid progenitor cell (LPC), granulocyte/erythroid/monocyte/megakaryocytic progenitor cell (GEMMPC); T cell progenitor cell (TCPC), B cell progenitor cell (BCPC), granulocyte/monocyte progenitor cell (GMPC), megakaryocytic progenitor cell (MPC), erythroid progenitor cell (EPC), basophil progenitor cell (BPC), eosinophil progenitor cell (EoPC); pre-T cell (Pre-T), pre-B cell (Pre-B), pre-neutrophil (Pre-N), pre-monocyte (Pre-M), pre-megakaryocyte (Pre-Meg), pre-red blood cell (Pre-RBC); T cell (T), B cell (B), neutrophil (N), monocyte (M), megakaryocyte (Meg), red blood cell (RBC), basophil (Baso), eosinophil (Eos). Note how despite the diagram for HSC differentiation being oversimplified it is far more detailed and complicated than the representations for ESCs and MSCs. **Panel D**: Currently, MSCs are known only to be capable of differentiating to the fully differentiated cell types indicated. Intermediate progenitor and pre-differentiated cell states are likely to exist but have yet to be well defined. It is also not certain if MSCs are capable of self-renewal in vivo. Although there are indications MSCs can differentiate to cell types other than those depicted, the circumstances of such evidence are so artificial it is not clear if it is a normal/natural ability.

were unable to self renew, or at least maintain their numbers, the recipient of a bone marrow transplant (BMT) would only make blood cells for a short time—essentially until their non-self renewing HSC supply was exhausted. It is the ability of stem cells to self renew that distinguishes them from progenitor cells.

Progenitor cells are precursors and in most cases are the intermediate step between stem cell and fully differentiated cells. There are early and late progenitor cells where late progenitors are generally restricted to a particular lineage while early progenitors are still capable of differentiating along multiple lineages (though generally more limited than the stem cells from which they came). Early or late, they do not self renew and thus are exhausted if not replaced by stem cells.

Another attractive feature of HSCs is their ease of isolation and manipulation. Aspiration of bone marrow is relatively quick and only mildly more complicated than a blood donation.

HSCs can then be enriched and in some circumstances expanded prior to use. With the ability to obtain a relatively pure population of HSCs, they can be targeted for gene therapy in addition to standard transplantation. They are then limited only by their compatibility with the recipient and/or the ability to deliver a functional gene in sufficient quantity.

Also present in the marrow, and intimately linked to HSC biology, is a population of bone marrow stromal cells that when isolated are capable of differentiation down multiple mesenchymal lineages to become osteocytes, myocytes, tenocytes, adipocytes, and perhaps other cell types [Caplan and Bruder, 2001]. Due to their similarity to HSCs, multipotential bone marrow stromal cells have been somewhat prematurely named mesenchymal stem cells (MSCs). MSC-like cells have also been isolated from other tissue though it remains to be seen if the marrow derived MSCs and the MSC-like cells from other tissues are a common cell or merely different cell populations with a similar capacity to differentiate along multiple lineages. Because they appear to have stem cell-like characteristics similar to HSCs, current models of MSC biology look much like their HSC counterpart (Fig. 1, panel D). It must be stressed, however, that these cells have yet to live up to the stem cell moniker in any of the current models, especially as it relates to self-renewal. Despite a possible lack of stem cell behavior, MSCs have demonstrated interesting biological activities.

In this review, issues surrounding the biology and use of mesenchymal stem cells in comparison to other stem cells are addressed with particular emphasis on ultimate endpoints of stem cell research in general.

STEM CELL PARADIGM

HSCs have proven clinically useful because they can be isolated, transplanted, and effectively reconstitute the hematopoietic compartment, giving rise to long-term replacement of the entire host hematopoietic system. This appears to work in large part because the hematopoietic system has remarkable proliferative capacity as part of its normal job of making millions of cells an hour in the high turnover environment of the immune and blood systems. Thus, when these cells are taken from the host and placed in a recipient they are primed to take over for the host cells that are dying out from marrow ablating treatments. Connective tissues do not have such a high turnover rate, in fact it is just the opposite. In vivo, connective tissue cells have a much lower turnover rate than hematopoietic cells. MSCs have shown remarkable proliferative capacity, at least, in terms of the number of population doublings but only in vitro [Bruder et al., 1997]. Coupled with the relative resistance of stromal cells to ablation by regimens that destroy HSCs, the ability to ablate and reconstitute cells of this type is significantly impaired. This may explain, in part, why attempts to transplant MSCs yield strikingly low levels of engrafted cells [Keating et al., 1998; Pereira et al., 1998; Horwitz et al., 1999; Devine et al., 2001]. Replacing a significant proportion of connective tissue cells generally or in specific tissues is unlikely without some mechanism to selectively remove the current resident cells and encourage repopulation by the transplanted cells, similar to what has been done with genetically modified HSCs [Davis et al., 2000; Sawai et al., 2001]. One exception may be the formation of granulation tissue in response to a wound. Wound tissue is a connective tissue comprised primarily of fibroblasts and collagen and is produced de novo in order to heal a wound. Because of this, wound repair may be an ideal tissue for introducing MSCs in order to achieve a therapeutic outcome [Gazit et al., 1999; Lee et al., 2001].

WHAT'S KNOWN

Unfortunately, there is relatively little information about the normal biology of MSCs. MSCs are critical to hematopoiesis and support of HSCs. It is known that MSCs are in intimate contact with HSCs and produce a plethora of cytokines and extracellular matrix beneficial to and/or required for HSC function in the bone marrow microenvironment [Caplan and Bruder, 2001]. The list of cytokines alone is long and includes such major hematopoietic determinants as IL-6, -7, -8, -11, -12, -14, -15, M-CSF, LIF, Flt-3 ligand, and SCF. MSCs can be induced to produce IL-1α, LIF, G-CSF, and GM-CSF by IL-1α. MSCs are also strongly associated with endosteal cells and osteoblasts. There is no evidence MSCs circulate in the blood system as part of their normal biology [Lazarus et al., 1997], although putative MSC-like cells have been identified [Lazarus et al., 1997].

Little else is definitively known about the normal biology of MSCs.

TRANSPLANTATION

Current models of MSC transplantation, even under the best circumstances, indicate that when MSCs are delivered systemically there is a very low level of engraftment—reports range from trace amounts at the limit of PCR detection to approximately 3%, depending upon the tissue [Pereira et al., 1998; Devine et al., 2001]. When delivered directly to a tissue, most of the cells appear to engraft but irrespective of the delivery method, the engrafted cells undergo significant loss and detection of long-term surviving cells, if any, is limited to a rather small population. In fact, engineered implants are generally considered resorbable, not permanent, and serve only to provide a scaffold upon which host tissue will eventually take over. This result runs contrary to the idea MSCs are stem cells as they would be expected to continuously contribute cells to tissue(s) and the number of donor cells in those tissues should go up or remain steady over time. Still, with such low engraftment levels, it is possible a diminution of donor cell numbers is merely representative of competitive disadvantage. Certainly, implants and MSC infusions will compete with the influx of GT forming host cells and/or normal turnover of cells in a given tissue. This is especially important given an emerging picture of transdifferentiation among stem cell-like cells that have been identified in various tissues [Slack, 2001; Uchida et al., 2001; Zhou et al., 2001a]. These stem cell-like cells may be one cell type able to compete directly with MSCs delivered to sites outside the marrow.

Much of the work on stem cell-like cells present in various tissues has been sparked by the association of a stem cell phenotype with cells capable of relatively greater Hoechst dye efflux activity—thus providing a relatively simple means of identifying putative stem cells in various tissues [Jackson et al., 2001; Uchida et al., 2001; Zhou et al., 2001a]. By pumping out the dye efficiently, these cells are segregated into a population that moves out to the side, relative to the main population of cells, on scatter plots and these cells have been termed a "side population" or SP cells. The most striking example of stem cell-like behavior and transdifferentiation from nonhematopoietic tissue cells comes from muscle and brain cells that appear capable of functioning as hematopoietic stem cells [Bjornson et al., 1999; Jackson et al., 1999; Seale and Rudnicki, 2000; Vescovi et al., 2001]. However, it is not entirely clear whether SP or other stem cell-like cells resident in various tissues are part of a separate stem cell population which remains relatively primordial and are shared among many tissues as a kind of universal stem cell population, or whether these are tissue specific stem cells that can transdifferentiate into stem cells for other tissues under the right conditions. It is also unclear, given HSCs are known to circulate, whether the cells from nonhematopoietic tissue that reconstituted the hematopoietic system were originally HSCs that lodged in extra-marrow tissue (Table I). One thing is clear; our current models of differentiation and cell fate determination will need to be revised.

While HSCs, and other stem cells apparently capable of functioning as HSCs, are able to reconstitute the hematopoietic system and to some extent provide cells for nonhematopoietic tissues, the circumstances are always in the context of repair of injured tissue. Replacement of the hematopoietic system occured after ablation and demonstration of HSCs contributing to other tissues occured most readily when cells were directly injected, thus producing a puncture wound. Interestingly, MSCs, when delivered as part of an engineered tissue trans-

TABLE I. Stem Cells, Their Sources, and the Cell Types/Lineages They Can Produce

Stem cell	Source	Cell types/lineages
Hematopoietic	Bone marrow	All blood lineages, immune system lineages and perhaps brain, muscle and lung cell types
	Mobilized peripheral blood	Myeloid
	Umbilical cord blood	Lymphoid
Mesenchymal	Bone marrow	Osteoblasts, adipocytes
	Adipose tissue	Myocytes, tenocytes, other?
Neural	Neonatal or adult brain	Neurons, astrocytes, oligodendrocytes, hematopoietic
Muscle	Muscle tissue, HSCs?	Myocytes, hematopoietic?
Embryonic	Embryos	All cell types

plant using a support matrix, appear quite capable of stimulating injury repair under circumstances where the injury would either heal poorly or not heal at all. One particularly useful model relies on repair of a bone segmental defect [Bruder et al., 1998; Gazit et al., 1999; Moutsatsos et al., 2001]. In this model, several groups report MSCs are capable of populating a repair matrix and that use of this MSC populated matrix, especially when combined with the introduction of a bone morphogenic protein (BMP) gene to the MSCs, produces significant healing of bone segment defects that would otherwise not heal properly. As successful as this approach appears to be, it still results in significant or complete loss of the donor MSCs with little or no long-term survival of donor cells.

Loss of donor MSCs may be related to rejection of the cells by the host immune system, at least in some models. But this is somewhat contradicted by mounting evidence that MSCs can downregulate or evade immune responses. When transplanted with HSCs, MSCs may ameliorate graft versus host disease [Koc and Lazarus, 2001]. When bone fragments are transplanted in addition to BMT, autoimmune disease may be abrogated in one model [Ishida et al., 1994]. There are no clear examples where MSCs have engrafted at high levels and functionally replaced a significant portion of tissue, certainly nothing approaching the complete or near complete replacement of the hematopoietic system by HSCs.

CELL THERAPY

As a cell therapy product, MSCs may be useful all by themselves (e.g., as a treatment for osteogenesis imperfecta) [Horwitz et al., 1999] or in conjunction with a BMT (to speed engraftment) [Koc et al., 2000]. It is clear that MSCs in tissue culture are capable of differentiating along multiple lineages when placed in culture conditions that induce diffierentiation towards a particular lineage. It is also clear that MSCs can respond to a particular matrix environment and differentiate appropriately based on the matrix composition in vitro and to some extent in vivo (bone formation in hydroxyappatite cubes, etc.). Even though their in vitro proliferative capacity suggests they may be capable of self-renewal, in vivo they have not yet demonstrated a capacity to remain stem cells in an undifferentiated state—necessary for maintenance of the stem cell phenotype. In many ways, it appears these cells' primary feature is merely their ability to differentiate along particular lineages.

While having only the ability to differentiate along multiple lineages may not be ideal in terms of using them as true stem cells, it does make them attractive for use in cell based therapies. This may be the basis of their success in repairing the bone segmental defects, since by placing them in a support matrix conducive to bone formation, the implanted matrix/MSC mixture is already primed for producing bone and the gap can heal without relying on recruiting bone cells to the gap or to a cell free matrix. A priming effect can be demonstrated in other repair models and generally results in more rapid healing [Davidson et al., 1999]. This approach may be useful for connective tissues that traditionally do not heal well—bone, cartilage, and tendon. Presumably, these tissues do not heal well because they are, for the most part, avascular and relatively sparsely populated by cells. Therefore, they are excellent targets for the introduction of engineered tissues consisting of an organotypic support matrix seeded with MSCs capable of differentiating into the cell type appropriate to that matrix [Butler and Awad, 1999; Awad et al., 2000]. It may also help to prime the MSCs themselves by exposure to cytokines that promote differentiation towards the appropriate cell type, such as BMPs and/or transforming growth factors (TGFs) [Gazit et al., 1999; Gao et al., 2001]. Even if MSCs turn out to have no self-renewal capacity, they will likely have significant usefulness in tissue repair and tissue engineering contexts (Table II).

GENE THERAPY

Ideally, MSCs will be shown to have self-renewal capacity and to contribute cells to

TABLE II. Some Clinical Applications of Cell Therapy Using Stem Cells

Stem cell	Clinical application(s)
Hematopoietic	Many blood-based conditions; cancer
Mesenchymal	Connective tissue repair; improve HSC engraftment, tissue engineering, drug delivery
Neural	Parkinson's Disease, spinal cord and brain injuries
Embryonic	Only speculated—all of the above and more?

mesenchymal tissues long-term in vivo. This will facilitate their use as a target for gene therapy by providing an unlimited, continuous source of gene modified cells in vivo. However, short-term survival can be a significant benefit when continuous expression is not desired for safety or because expression is not needed long-term. If MSCs can function as true stem cells, it will also allow the targeting of genetic disorders of connective tissues, like osteogenesis imperfecta, for autologous correction rather than allogeneic correction as is currently being attempted in a cell therapy based approach [Horwitz et al., 1999; Niyibizi et al., 2001]. Even without self-renewal in vivo, MSCs may be useful for more general gene therapeutic approaches, since MSCs appear to downregulate certain immune responses making them somewhat immune privileged and thus there is a reduced likelihood the transgene product may become immunogenic. Since connective tissue cells have a relatively low turnover rate, an introduced gene product may be produced for a longer period than might otherwise be achieved by other delivery methods. In addition, the ability to manipulate MSCs ex vivo means the transduction can be controlled and the transgene dose delivered with precision when compared with in vivo transduction methods where vectors are directly injected into tissues. MSCs are amenable to temporary transfection with DNA vectors or transduction by adenoviral, retroviral, and lentiviral vectors making them suitable for gene therapy using a multitude of approaches [Caplan, 2000].

Allay et al., 1997, retrovirally transduced the β-galactosidase gene into MSCs, seeded the transduced MSCs onto hydroxyappatite artificial bone cubes and implanted the MSC seeded cubes into mice. They found the β-gal gene was expressed in vivo even after the MSCs differentiated into osteocytes.

Bartholomew et al., 2001 transduced the EPO gene into MSCs and transplanted the MSCs directly via intramuscular injection or by implantation of MSC loaded immunoisolation devices. They found EPO was produced in sufficient quantity to be detected in serum for up to 137 days. Increased EPO expression correlated with improved hematocrit.

Other groups have introduced clinically relevant genes and demonstrated temporary effects of transgene expression. However, in all cases transgene expression peaked a week or two after delivery of the modified MSCs and eventually dropped off to very low or non-detectable levels. Transgene silencing is not likely to result in complete loss of expression, since expression can be maintained after differentiation [Allay et al., 1997]. This indicates either the transgene containing cells or the MSCs in general are being eliminated over time either directly by immune surveillance or indirectly by competitive repopulation from host/unmodified cells. Competitive repopulation is one mechanism that prevents gene therapy of HSCs from being more successful, since the relatively rare gene modified stem cell tends to be outnumbered by the unmodified stem cells. Also, there may be a disadvantage associated with having the transgene expressed constitutively in all cell types derived from the stem cell. For this reason, lineage specific transgene expression systems are being explored.

Gene transfer into HSCs would potentially result in transgene expression in a high proportion of any blood cell lineage. Restricting transgene expression to the specific lineage required has been under investigation primarily for disorders of erythrocytes and platelets [Wilcox et al., 1999; Moreau-Gaudry et al., 2001; Tan et al., 2001]. Targeting expression to the myeloid, lymphoid, or dendritic cell lineages has not been widely studied but could be useful for gene therapy of immunological defects, such as X-linked SCID and chronic granulomatous disease, or infectious diseases and cancer.

β-globin expressed exclusively in the erythrocyte lineage for β-thalassemia has been shown using the β-globin promoter together with core elements of the β-globin locus control region [Tan et al., 2001]. This was also demonstrated using the autoregulatory element of the GATA-1 erythroid-specific promoter for β-globin expression [Grande et al., 1999], and an ankyrin-1/β-globin promoter for ferrochelatase expression [Richard et al., 2001]. The integrin αIIb promoter has been used to restrict integrin β3 subunit expression to megakaryocytes resulting in in vitro correction of the platelet defect in Glanzmann disease [Wilcox et al., 2000].

The primary obstacle to successful treatment of hematopoietic disorders using lineage-restricted expression of the transgene has been the ability to transduce sufficient numbers of HSCs so that a large proportion of a particular lineage is expressing the therapeutic gene.

TABLE III. Some Gene Therapy Applications for Stem Cells

Stem cell	Gene	Gene therapy application
Hematopoietic	Phox	Chronic granulomatous disease
	MGMT	Bone marrow protection from chemotherapy
	Globins	Thallesemias, sickle cell disease
Mesenchymal	Many	Production of secreted therapeutic proteins
	Collagen	Osteogenesis imperfecta
	Erythropoietin	Anemia
	Insulin	Diabetes
Neural	Speculated	Parkinson's disease, other neurological
Muscle	Dystrophin	Muscular dystrophy
Embryonic	Any?	Theoretically unlimited?

Expression difficulties deal principally with maintaining long-term expression, which is a general concern in gene therapy. Identification of additional lineage specific promoters could help in this regard as well as in the ability to 'select' the magnitude of transgene expression based on therapeutic requirements. Theoretically, MSCs should provide essentially the same opportunities as HSCs for cell-type specific gene expression, only for mesenchymal cell types. This will be critical if MSCs are shown to provide cells to several mesenchymal tissues. Certainly it would not be useful to have uncontrolled expression of type I collagen in adipose or muscle tissue when it is only needed in bone as in osteogenesis imperfecta (Table III).

ENDPOINTS

It will not be enough to rely solely on HSCs, MSCs, or even ESCs as a source of cells for cell, gene, or tissue engineered therapies. The knowledge endpoint for stem cell research is to define the mechanisms by which stem cells maintain themselves and differentiate. This knowledge will need to be combined with information on how differentiated cells organize to create a tissue or organ. The ultimate practical endpoint, then, is the ability to manipulate these mechanisms in order to produce a desired therapeutic outcome. This will involve intricately timing cellular influences using a variety of methods; externally generated in the form of EM radiation exposures [Aaron and Ciombor, 1996], targeted pharmacological interventions [Noshi et al., 2001; Zhou et al., 2001b], physical elements [Aaron and Ciombor, 1996; Arnoczky, 1999], biologically engineered materials [Arnoczky, 1999; Butler and Awad, 1999], genetic manipulations [Caplan, 2000; Moutsatsos et al., 2001], and other factors as yet undefined. Attempts to achieve specific and controlled differentiation have been relatively limited [Wei et al., 1995; Fairchild et al., 2000; Caplan and Bruder, 2001] but are good examples of what can be achieved with refinements in stem cell manipulations and an understanding of stem cell biology.

The potential versatility of stem cells, HSCs, MSCs, or ESCs, makes them inherently exciting and valuable to biologists, preclinical researchers, and clinicians alike. Biologists want and need to elucidate the roles and mechanisms by which stem cell populations maintain tissues, how they stay stem cells, what controls and drives their differentiation, and what factors govern the limited range of lineages to which they contribute versus what mechanisms may allow them to cross lineages. Preclinical researchers need to take the biology of stem cells and find ways of manipulating the stem cells in disease models in order to produce a clinically relevant outcome. Clinicians will need to adapt therapeutic regimens to account for the biology of stem cells and translate preclinical models into effective therapies. The promise of understanding stem cell biology is great and the potential applications are far greater. Stem cells may be the keys to unlocking the body's ability to regenerate rather than merely heal. This is because it is the complete understanding of stem cell biology that will provide ways of manipulating that biology and allowing application of stem cells for the purpose of replacing or regenerating human tissues, organs, and even entire limbs. This may be an ambitious goal, but one that should be achievable with sufficient effort and support of stem cell research on many multidisciplinary fronts.

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