REVIEW

Multipotential differentiation of adipose tissue-derived stem cells

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Abstract. Tissue engineering offers considerable promise in the repair or replacement of diseased and/or damaged tissues. The cellular component of this regenerative approach will play a key role in bringing these tissue engineered constructs from the laboratory bench to the clinical bedside. However, the ideal source of cells still remains unclear and may differ depending upon the application. Current research for many applications is focused on the use of adult stem cells. The properties of adult stem cells that make them well-suited for regenerative medicine are (1) ease of harvest for autologous transplantation, (2) high proliferation rates for *ex vivo* expansion and (3) multilineage differentiation capacity. This review will highlight the use of adipose tissue as a reservoir of adult stem cells and draw conclusions based upon comparisons with bone marrow stromal cells. (Keio J Med 54 (3): 132–141, September 2005)

Key words: adipose tissue-derived stem cells, cell differentiation

Introduction

White adipose tissue has the ability to dynamically expand and shrink throughout the life of an adult. This capacity is mediated by the presence of vascular and non-vascular cells that provide a pool of stem and progenitor cells with unique regenerative capacity. This review will describe the recent preclinical research focused on these stem cells and identify potential clinical applications.

Over the past 25 years bone marrow mesenchymal stem cells (also frequently referred to as marrow stromal cells and herein referred to as MSCs) have been the subject of considerable research. These cells have many properties that suggest considerable potential utility in cellular therapy for a variety of disorders. For example, their ability to differentiate into osteoblasts, both *in vitro* and *in vivo*, has led to their clinical use in pilot studies of an inherited bone disorder, Osteogenesis imperfecta[1] while their ability to promote revascularization following ischemic injury has led to preclinical studies in large and small animal models of myocardial ischemia.[2,3]

Like marrow, adipose tissue is a mesodermally-derived organ that contains a stromal population containing microvascular endothelial cells, smooth muscle cells and stem cells.[4] These cells can be enzymatically digested out of adipose tissue (commonly from lipoaspirate) and separated from the buoyant adipocytes by centrifugation. A more homogeneous population emerges in culture under conditions supportive of MSC growth. This population (termed Adipose Tissue-Derived Stem Cells, ADSCs) shares many of the characteristics of its counterpart in marrow including extensive proliferative potential and the ability to undergo multilineage differentiation.[5,6]

Clonogenic assays have typically been used to quantify MSCs in marrow.[7–10] In these assays (colony forming units, CFU-F), cells are plated at $\sim 1,000/cm^2$ and grown for two-three weeks. Colonies of more than 50 cells are then quantified. Using these assays, the number of MSCs in bone marrow is generally found to be approximately 1 in 25,000 to 1 in 100,000,[7–10] although many authors have found this frequency is influenced

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by factors such as age, gender, presence of osteoporosis, and prior exposure to high dose chemotherapy or radiation.[7,11–13] Our unpublished data suggest that the average frequency of ADSCs in processed lipoaspirate (56 donors, median age 49) is ∼2% of nucleated cells (manuscript in preparation). As with bone marrow stem cells, yield is dependent upon donor age, body mass index and tissue harvest site. This finding is consistent with previous reports showing rapid growth of ADSCs from cultures initiated at considerably lower cell density than that typically used with marrow.[6,14] Further, our studies have indicated that the yield of ADSCs is approximately 5,000 CFU-F per gram of adipose tissue. This compares with estimates of approximately 100–1,000 CFU-F per milliliter of bone marrow.

The significance of this difference for stem cell therapy is clear. Donor site morbidity limits the amount of marrow that can be obtained and thereby extends the time in culture required to generate a therapeutic cell dose. Thus, the volume of human marrow taken under local anesthesia is generally limited to no more than 40 ml and yields approximately 1.2×10^9 nucleated cells.[15] Obtaining a larger volume necessitates the use of general anesthesia, increases donor site morbidity,[16,17] and further dilutes the stem cell fraction with stem cell-free blood.[15] At the stem cell frequency cited above,[7,8] this 40 ml of marrow will contain approximately 2.4×10^4 MSCs in a skeletally mature adult. By contrast, a typical harvest of adipose tissue, under local anesthesia, can easily exceed 200 ml and yield ∼2×10^8 nucleated cells per 100 ml of lipoaspirate.[18] Thus, 200 ml lipoaspirate will typically yield in excess of 1×10^6 stem cells; a differential of approximately 40-fold more than that present in 40 ml of marrow.

Cell Surface Characterization

The cell surface phenotype of human ADSCs is quite similar to MSCs (see Table).[5,19] It is worth noting some of the key similarities and differences between these cells. For instance, CD105, STRO-1 and CD166 (ALCAM) are three common markers used to identify cells with multilineage differentiation potential and are consistently expressed on ADSCs and MSCs.[20–26] Also, CD117 (the stem cell factor receptor) has been shown to be expressed on an array of totipotent or pluripotent cells including embryonic stem cells, hematopoietic stem cells, MSCs and ADSCs.[27–29] In addition to these multipotent markers, ADSCs and MSCs display numerous other molecules including CD29 (beta-1 integrin, which plays a critical role in therapeutic angiogenesis[30]), CD44 (hyaluronate receptor, which is crucial in the development of neoextracellular matrix and plays a role in numerous pathologic and physiologic events) and CD49e (alpha-5 integrin, important

Table The Cell Surface Phenotype of ADSCs is Similar to That of Bone Marrow MSCs

Surface marker	Expression pattern	
	ADSCs	MSCs
CD9	+	+
CD10	+	+
CD13	+	+
CD29	+	+
CD31	−	−
CD34	−	−
CD44	+	+
CD45	−	−
CD49d	+	−
CD49e	+	+
CD54	+	+
CD55	+	+
CD59	+	+
CD90	+	+
CD105	+	+
CD106	−	+
CD117	+	+
CD146	+	+
CD166	+	+
STRO-1	+	+

for cell adhesion to fibronectin). ADSCs also express high levels of CD54 (ICAM-1) when compared with BM-MSCs.[19] ICAM-1 is a member of the immunoglobulin supergene family and can be up-regulated in response to numerous inflammatory mediators and cytokines.[31]

ADSCs lack the expression of known hematopoietic and endothelial markers such as CD3, CD4, CD11c, CD14, CD15, CD16, CD19, CD31, CD33, CD38, CD56, CD62p, CD104, and CD144. Also, less than 1% of ADSCs express the HLA-DR protein and the majority express MHC Class I molecules,[18] suggesting their potential for allogeneic transplantation.[32] One difference in surface marker expression appears to be the reciprocal expression of VLA-4 (CD49d/CD29) and its cognate receptor VCAM-1 (CD106). Thus, we have observed expression of VLA-4 but not VCAM-1 by ADSCs from the majority of donors. This is the reverse of the expression pattern of these molecules by MSCs.[19] While the nature of this difference needs further investigation, this observation is intriguing since these molecules are involved in hematopoietic stem and progenitor cell homing to and mobilization from the bone marrow.[33,34]

Multilineage Differentiation Capacity

We and others have demonstrated the ability of adipose tissue-derived stem cells to undergo differentiation

ADSCs

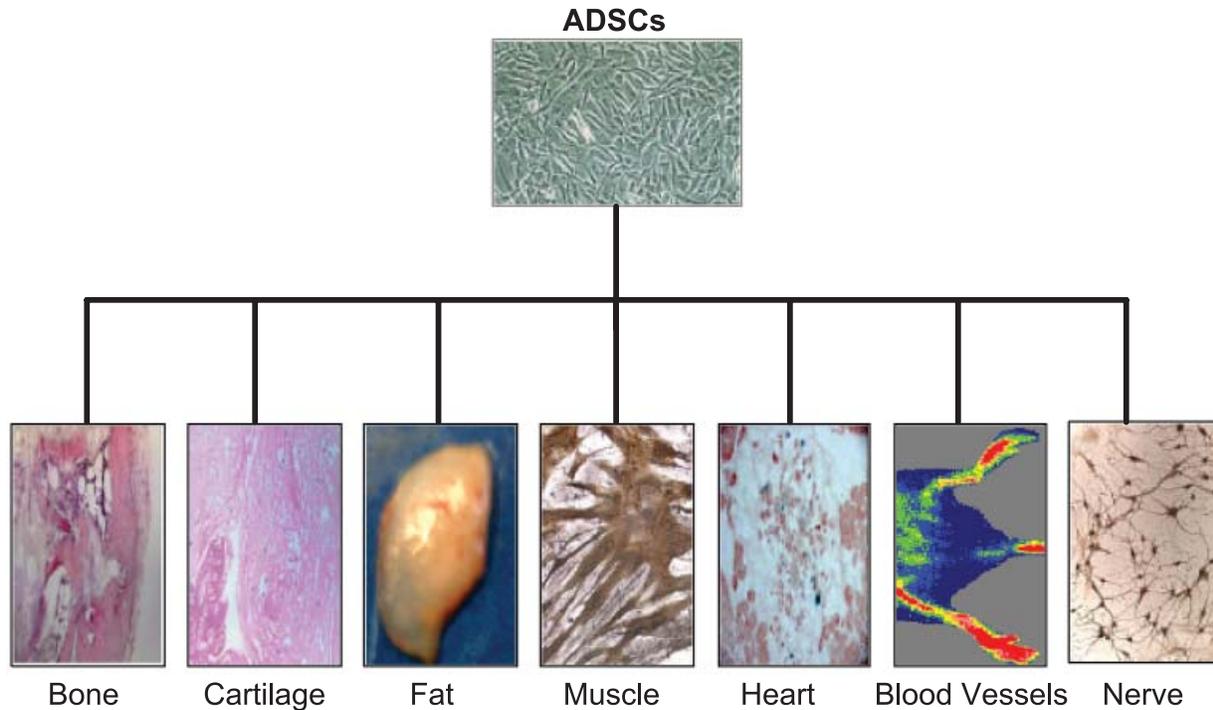| Bone | Cartilage | Fat | Muscle | Heart | Blood Vessels | Nerve |

Figure   Adipose Tissue-Derived Stem Cells are multipotent, extending beyond the traditional mesenchymal lineages.

along classical mesenchymal lineages: adipogenesis, chondrogenesis, osteogenesis, and myogenesis.[4–6,35–43] Non-mesenchymal lineages have also been investigated and confirm the transdifferentiation ability of ADSCs[44–47] (see Figure). Our data using single cell-derived clonal populations demonstrates that at least part of this plasticity resides in a population of multi-potential cells.[5] In most respects, these data demonstrate a set of functional properties that is very similar, though not always identical, to that of MSC.

Mesenchymal lineages

Adipogenesis

Given the origin of ADSCs it is not surprising that, when cultured in adipogenic medium, ADSCs express several adipocytic genes including lipoprotein lipase, aP2, PPAR(gamma)2, leptin, Glut4, and develop lipid-laden intracellular vacuoles, the definitive marker of adipogenesis.[4,5,40,48] Despite certain donor-to-donor qualitative differences in adipogenic potential,[48] the pattern of expression of these genes appears to be very similar, if not identical, to that observed for adipogenic differentiation in MSCs.[23,49,50]

The *in vivo* capacity of these cells to differentiate into cells of the adipocytic lineage has also been demonstrated in studies involving implantation of cell-seeded natural (collagen and hyaluronic)[51–53] or syn-

thetic bioresorbable (polylactic acid, polyglycolic acid)[54,55] scaffolds. It is important to note that these studies generally agree that robust ectopic *in vivo* adipogenesis requires *in vitro* pre-differentiation of ADSCs prior to implantation. This requirement may be eliminated by co-implantation of cells with a source of adipogenic stimuli. Thus, Yuksel and colleagues have demonstrated ectopic adipogenesis at the site of implantation of microbeads containing insulin and insulin-like growth factor 1.[56] This suggests the recruitment of adipocytic stem and progenitor cells to the site of implantation, however it is not clear if this is a local recruitment or derives from distal compartments of such progenitors in fat and/or marrow.

Osteogenesis

The ability of MSCs to give rise to osteoblasts is well known.[57–60] Observations of patients with a rare disorder, progressive osseous heteroplasia, in which calcified nodules form in subcutaneous adipose depots, provided physiological evidence that similar cells may exist in adipose tissue.[61,62] In the past decade, several groups have isolated cells from adipose tissue of humans and other species capable of differentiating into osteoblasts *in vitro*.[5,36,39,42,63–67] Under osteogenic conditions, similar to those used for MSCs, ADSCs are observed to express genes and proteins associated with an osteoblasts phenotype, including alkaline phospha-

tase, type I collagen, osteopontin, osteonectin, osteo-calcin, bone sialo protein, RunX-1, BMP-2, BMP-4 BMP receptors I and II, PTH-receptor.[5,39] ADSCs are also able to form mineralized matrix *in vitro* in both long term 2-D or 3-D osteogenic cultures.

Using a variety of supportive scaffolds, human ADSCs can form bone in immunodeficient rodent ectopic bone models.[35,55,68] Histologic analysis of these implants revealed cells staining positive for the human nuclear antigen, confirming that the cells making the new bone are donor derived. ADSC-derived were found lining the regions of ectopic bone and also embedded within the newly formed bone. In a murine critical size calvarial defect model, ADSCs were able to regenerate cranial bone, which spanned the defect site, within 8 to 12 weeks of implantation.[14] Bone formation occurred via intramembranous ossification, confirmed by a lack of chondrogenic matrix. Since the calvarium normally develops through this mechanism, ADSCs differentiated appropriately for cranial membranous bone repair. Overall, the rate and extent of bone formation was comparable to that of MSCs.

These findings supported a recent clinical report describing successful treatment of a large, bilateral calvarial defect in a seven year old girl. Repair was achieved through an approach in which autologous adipose-derived cells, iliac crest bone, and fibrin glue were combined with a resorbable mesh.[69] CT scans taken 3 months after surgery showed marked ossification throughout the defect. Further, the child no longer has to wear a protective helmet. This anecdotal report suggests that use of adipose-derived cells was safe and that they may have contributed to healing of a large, previously refractory defect.

Chondrogenesis

High density micromass cultures of ADSCs and MSCs generate cellular nodules that produce large amounts of cartilage-related extracellular matrix molecules including sulfated proteoglycans, collagen II and IV, PRELP and aggrecan.[4,5,23,36,38,40,41,70] ADSCs seeded onto alginate discs and implanted into immuno-deficient mice exhibit prolonged (12 weeks) synthesis of cartilage matrix molecules including collagen II, collagen VI, and aggrecan.[38] The identification of which cells (ADSCs or MSCs) are better for engineering cartilage is still under debate. We have analyzed ADSCs and MSCs from the same patients under identical conditions and found that ADSCs had greater chondrogenic ability than MSCs.[6] However, Winter *et al.* demonstrated that, while ADSCs and MSCs are not significantly different in their ability to undergo chondrogenesis in two-dimensional cultures, MSCs exhibited enhanced chondrogenesis in three dimensional cultures.[41] This is in contrast to a recent *in vivo* study of mature cartilage development from ADSCs in a rabbit osteochondral defect model.[43] In this study the authors noted that the repair induced by ADSCs was superior to that derived from osteochondral autografts. Specifically, the Pineda score, which assesses four different parameters of cartilage repair, was greater for ADSC-derived grafts than for osteochondral grafts at each time point examined. ADSC grafts also showed superior performance in creep indentation biomechanical testing performed at 24 weeks. However, it should be noted that performance at 24 weeks was still inferior to intact cartilage. Nonetheless, these data indicate that the deficit observed by Winter *et al.* may be a culture artifact rather than an inherent limitation in the osteochondral capacity of ADSCs.

Non-mesenchymal lineages

Skeletal myogenesis

Culture of ADSCs and MSCs in the presence of dexamethasone, hydrocortisone, and/or 5 azacytidine results in a time-dependent pattern of expression of muscle-related genes that is consistent with normal myogenesis. This is defined by early expression of key master regulatory factors MyoD1 and myf5, myf6, and myogenin followed by later expression of myosin heavy chain.[5,37,71] This process is associated with characteristic changes in cell morphology including generation of long, multinucleate, MyoD1-positive cells early in culture and bundles of myosin heavy chain-positive myofibrillar structures appearing after two weeks.

Thus far, only one study has investigated the use of ADSCs to restore functional capacity of damaged skeletal muscle *in vivo*. Bacou and colleagues demonstrated that ADSCs transplanted into injured regions of rabbit skeletal muscle increased muscle weight and fiber cross section area, and significantly raised the maximal contractile force compared with damaged control muscles.[72] The transplanted cells were identified in the region of damaged tissue and some expressed the skeletal muscle markers merosin, myogenin and multiple isoforms of myosin heavy chain, suggestive of myogenic differentiation.

Cardiac

Cardiovascular disease (CVD) has emerged as a leading cause of morbidity and mortality, accounting for approximately one-third of all deaths in 2002.[73] Current therapies for CVD are focused on revascularization, followed by drug therapy to mitigate the deleterious remodeling that ensues post ischemia. While this therapeutic regimen has had a substantial impact

on how CVD is treated, there is still a necessity to further advance treatment options. Cell therapy for cardiovascular diseases was originally thought to focus on the regeneration of injured myocardium. The use of progenitor/stem cells has changed this paradigm by potentially contributing to multiple mechanisms for cardiac repair. These mechanisms include, but are not limited to: increasing blood flow to the ischemic tissue (either by differentiating into neovasculature or secreting factors to recruit or expand endogenous vasculature), reducing cardiac myocyte apoptosis through a paracrine-mediated response, regulating the inflammatory milieu (which can alter the negative remodeling the ensues following acute ischemic injury), or by recruiting endogenous stem cells (i.e. from the heart[74] or bone marrow[75]) to assist in regenerating the injured tissue.

While all of these paths could potentially augment current therapies, regeneration of lost myocardium is still the ideal outcome, particularly in congestive heart failure, which involves currently irreversible loss of contractile function. Both ADSCs[45,76,77] and MSCs[78-81] have been shown to be capable of *in vitro* differentiation into cardiac myocytes. The most compelling data was obtained by Planat-Bernard *et al.* in which fresh adipose-derived cells were plated into semisolid culture. After three weeks, colonies of spontaneously beating cells were observed. These cells exhibited several molecular, electrophysiologic, and pharmacologic properties of cardiac myocytes.[76] However, there is a lack of data addressing the ability of ADSCs to regenerate damaged myocardium *in vivo*. In work presented at the European Society of Cardiology in Munich, Germany in 2004, Valina *et al.* presented data showing that delivery of either ADSCs or MSCs through the left coronary artery following an acute myocardial infarction in pigs resulted in improved left ventricular function after 4 weeks compared to saline-treated controls. Upon histologic analysis, the group identified donor ADSCs at the site of infarction, and that these cells expressed cardiomyocytic markers.

Neurogenesis

In vitro differentiation along the neuronal lineages has also been demonstrated for both ADSCs[5,46,82] and MSCs.[83-86] Thus, treatment of rat and human MSCs or ADSCs with beta-mercaptoethanol results in rapid transition of cells to a neuronal morphology (a condensed cell body with multiple neuron-like outgrowths), and expression of neural markers including nestin, neuron-specific enolase (NSE), and neuron-specific protein (NeuN), all of which are early markers of the neuronal lineage. Similar results are seen with alternate inductive conditions such as isobutylmethy-

lxanthine (IBMX) and dibutyryl cAMP or forskolin and butylated hydroxyanisole. We have also detected expression of trkA (a receptor for NGF) and the presence of voltage gated potassium channels. However, to date, detection of neural markers in *in vitro* differentiated ADSCs and MSCs has been restricted to these early genes; no expression of markers characteristic of mature neurons, oligodendrocytes, or astrocytes has been described. This may suggest that the expression of these markers is the result of disordered gene expression resulting from the toxic inductive stimulus or that the induction is unmasking an inherent neuronal potential that is only partially supported by the culture conditions.

The latter interpretation is supported by *in vivo* studies in which ADSCs and MSCs have been implanted into the CNS of experimental animals. Zhao *et al.*[87] have demonstrated that implantation of MSCs into the cortex of rats following ischemic injury resulted in significantly improved performance in a limb placement test and that the implanted cells had undergone an *in vivo* change in marker expression consistent with differentiation along astrocytic, oligodendrocytic, and neural lineages. However, there was no evidence for incorporation of these cells into the cerebral architecture. Therefore, it is possible that the observed functional improvement was due to an indirect mechanism, for example, paracrine expression of angiogenic and/or anti-apoptotic factors by the implanted cells would promote survival of functionally compromised but viable host tissue.[88,89] Other studies have also demonstrated engraftment of MSCs into the region in the absence of injury albeit with modest evidence of differentiation.[90,91] Nonetheless, these data provide substantial support for a potential role of MSCs in direct or indirect (gene-modified) therapy for the CNS.[92,93]

ADSCs have also been applied in the setting of experimental stroke. Thus, Kang *et al.* directly implanted ADSCs into the brain of rats following 90 minutes of middle cerebral artery occlusion.[94] In some studies, the ADSCs were transduced with either the lacZ gene or the gene encoding brain-derived neurotrophic factor (BDNF) as both a marker and a potentially therapeutic agent. Marked cells were seen throughout the infarct area 14 days after implantation and a fraction of these cells co-expressed MAP2 (4% of marked cells) or GFAP (9% of marked cells) suggesting a degree of neural differentiation. No data were presented with regard to potential fusion between donor and recipient cells. As with the MSC studies, ADSC-treated animals showed significant improvement in neurologic testing with the animals receiving BDNF-transduced cells exhibiting significantly better recovery of function than those treated with unmodified ADSC. The same group also examined the co-culture of human ADSCs and murine NSCs, and observed that when NSCs were cul-

tured on mitomycin-treated ADSC-monolayers, their proliferation was decreased, but neural differentiation was increased. However, when NSCs were cultured with ADSC-conditioned medium or co-cultured with permeable filter on which ADSCs were grown, the proliferation of NSCs significantly decreased and glial differentiation increased, but their neural differentiation was not affected. Thus Kang *et al.* concluded that direct physical contact between ADSCs and NSCs is required for induction of neural differentiation.[95]

Other applications

Therapeutic angiogenesis

Restoring blood flow to ischemic tissue has proven instrumental in the treatment of patients with acute myocardial infarctions. However, this application is not the only one that could greatly benefit by increased angiogenesis. Therapeutic angiogenesis, potentially from ADSCs and MSCs, has an extremely broad range of clinical applications under investigation, such as ischemic cardiomyopathy, peripheral vascular disease, ischemic stroke, acute tubular necrosis, diabetic retinopathy, ischemic encephalopathy, traumatic spinal cord injuries, and transplant related ischemia. Both ADSCs and MSCs have been shown to increase angiogenesis to ischemic tissue; however the underlying mechanism remains unclear. Both cell types have been shown to excrete substantial quantities of angiogenic growth factors, including VEGF, PlGF, bFGF, angiogenin, GM-CSF, MCP-1 and SDF-1alpha.[96,97] Both cell types have also been shown to be capable of endothelial differentiation.[44,98]

Miranville *et al.* have presented data supporting the presence of cells within adipose tissue that differentiated into endothelium.[99] Thus, $CD34^+/CD31^-$ cells within adipose were shown to be capable of *in vitro* differentiation into cells that expressed both CD31 and von Willebrand factor, both markers of mature endothelium. Most importantly, the authors demonstrated the ability of these cells to improve blood flow and capillary density in a NOD-SCID mouse model of hind limb ischemia. These data are confirmed by another study showing that delivery of ADSCs to immunodeficient animals following induction of severe hind limb ischemia results in accelerated restoration of perfusion.[96] As an interesting side note, Miranville *et al.* also reported that approximately 18% of $CD34^+/CD31^-$ cells co-expressed ABCG2, a protein associated with the side population (SP) stem cell phenotype; overall approximately 4% of all non-buoyant adipose-tissue-derived cells express ABCG2.[99] It should be noted that there, at present, are no data addressing the question of whether or not the endothe-

lial and mesenchymal differentiation capacity within adipose tissue reside within the same cells.

Gene delivery

A number of investigators have transduced ADSCs in order to facilitate tracking or to engender a therapeutic effect. Thus, Leo *et al.* used Ad-CMV-luciferase to allow non-invasive, real-time tracking of ADSCs in rat spine.[100] Similarly, Dragoo *et al.*[35] infected both MSCs and ADSCs with E1A-deleted-type 5 adenovirus constructs containing the BMP-2 (bone morphogenic protein-2) gene or the bacterial beta-galactosidase (lacZ) gene. LacZ gene transduction efficiency was 35% for MSCs and 55% for ADSCs. Ad-BMP2 infection resulted in levels of expression of BMP-2 protein that were three-fold higher than those derived from MSCs. Ad-BMP-2 infected ADSCs exhibited *in vitro* osteoblastic differentiation in the absence of exogenous osteogenic factors. They also exhibited robust ectopic *in vivo* production of bone when cells were implanted into a collagen sponge within the subcutaneous space.[35] Given the success of unmodified MSCs in treatment of osteogenesis imperfecta[101,102] these data support the potential for transplant of allogeneic or gene-modified ADSCs for genetic disorders of the skeletal system.

Kang *et al.*[94] have also used an E1A-deleted type 5 adenovirus to infect ADSCs. As described above, these studies employed transduction of a tracking gene (lacZ) and a potentially therapeutic gene Brain-Derived Neurotrophic Factor (BDNF) achieving 100% and 94% transduction efficiency, respectively. Transduced cells were implanted into areas of the brain that had undergone transient (90 minute) ischemia-reperfusion injury. Donor cells capable of continued expression of the transgene were retained to 30 days, the longest time point examined in this study.

Finally, our group has published results of a study comparing infection of ADSCs with oncoretroviral, and lentiviral vectors.[103] The primary lentivirus used was the VSV-G pseudotyped HIV-1 vector SIN18-Rh-MLV-E (VSV). Infection by the VSV-G pseudotyped MuLV oncoretrovirus SR(alpha)L-EGFP and a second lentivirus construct, RRL-PGK-EGFP-SIN18, was also used. Direct comparison of infection of the lentiviruses and the oncoretrovirus was possible due to common envelope protein and the similarity of the transcription level driven in transduced cells. Thus, we were able to infect ADSCs with the same number of EGFP transduction units (virus preparations standardized to drive the same level of GFP expression in control cells) and determine efficiency by flow cytometry three days and one week after infection. The lentiviral contructs resulted in 4–10-fold higher expression than the retroviral vector. The percentage of transduced cells was

not high (10–15%) but remained stable in culture over 100 days. Moreover, using a lentiviral vector with the cytomegalovirus promoter resulted in a transduction efficiency of >90% at a MOI of 14. Studies using lentiviral-infected cells (RRL-PGK-EGFP-SIN18; MOI 59) in which transduction efficiency was 98% at day 3 and >95% at day 100 allowed examination of gene expression during *in vitro* differentiation. Retention of marker gene (EGFP) expression was observed following both adipogenic and osteogenic differentiation.

Hematopoietic support

The ability to support hematopoiesis is another property of MSCs that may be important in clinical applications. Indeed, co-infusion of MSCs with grafts containing hematopoietic stem cells has been shown to enhance the rate of hematopoietic engraftment in human clinical studies.[104] Hematopoietic support is also important in transduction of CD34-positive hematopoietic stem and progenitor cells.[105–107] While no study to date has specifically examined the ability of ADSCs to support hematopoiesis, a recent study has claimed to demonstrate that adipose tissue contains a population of cells with hematopoietic stem cell activity; that is, a population of cells capable of rescuing lethally irradiated animals.[108] Thus, intraperitoneal transplant of 10^7 fresh adipose or marrow cells was associated with 40% survival following 10 Gy irradiation. Recovery of platelet and white blood cells counts was more rapid with marrow than for adipose tissue cells (8 weeks vs 10 weeks for return to normal levels). However, adipose-derived cell transplant resulted in a very low level of hematopoietic chimerism (1.7% marrow hematopoietic progenitor cells of donor origin). This suggests that the survival advantage conferred by adipose-derived cell transplant is due to enhancement of the recovery of *endogenous* hematopoietic stem cells from the otherwise lethal irradiation in a manner that is generally consistent with the human co-infusion study cited above.[104]

Summary

In summary, adipose tissue, like bone marrow, contains a population of cells that has extensive self-renewal capacity and the ability to differentiate along multiple lineages. The cells possessing this activity can be obtained in large numbers at high frequency from a tissue source that can be extracted in large quantities with minimal morbidity, unlike marrow. These cells can also be infected by adenoviral, oncoretroviral, and lentiviral vectors with moderate to high efficiency. Thus, adipose tissue appears to represent a potential clinically

useful source of cells for cellular therapy, tissue engineering and gene transfer applications.

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