

REVIEW

Terminal differentiation is not a major determinant for the success of stem cell therapy - cross-talk between muscle-derived stem cells and host cells

Burhan Gharaibeh^{1,2}, Mitra Lavasani¹, James H Cummins¹ and Johnny Huard^{1,2,3,*}

Abstract

We have found that when muscle-derived stem cells (MDSCs) are implanted into a variety of tissues only a small fraction of the donor cells can be found within the regenerated tissues and the vast majority of cells are host derived. This observation has also been documented by other investigators using a variety of different stem cell types. It is speculated that the transplanted stem cells release factors that modulate repair indirectly by mobilizing the host's cells and attracting them to the injury site in a paracrine manner. This process is loosely called a 'paracrine mechanism', but its effects are not necessarily restricted to the injury site. In support of this speculation, it has been reported that increasing angiogenesis leads to an improvement of cardiac function, while inhibiting angiogenesis reduces the regeneration capacity of the stem cells in the injured vascularized tissues. This observation supports the finding that most of the cells that contribute to the repair process are indeed chemo-attracted to the injury site, potentially through host neo-angiogenesis. Since it has recently been observed that cells residing within the walls of blood vessels (endothelial cells and pericytes) appear to represent an origin for post-natal stem cells, it is tempting to hypothesize that the promotion of tissue repair, via neo-angiogenesis, involves these blood vessel-derived stem cells. For non-vascularized tissues, such as articular cartilage, the regenerative property of the injected stem cells still promotes a paracrine, or bystander, effect, which involves the resident cells found within the injured microenvironment, albeit not through the promotion of angiogenesis. In this paper, we review the current knowledge of post-natal stem cell therapy and demonstrate the influence that implanted stem cells have on the tissue regeneration and repair process. We argue that the terminal differentiation capacity of implanted stem cells is not the major determinant of the cells regenerative potential and that the paracrine effect imparted by the transplanted cells plays a greater role in the regeneration process.

Introduction

Our research group has isolated, by a modified preplate technique [1,2], a population of muscle-derived stem cells (MDSCs) from murine post-natal skeletal muscle. These MDSCs were shown to have the ability to proliferate *in vivo* for an extended period of time and the capacity to self-renew, and to undergo multilineage differentiation *in vitro* and *in vivo* [2]. Current knowledge on the utility of MDSCs for enhancing repair in various musculoskeletal tissues and injured cardiac muscle and the potential for their use in clinical applications is

reviewed in several publications [3-5]. Our data show that these cells display a high transplantation capacity in skeletal muscle and exhibit only limited degrees of engraftment capacity in cardiac, bone, cartilage, and nerve tissues in respective animal model injuries. Their success in repairing damaged tissues is attributed, at least in part, to their resistance to stress and through the paracrine effect that they impart on host tissues. These paracrine effects can stimulate the mobilization of host progenitor cells, stimulate the production of new blood vessels via neoangiogenesis, and possibly modulate the host immune response.

The term paracrine mechanism, used herein, refers to effects that are not necessarily limited to the injury site but occur throughout the organism. In this review, we focus on studies involving MDSCs in enhancing repair and improving function in injured heart muscle and promoting repair of osteochondral defects in articular

*Correspondence: jhuard@pitt.edu

¹Stem Cell Research Center, Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA 15219, USA

Full list of author information is available at the end of the article

cartilage (AC), and explore the possibility that the repair is induced by host cell recruitment, angiogenic and/or anti-inflammatory activities, and not necessarily restricted to the differentiation of the implanted cells in host tissue.

Stem cell-mediated therapies for cardiac injuries

Cellular cardiomyoplasty (CCM), cell transplantation for cardiac repair, is an alternative therapeutic approach for the treatment of congestive heart failure [6,7]. Researchers have used a wide variety of cell types for CCM, including embryonic and neonatal rodent and porcine cardiomyocytes, fetal smooth muscle cells, AT-1 tumor cardiomyocytes, human adult and fetal cardiomyocytes, autologous adult atrial cells, and dermal fibroblasts [8-19]. Researchers have also identified more suitable donor cells for CCM. The most promising cell populations evaluated to date include skeletal muscle myoblasts [20-23], murine embryonic stem cells [24,25], bone marrow (BM)-derived stem cells, mesenchymal stem cells (MSCs) [17,26-28], purified (enriched) hematopoietic stem cells [29-31], blood- and BM-derived endothelial progenitor cells [32-35], and cardiac stem cells [36-40]. To date, donor cell populations used in CCM have demonstrated some beneficial effects for the heart, but various ethical, biological, and technical concerns limit their suitability for use in human patients. Segers and Lee [41] and Gersh *et al.* [42] reviewed reports on randomized and controlled studies in a large number of patients treated with different cell therapeutic strategies (autologous, natural, or bioengineered cell populations) and the modes of cell injection, and provided an evaluation of their suitability for clinical use. Other reports have summarized findings on CCM in animal models and clinical reports [41,43-44].

Promising data have been generated recently when skeletal muscle myoblasts were utilized in several CCM human trials [45,46]. The cells were transplanted via direct intramyocardial injection and intra-arterial injection [21-23,47-49] and despite the ability of the engrafted skeletal myoblasts to adapt to the cardiac microenvironment and improve cardiac performance in experimental animal models of cardiac injury [21,22,46-50], various limitations, including poor cell survival - similar to those encountered with myoblast transplantation into skeletal muscle - have hindered the overall applicability of this therapeutic approach [51-53].

Our group has used MDSCs in cardiac transplantation experiments, which display an improved transplantation capacity when compared with myoblasts. MDSC engraftment was 25-fold higher than myoblasts in diseased hearts (with a mean of 53 donor MDSCs versus 2 donor myoblasts found within the injected hearts at 12 weeks post-injection) [54]. Importantly, they elicited significant

improvements in cardiac function by exhibiting superior cell survival and improving angiogenesis [54-56], most likely due to their secretion of vascular endothelial growth factor (VEGF) [57].

Although the exact origin of mouse MDSCs remains to be determined, these cells express the endothelial cell marker von Willebrand factor and can spontaneously participate in blood vessel formation after being injected into skeletal and cardiac muscle [58]. Their participation in blood vessel formation appears to be due to their expression of VEGF and by the fact that they can also differentiate toward an endothelial cell lineage [2,54]. The latter results suggest that a relationship exists between mouse MDSCs and endothelial cells. Other types of stem cells derived from the walls of blood vessels, including mesoangioblasts and perivascular cells, appear to share similarities with MDSCs, which also supports our hypothesis that a relationship exists between MDSCs and endothelial cells. We have also isolated several populations of muscle-derived cells from human skeletal muscle by fluorescence-activated cell sorting (FACS) that co-express myogenic (CD56 and Pax7) and endothelial cell markers (CD34, von Willebrand factor, ulex, and VE-cadherin) both *in vitro* and *in vivo* [59-61]. We observed that certain types of cells that constitute the walls of blood vessels in adult human muscle (endothelial cells, myo-endothelial cells, and pericytes) appear to be very early myogenic progenitor cells that have high myogenic potential, and regenerative capacities in both skeletal and cardiac muscles [59,61-63], much like that exhibited by murine MDSCs [2]. We have recently observed that a greater improvement in left ventricular function was observed after the intramyocardial injection of myo-endothelial cells when compared to hearts injected with myoblasts [63]. Transplanted myo-endothelial cells generated relatively good engraftments within the infarcted myocardium and also stimulated angiogenesis, attenuated scar tissue formation, and supported the proliferation and survival of endogenous cardiomyocytes more effectively than transplanted myoblasts or endothelial cells [63]. In a different set of preliminary studies, we also observed that the injured hearts injected with skeletal muscle-derived pericytes displayed significant improvements in cardiac contraction, greater neoangiogenesis, and a significant reduction in scar area formation when compared with hearts injected with phosphate-buffered saline [64].

These latter findings suggest that myo-endothelial cells and pericytes likely represent the human counterpart to murine MDSCs and consequently comprise a potential therapeutic cell source that could provide valuable benefits for patients suffering from myocardial infarction [64]. We have observed that after the implantation of murine MDSCs and human muscle-derived cells

(myo-endothelial cells and pericytes), the induction of heart repair is mediated mostly by the host cells. Indeed, we have observed that only a small fraction of the donor cells can actually be found within the regenerated heart tissue, indicating that the host cells must be largely contributing to the cardiac repair process [54,63]. These results indicate that the injected cells may act as a reservoir of secreting molecules that play a role in the repair process without actively differentiating toward a cardiomyocyte lineage or by fusing with host cardiac cells.

The cell-mediated paracrine and bystander effects on cardiac repair have also been observed with other stem cell types, including BM-derived cell populations [65-69], hematopoietic cells [70], adipose-derived stem cells [71], endothelial progenitor cells [72], human blood endothelial cells [73], and kidney-derived MSCs [74]. These reports support the hypothesis that the beneficial effect seen with MDSCs is likely due to the increased secretion of paracrine factors and not primarily due to the differentiation capacity of the donor cells toward a cardiac phenotype (for reviews refer to [28,75]), especially since the cardiac differentiation of these stem cells after implantation remains extremely low (Table 1).

In support of this paracrine effect hypothesis, we previously reported that inhibiting angiogenesis by injecting genetically manipulated MDSCs that express the anti-angiogenic protein sFlt-1 reduces the regeneration capacity of MDSCs in injured heart. The findings of this study demonstrated that most of the cells contributing to the repair process were indeed chemo-attracted to the injury site by the injected cells [57]. Although the paracrine action of the donor stem cells is widely accepted, the origin of the host cells that participate in the repair process remains largely unknown. Further experiments are underway to determine the type of cells and their origins. Likely candidates for the host cells involved in the repair process include, but are not limited to, BM-derived cells, vascular-derived endothelial progenitor cells, inflammatory cells and resident tissue stem cells. Since it is well established that the vascular supply plays a major role in cardiac tissue repair, it is logical to speculate that blood vessel-derived cells are also involved in the repair process after cardiac injury. Indeed, it has already been shown [76-78] in other animal injury models that tissue repair induces the mobilization and incorporation of BM-derived endothelial progenitor cells, suggesting that some of the chemo-attracted host cells are perhaps derived from endothelial progenitor cells. It is important to bear in mind that promoting angiogenesis will bring more blood vessel-derived cells to the injury area, but also cells derived from the BM and bloodstream; therefore, caution needs to be taken when reporting these research findings.

These results with MDSCs and other stem cell types (Table 1) strongly support the fact that a stem cell's multipotent capacity - in this case the ability to differentiate into a cardiac lineage - is not a pre-requisite for the cell's ability to readily aid in the repair of an injured heart. We cannot exclude that a stem cell's ability to readily undergo cardiac differentiation does not provide additional beneficial effect for cardiac repair; however, this still remains to be verified experimentally. More importantly, we have observed that the ability of MDSCs to resist environmental stresses, including oxidative and inflammatory stresses, through the cells' high antioxidant capacity (via glutathione and superoxide dismutase), plays a major role in the high regenerative capacity of MDSCs in various tissues, including the heart [79,80]. Moreover, we have observed that treating MDSCs with the reducing agent glutathione significantly reduced their ability to repair the heart, supporting our driving hypothesis that a cell's ability to survive within the injured tissue is more important than its terminal differentiation potential [79]. A population of stem cells that could survive better in this type of harsh environment could enhance the regeneration process, potentially through an increase in their paracrine effect (for example, increased angiogenesis).

A recent study showed that preconditioning BM-derived MSCs prior to their transplantation enhanced their capacity to repair infarcted myocardium, which was attributable to an improvement of donor cell survival and an increase in angiogenesis/vascularization and pro-angiogenic factors [81]. In addition, Pasha *et al.* [82] reported that preconditioning cells with the chemokine stromal-derived factor 1 alpha (SDF-1) could significantly enhance BM-derived MSC survival, vascular density, engraftment, and myocardial function [82]. MSCs derived from adult BM genetically modified with the anti-apoptotic gene *Bcl-2* enhanced cell survival, engraftment, revascularization, and functional improvement in a rat left anterior descending ligation model of myocardial infarction via an intracardiac injection [83]. Taken together, these results suggest that a cell's ability to survive the harsh microenvironment within the injured heart represents a major determinant for its regenerative capacity. Surviving cells can eventually promote the repair process primarily through a paracrine effect that involves angiogenesis, especially for cardiac repair (Figure 1).

Finally, it is important to state that stem cells with cardiomyocyte properties (such as cardiac stem cells, embryonic stem cells or genetically engineered cells with cardiomyocyte inducers) are perhaps more likely to terminally differentiate into cardiomyocytes and participate in heart repair than stem cells incapable of differentiating toward a cardiac lineage. It is possible that

Table 1. Literature showing the tissue-regenerative abilities of different stem cells through a paracrine effect

Stem cell source	Animal model	Resident tissue	Disease model	Number of cells injected	Route of transplantation	Time of transplantation post-injury	Cell engraftment	Outcome	Reference
ASCs	Rat	Heart	Myocardial infarction	5×10^6	Intramyocardial	1 week	0.5% at 30 days	Paracrine effect underlying mechanism behind increased capillary density	[71]
BM	Rat	Heart	Myocardial cryo-damage	2×10^7	Infusion via femoral vein	1 week	14 cells per field	Stem cells modulate inflammatory response	[65]
MSCs	Rat	Heart	Myocardial infarction	1.6×10^7	Intramyocardial	1 week	Few	Paracrine effect, angiogenesis, and cytoprotection	[68]
SM	Nude rat	Heart	Myocardial infarction	5×10^6	Intramyocardial	10 days	Few cells at 30 days	Myoblast-secreted factors induced cardiac repair	[122]
SM-CD133+	Nude rat	Heart	Myocardial infarction	5×10^5 to 5×10^6	Intramyocardial	10 days	Few cells at 30 days. CD133+ detected only by PCR	Both myogenic and bone marrow-derived cells improved cardiac function though the myogenic cells were superior	[123]
BM	Mouse	Muscle	Femoral artery ligation (hindlimb ischemia)	1×10^6	Intramuscular	24 hours	Few	Cells secrete arteriogenic cytokines	[66]
EPCs/OECs	Nude mouse	Muscle	Hindlimb ischemia	2×10^5	Intramuscular	3 to 6 hours	33% of new vessels contained donor cells	Neovascularization through cytokines	[73]
EPCs	Rabbit	Cerebral arteries	Common carotid artery denudation	5×10^5	Injection into arterial lumen	Immediately	Few colonies	Stem cells promote vasoprotection	[124]
EPCs	Rabbit	Basilar arteries	No injury	5×10^5	Injection into cisterna magna	NA	Few colonies	Stem cells promote vasoprotection by cyclogenase-2 via paracrine activity	[72]
MSCs	Mouse	Kidney	Renal ischemia (kidney clamping)	10^5	Intra-arterial infusion	Simultaneous with kidney clamping	<1 cell per whole kidney section	Vasculotropic and paracrine effects	[125]
MSC (4E)	Mouse	Kidney	Renal ischemia (kidney clamping)	10^6	Intravenously via tail injection	24 hours	9 to 45% at 15 to 30 days	Paracrine angiogenic chemoattraction and neovascularization	[74]
BM-MSCs	Mouse	Skin	Excisional wound splinting	Conditioned media from cells	Subcutaneous	Immediately	NA	Conditioning media enhance wound healing by recruiting macrophages and endothelial cells	[67]
Chondrocytes/perosteal cells	Miniature pig	Joint cartilage	Superficial chondral lesions	5×10^5	Press-fitting into cavity	Immediately	0% in the repair area after 12 weeks	Donor cells secrete BMP2 and recruited bone marrow-derived cells to the injury site	[114]
BM	Sheep	Cartilage	Full thickness cartilage defects	NA	Implants with cells	Immediately	NA	No improvement on tissue repair with increased cell loading	[126]
Autologous MSCs	Lamb	Cartilage	Tibial growth plate defect	4×10^6 in gel foam	Gel foam scaffold	5 minutes	NA	MSC cells can be used to regenerate growth plate	[127]
MSCs from fat pad	Rabbit	Cartilage	Osteoarthritis	1×10^6	Intra-articular injection	12 weeks	NA	Infrapatellar fat pad-derived cells can be used to repair osteoarthritis	[128]
hADSCs	Rat	Bone	Calvarial defect	1×10^6	Cell plus scaffold implant	Immediately	NA	Donor cells detected by FISH. Alendronate enhances hADSC osteogenic ability	[129]

Continued overleaf

Table 1. Continued

Stem cell source	Animal model	Resident tissue	Disease model	Number of cells injected	Route of transplantation	Time of transplantation post-injury	Cell engraftment	Outcome	Reference
hESCs-CTP	Mouse	Tendon	Full thickness Achilles tendon injury	NA	Engineered tendon graft	Immediately	NA	Tendon graft made of CTP had the ability to repair tendon	[130]
BM	Goat	Meniscus	Osteoarthritis by ACL transection and meniscectomy	10×10^6	Intra-articular injection	6 weeks	High level	GFP labeled donor cells detected especially at the surface of tissues	[131]
Allogenic BM-MSCs	Human	Systemic	Osteogenesis imperfect disorder	5.7×10^8 to 7.5×10^8 cells/kg	Intravenous infusion	NA	1.5 to 2.0% after 3 months	Increased bone density in osteogenic imperfecta patients	[132]

ACL, anterior cruciate ligament; ASC, adipose-derived stem cell; BM, bone marrow; BM-MSC, bone marrow-derived mesenchymal stem cell; BMP, bone morphogenetic protein; EPC, endothelial progenitor cell; FISH, fluorescent *in situ* hybridization; GFP, green fluorescent protein; hADSC, human adipose-derived stem cell; MSC, mesenchymal stem cell; MSC (4E), kidney-derived mesenchymal stem cell; NA, not available; OEC, outgrowth endothelial cell; SM, skeletal myoblast; SM-CD133+, skeletal myoblast and bone marrow-derived CD133+; hESC-CTP, connective tissue progenitor from human embryonic stem cell.

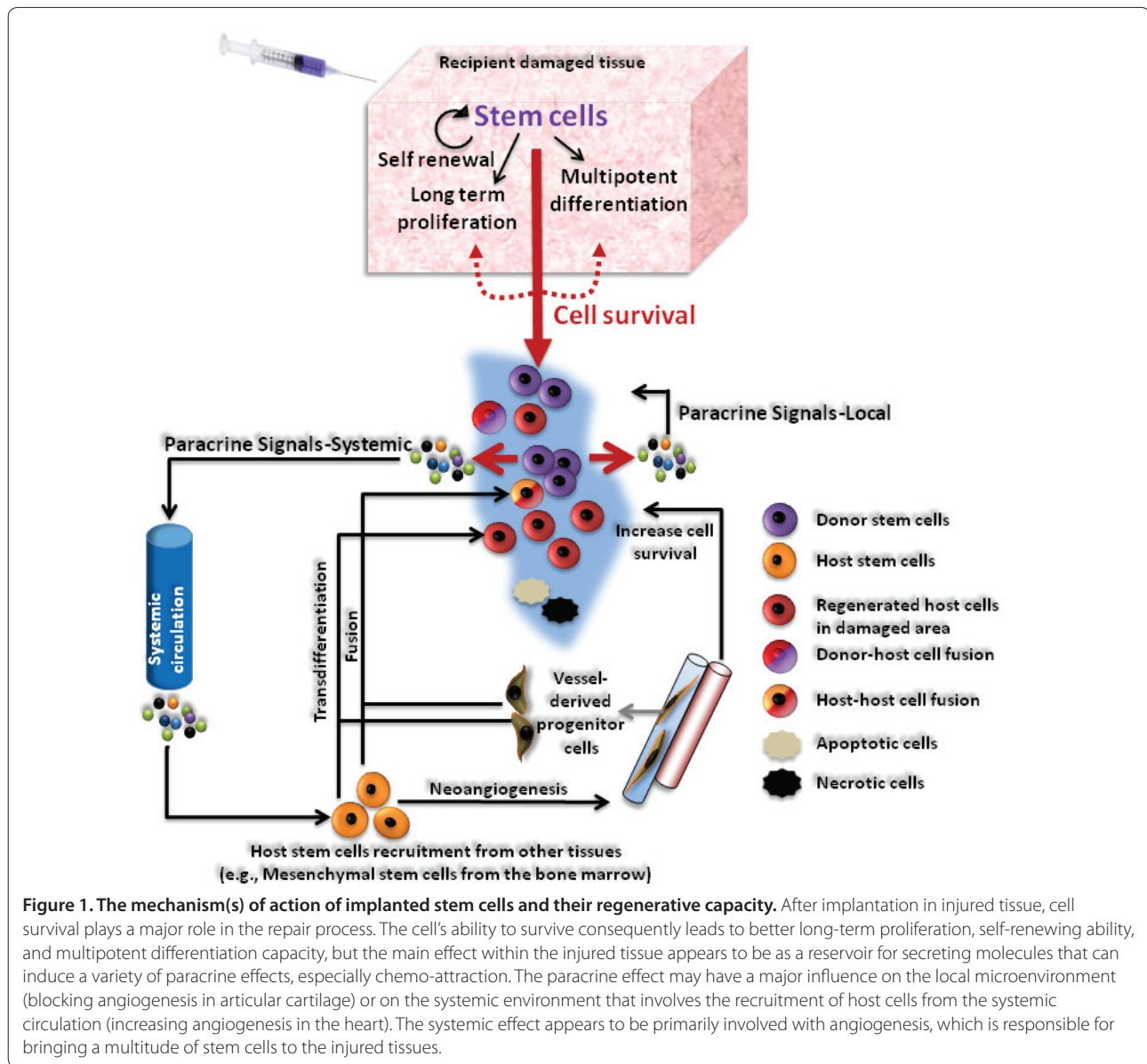
cardiac differentiation of stem cells prior to their transplantation could improve cardiac function due to their ability to integrate more effectively with the host myocardium. It will also be important to determine whether differentiating the cells toward a cardiac lineage prior to their implantation could influence the paracrine effect of the stem cells and how this would influence their action in the cardiac repair process. Thus far, however, from our observations and reports in the literature (Table 1), very few post-natal stem cells have been shown to adopt a cardiomyocyte phenotype, yet the vast majority of transplanted stem cell types have been shown to improve cardiac function.

Stem cell therapy for articular cartilage repair

In this section, we investigate the paracrine effect of stem cell therapy in the AC repair process of osteoarthritis (OA), where the cells used (MDSCs) have the ability to undergo chondrogenic differentiation but the paracrine effect of the donor cells on the promotion of angiogenesis is not required, but, in fact, needs to be inhibited. Therefore, it is important to determine, in this situation, whether the paracrine effect of the stem cells on the local microenvironment also plays a major role in the regenerative capacity of the stem cells for AC repair, without relying on angiogenic-related cells (blood vessel and circulation-derived stem cells).

OA is a chronic degenerative joint disorder with worldwide impact that is primarily characterized by AC destruction and osteophyte formation. One of the chief mediators of OA is inflammation and angiogenesis. Yin and Pacifici [84] demonstrated that VEGF treatment during early limb bud development in chick embryos causes excess vascularization and consequently reduced condensation of the chondrogenic mesenchyme. In the growth plate, VEGF has been reported to play an essential role in cartilage vascularization and absorption of hypertrophic chondrocytes, which together lead to endochondral ossification [85-87]. On the other hand, when VEGF was blocked with the soluble receptor protein (sFlt-1), it led to the expansion of a zone of hypertrophic cartilage and the inhibition of cartilage resorption [86]. Similar to endochondral ossification, osteophyte formation during OA development has been reported to involve VEGF signaling [88]. For an extensive review on the relationship between angiogenesis and OA in humans and animal model studies, we refer our readers to the review by Ashraf and Walsh [89], who outlined the complexity of OA and the interrelationship between angiogenesis, inflammatory processes, damage, innervation, and pain perception in the joint.

Recent data reveal that the expression of VEGF and its receptors (Flt1 and Flk1) in osteoarthritic cartilage reflects the ability of VEGF to enhance catabolic



pathways in chondrocytes by stimulating matrix metalloproteinase activity and reducing tissue inhibitors of matrix metalloproteinases (TIMPs) [90-93]. These data suggest that, apart from the effect of VEGF on cartilage vascularization and proliferation of cells in the synovial membrane, chondrocyte-derived VEGF promotes catabolic pathways in the cartilage itself, thereby leading to a progressive breakdown of the AC extracellular matrix.

Since AC is a tissue type that is poorly supplied by blood vessels (avascular), nerves, and the lymphatic system, it has a very limited capacity for repair after injury. Although several therapies have been used to treat OA, no widely accepted treatments have been established, with the exception of arthroplasty. For this reason, tissue engineering techniques aimed at repairing AC have

been extensively studied and chondrocyte transplantation is currently performed in clinics [94-96]. The current most effective OA treatment, besides arthroplasty, is the use of autologous chondrocyte transplantation. However, this treatment has several limitations, including the use of neighboring healthy donor cartilage, difficulty in treating large-scale defects, limited expansion capacity of the primary chondrocytes, the need for a periosteal patch to maintain engineered cartilage, and the fact that, in most cases, only 30 to 40% of the defect regenerates AC, with the remaining defect being filled with fibrocartilage [97-99]. In light of these limitations, it is important to find other sources of cells that are abundant and capable of chondrogenic differentiation. Stem cells are more attractive than primary chondrocytes because of their

superior capacity for self-renewal, proliferation, and survival following microenvironmental stress. Recently, stem cell-based therapies have been used clinically for cartilage repair [100,101]. Several studies have suggested that stem cells can undergo chondrogenesis and repair AC in experimental cartilage injury models (osteocondral lesions), including studies using MDSCs [102]. We have already reported that bone morphogenetic protein (BMP)4-transduced MDSCs improve cartilage regeneration in *in vitro* pellet cultures and in an *in vivo* cartilage defect model (osteocondral defect) [102]. We have also shown recently that human muscle-derived cells (myo-endothelial cells and pericytes) can undergo chondrogenic differentiation *in vitro*, albeit to a different extent [61,62].

Since the expression of VEGF by chondrocytes in the osteoarthritic joint has been related to cartilage destruction [88,90,91,93,103-105] and the induction of arthritis (especially when the dosage reached a certain threshold) [106-108], it is likely that blocking VEGF would prove to be a beneficial means of preventing or delaying the progression of OA. This hypothesis was recently supported by the fact that treatment with sFlt-1 (a VEGF antagonist) decreased the severity of arthritis in a murine model [86,109,110] and our recent observation that the injection of MDSCs expressing both BMP4 and sFlt-1 improved AC repair in a more effective manner than MDSCs expressing just BMP4 [111,112]. Our results suggest that sFlt-1/BMP4-transduced MDSCs, which were transplanted intra-capsularly into an OA rat model, enhanced AC regeneration via BMP4's autocrine/paracrine effects, and contributed to an appropriate environment that prevented chondrocyte apoptosis by blocking the intrinsic VEGF catabolic pathway and by extrinsic VEGF-induced vascular invasion. Treatment of MDSCs with sFlt-1 and BMP4 combined is potentially an effective therapy for OA repair that may improve the quality and persistence of regenerated AC [111]. Since the cells were injected into the joint fluid, most of the injected cells do not primarily contribute to the regeneration of the AC through their differentiation into chondrocytes; instead, they chemo-attract host cells to the injury site, which are the cells primarily seen in the regenerated tissue. We are performing experiments to determine the origin of these host cells that participate in the repair process by testing the role of inflammatory/immune, BM and synovial cells. Since we have observed that the injection of muscle-derived cells (isolated from rabbit skeletal muscle) in the joint fluid of rabbits leads to a massive attachment of the injected cells in the synovium [113], we posit that synovium-derived cells are implicated in the repair process.

Although the mechanism behind the beneficial effect of blocking angiogenesis in AC repair is still not fully

elucidated, these results highlight the importance of controlling the local microenvironment by reducing angiogenesis. Therefore, the reduction of angiogenesis eliminates the mobilization of blood vessel wall- and circulation-derived progenitor cells and thus their recruitment and differentiation toward a chondrogenic lineage, which demonstrates the paracrine effect that the implanted stem cells exert on the local microenvironment for optimizing the AC repair process (Figure 1).

Recently, Gelse and colleagues [114] reported that transplanted rib chondrocyte spheroids could repair a cartilage defect in a miniature pig model by producing BMP2 and attracting the host's BM-derived cells. The transplanted chondrocyte spheroids provided a stimulatory paracrine effect that induced the in-growth and chondrogenic ability of the host BM-derived cells. This paracrine effect was observed to be far more important to the repair process than the direct differentiation of the transplanted cells into chondrocytes. Although the transplanted cells enhanced the tissue repair process, these experiments further validate our hypothesis that the AC repair process, even using stem cells other than MDSCs, also relies on the paracrine effect that the donor cells impart on the host cells.

The microenvironment influences the fate of stem cells

BMP4-transduced MDSCs can undergo osteogenesis and promote bone repair when injected into a bone defect [115-117], which is difficult to explain given that similar BMP4-transduced cells can promote the repair of AC when injected into an osteocondral defect. This phenomenon, however, is a good example of how the microenvironment influences the regenerative ability of the transplanted stem cells. After a bone or AC injury occurs, a multitude of signals are released at the site of injury. It is likely that the chemotaxis of host cells is enhanced by growth factors and cytokines produced by the donor cells, which are in turn affected by their interaction with the extracellular matrix at the injury site. Speculatively, stem cells injected into the site could aid and enhance the mediation of the repair process; however, knowing that the repair process relies primarily on host cell participation, it is easier to understand why BMP4-expressing MDSCs have a beneficial effect on both bone and AC repair since the repair process does not rely on the terminal differentiation of the donor cells *per se*. Furthermore, Blanke *et al.* [118] have shown that successful repair of cartilaginous tissue after transplantation of chondrocytes was associated with their production of thrombospondin-1 and chondromodulin-I anti-angiogenic proteins. They found that tissues resisted ossification when the chondrocytes produced detectable levels of anti-angiogenic proteins,

which counteracted the angiogenic activity of endothelial cells. It is very likely that MDSCs react in a similar manner to the local environmental cues and produce anti-angiogenic proteins similar to thrombospondin-1 and chondromodulin-I when in a cartilaginous microenvironment, a hypothesis that will need to be further investigated in future studies.

Another example that demonstrates the influence of the microenvironment is the fact that the regenerative capacity of stem cells has been shown to be influenced by the age of both the host- and the donor-derived stem cells [119]. The results showed the rejuvenation of aged progenitor cells after their exposure to a young systemic environment where a young and aged mouse had their circulatory systems linked by heterochronic parabiosis. These findings highly implied that the proliferative property of satellite cells obtained from old mice is restored after incubation with the serum from a young animal. These results further support our hypothesis that the regenerative process of given stem cells is strongly influenced by signals found within the microenvironment.

Furthermore, we have recently observed that the regenerative capacity of stem cells also appears to be influenced by the gender of the donor cells and the host. In fact, we reported that female MDSCs are more myogenic and can promote muscle regeneration in a more effective manner than male MDSCs [120]. On the other hand, male MDSCs were shown to be more osteogenic and chondrogenic and promoted both bone and AC repair in a more effective manner than their female counterparts [111,121]. Although the mechanisms behind these gender differences are still unclear, we have shown that the host microenvironment is also influenced by the gender of the animal and plays a role in the efficiency of the repair process. Indeed, we have reported that bone formation mediated by male MDSCs is superior in a male host versus that of a female host [121]. Interestingly, female MDSCs produce better bone when injected into a male host when compared to a female host. These results further support our hypothesis that the microenvironment influences the fate and regenerative potential of the injected stem cells.

Conclusion

Although it has been speculated for numerous years that the high regenerative potential of stem cells is due to their terminal differentiation capacity, current findings appear to indicate that very few donor cells actually differentiate and participate in the regeneration of these injured tissues; instead, the vast majority of the cells reconstituting the regenerated tissues are host-derived. These findings are further supported by recent results showing that when the paracrine signaling of the implanted stem cells is interrupted (that is, by blocking

VEGF and angiogenesis), there is a reduction in the regeneration and repair capacity of the injured tissues, as is the case for cardiac muscle repair. Although it is still unclear which host cells are involved in the repair processes after stem cell transplantation, blood vessel cells, immune and inflammatory cells and resident cells at the site of the injury (especially for AC damage), appear to play a role in the regeneration/repair process. It is quite clear, however, that the terminal differentiation of stem cells does not represent a major determinant for the success of stem cell therapy; instead, it appears that donor cell survival and the cells' paracrine effect play much more critical roles in the success of stem cell therapy. This finding challenges current dogma indicating that embryonic stem cells may have an advantage over adult-derived stem cells because of their higher level of multipotentiality. We therefore put forward the proposition that it is the stem cell's superior cell survival capacity that leads to its increased ability to chemo-attract host cells through the secretion of certain growth factors and chemokines and this is the key important feature for successful stem cell therapy, more so than stem cells' terminal differentiation capacity.

Future directions

The paradigm shift in evaluating stem cell engraftment based on their terminal differentiation into host cells underscores the need to understand the biology of stem cells to fully utilize their potential. Furthermore, we are increasingly aware that stem cells alone are not sufficient for a long lasting regenerative effect. The ultimate goal would thus be the generation of tissues incorporating stem cells, scaffolds and biological materials that permit communication with host tissues, allowing optimal remodeling and improved functionality. Future regenerative schemes may include cells and a complex milieu of factors based on a rigorous understanding of stem cells' paracrine secretions. Computer-aided bioreactors, bioprinters, artificial or decellularized organs and other bio-devices could also benefit from knowledge of stem cells' paracrine activities.

Abbreviations

AC, articular cartilage; BM, bone marrow; BMP, bone morphogenetic protein; CCM, cellular cardiomyoplasty; MDSC, muscle-derived stem cell; MSC, mesenchymal stem cell; OA, osteoarthritis; VEGF, vascular endothelial growth factor.

Competing interests

JH has received remuneration as a consultant from Cook MyoSite Inc., Pittsburgh, USA over the past 5 years, the other authors declare that they have no competing interests.

Acknowledgements

This work was supported in part by a Department of Defense Contract (W81XWH-08-0076), the National Institute of Health (RO1 DE013420-09), the William F and Jean W Donaldson Chair at the Children's Hospital of Pittsburgh, the Henry J Mankin Endowed Chair in Orthopaedic Surgery at the University

of Pittsburgh and the Orris C Hirtzel and Beatrice Dewey Hirtzel Memorial Foundation.

Author details

¹Stem Cell Research Center, Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA 15219, USA. ²Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15219, USA. ³Departments of Molecular Genetics and Biochemistry, Physical Medicine and Rehabilitation, and Pathology, University of Pittsburgh, Pittsburgh, PA 15219, USA.

Published: 8 July 2011

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doi:10.1186/scrt72

Cite this article as: Gharaibeh B, *et al.*: Terminal differentiation is not a major determinant for the success of stem cell therapy - cross-talk between muscle-derived stem cells and host cells. *Stem Cell Research & Therapy* 2011, **2**:31.