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Is Adipocyte Differentiation the Default Lineage for Mesenchymal Stem/Progenitor Cells after Loss of Mechanical Loading? A Perspective from Space Flight and Model Systems

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Abstract

Mesenchymal stem/progenitor cells (MSC/MPC) are found in many tissues and fluids including bone marrow, adipose tissues, muscle, synovial membranes, synovial fluid, and blood. Such cells from different sources can proliferate and differentiate into different lineages (e.g. osteogenic, chondrogenic and adipogenic) after suitable stimulation. However, details regarding the regulation of MSC/MPC proliferation and differentiation status are still unclear and it is likely that regulation involves both biological and mechanical influences in the different environments. It has been noted that in humans and preclinical animal models that exposure to microgravity/space flight or prolonged bed rest (a surrogate for microgravity) can lead to infiltration of skeletal muscle and bone marrow with fat. Similarly, in preclinical models treated with multiple intramuscular injections of Botulinum Toxin A to induce muscle weakness and atrophy, there is also an infiltration of the muscle with fat. The origins and basis for these fat deposits are largely unknown, but there is a possibility that the altered mechanical and biological environments lead to dysregulation of MSC/MPC and progression to preferential differentiation towards the adipocyte lineage. Furthermore, loss of MSC regulatory control by either mechanical and/or biological factors may also contribute to their involvement in obesity development and progression. Thus, the utility of using MSC/MPC from some sources for tissue engineering purposes may be compromised and further research regarding optimal loading for tissue engineering purposes is likely warranted.

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Keywords

Mesenchymal Stem Cells, Adipose-Lineage, Microgravity, Bed Rest, Muscle Weakness, MSC Regulation, Adipose Tissue

1. Introduction

1.1. Mesenchymal Stem/Progenitor Cells

Mesenchymal stem/progenitor cells (MSC/MPC) are found in a number of tissues and compartments including blood, placenta, bone marrow, synovial membrane, synovial fluid, fat (infrapatellar fat pad of the knee, subcutaneous fat, abdominal fat) (reviewed in [1] [2]). Such cell populations are heterogeneous [1] [2] with respect to proliferation and differentiation [3], and MSC/MPC from different compartments (e.g. bone marrow and other compartments) exhibit different epigenetic signatures, indicating that the local environment influences the MSC/MPC populations (discussed in [1] [2]). MSC/MPC populations in different compartments appear to be able to migrate to other sites via the cardiovascular system (reviewed in [4]-[6]; and others), but the stimuli for such migratory patterns is not well defined as yet.

The MSC/MPC from different tissues and compartments appear to differ with respect to their predilection to differentiate towards different lineages (e.g. bone, cartilage, adipose/fat cells) ([3]; and others). Thus, those in the bone marrow appear to prefer to differentiate towards bone cell phenotypes, while those in the synovial membranes or synovial fluid of the knee prefer chondrogenesis ([3] [7]; and others). With MSC from sources such as fat and fat pads, the phenotype of the cells appears to be more anti-inflammatory than those from other sources, and they are being widely investigated for applications such as osteoarthritis, rheumatoid arthritis, and others (reviewed in [8]).

1.2. Control of MSC Numbers, Distribution, and Activity

Levels of MSC in blood are reported to decline with age (discussed in [9] [10]; and others). In addition, numbers of MSC in skeletal muscle are reported to be influenced by the sex of animal, with females having more MSC than males [11]. Gender dimorphisms in progenitor and stem cell functions have also been noted [12]. In rats, it has been reported that MSC can be induced to emigrate from the bone marrow into the blood stream, and then home towards sites of need via specific mediators (e.g. the neuropeptide Substance P) from such injury sites [13]. Interestingly, injection of large numbers of MSC into the blood stream (discussed in [5]) or into the injured knee joint [14] or other tissues [15] leads to very small numbers of the injected cells actually residing at the sites for an extended period of time. Thus, such populations are likely very heterogeneous with regard to homing, localization and fate, for reasons which are still mostly unclear (discussed in [1] [2]).

Some of the response patterns noted may be the result of specific features of the different environments both biologically and mechanically. For instance, one might expect that MSC would be able to initiate repair following an injury to a connective tissue. However, it was found that an inflammatory environment resulting from an injury leads to impaired function of MSC within a knee joint [7] [16]. Furthermore, while it might be expected that all environments exert some biomechanical influences on MSC (pulsatile loading in blood, intermittent compression in synovial fluid, aspects of shear in bone marrow due to loading of the bone), the mechanical environment of cells in synovial fluid or the infrapatellar fat pad would likely be quite different from that in subcutaneous fat. Thus, MSC are able to exist and function in quite diverse biological and mechanical environments.

That the biological environment can impact MSC regulation can be drawn from the literature. For instance, normal MSC from synovial fluid are able to differentiate along different lineages *in vitro* in response to specific stimuli [3], and in fact are capable of aggregating as they differentiate towards the chondrogenic lineage [16] [17]. However, MSC/MPC isolated from individuals with early osteoarthritis or following an injury leading to an inflammatory state are reported to have lost this attribute [17]. Similarly, MSC isolated from ovine knee synovial fluid are compromised if the knee has been subjected to an injury [3].

An additional biological contributor to MSC regulation in different environments that has not received much

attention is the nerve supply and mediators contained in such elements. Nearly all tissues are innervated (with articular cartilage a notable exception), including fat pads, bone, bone marrow, synovial membranes and joint capsules to name a few. Obviously, fluid components such as blood or synovial fluid are not innervated, but synovial fluid can be exposed indirectly to nerves via released mediators and neural influences on cells of the microcirculation are well known. However, while the nerve supply in many tissues is modest at best, it is clear that cells such as mast cells often co-localize with nerves and are capable of amplifying neural signals (reviewed in [18]-[21]). Thus, the impact of a few nerves on other resident cells may be a direct influence (e.g. nerve elements on MSC directly), or indirectly via amplification through other cells (e.g. nerve—mast cell-MSC). Some of this discussion is speculative at the present time, as nerve-MSC interactions and regulatory considerations have not been explored in any detail.

On the larger scale, the relative interplay between biological and mechanical variables in maintaining MSC in a "meta-stable" state in various tissues and fluids, is not well characterized (discussed in [22]). Thus, the question of how MSC numbers are stabilized in various tissues, and how the cells are kept in the MSC/MPC state in the face of a large number of potential activators and inhibitors, has not been addressed well in the literature, in fact such questions have not been addressed in the available literature based on this author's search of databases. This situation leads to several questions including the following: 1) what are the cellular mechanisms that maintain a balance between the MSC "state"?; 2) how is the need to replenish MSC when they are used for tissue repair or response to injury regulated?; and 3) if such regulatory systems become compromised (e.g. via aging, abnormal environments, or chronic diseases), what are the consequences with regard to MSC integrity? What follows is a discussion regarding potential answers to such questions, and possible research directions to address current gaps in our understanding.

2. Skeletal Muscle Responses to Botulinum Toxin A (BoToxA) in Preclinical Models

As noted through studies of animal models, MSC are present in the perivascular compartment of many skeletal muscles [23]-[25], with some sex differences in numbers and activity also noted (discussed in [23]-[25]). These MSC can be differentiated into the usual three lineages (osteogenic, chondrogenic, adipogenic) after isolation, as well as possibly others (e.g. myogenic, neural).

Injection of skeletal muscles of the rabbit hind leg with repeated doses of BoToxA leads to the rapid atrophy of the muscles with loss of function [26] [27]. Subsequent to the atrophy, there is a "fatty infiltration" of the muscles which persists for some time (discussed in [28]). The source or origins of the fatty infiltrate has not been characterized, and the findings are more descriptive than mechanistic thus far. Therefore, based on the temporal relationships, following BoToxA injection the overt atrophy occurs rapidly, and then in response to prolonged loss of mechanical integrity of the muscle, a fatty infiltrate occurs somewhat later, but which can be protracted. The fate of the muscle-associated MSC has not been addressed in this situation based on a search of the literature. In addition, the fate of MSC within existing fat pads of the knee, such as Hoffa's fat pad, during such BoToxA-induced atrophy has not been described, but these issues are currently under investigation (unpublished).

3. "Fatty Infiltrate" Responses to Micro-Gravity or Prolonged Bed Rest

Rapid atrophy of muscle and bone upon exposure to micro-gravity through space flight has long been known (reviewed in [29]-[31]; and others). Such loss occurs throughout the body, but appears to be focused on a subset of muscles of the lower extremities which are apparently the most influenced by the 1G-mediated ground reaction forces on earth. While the muscle loss can be at least partially prevented by counter measures, bone loss is less influenced by existing modalities (reviewed in [32]). Interestingly, the extent of bone and muscle loss by those experiencing micro-gravity in space flight is quite individualized, with some people losing tissue mass at rates several times faster than others (e.g. 2%/month vs 0.2%/month) (discussed in [33]). Thus, there appears to be a genetic component to the rate of loss that is not overtly expressed when in the 1G environment (possibly silent mutations that are masked by the 1G environment). Some authors have suggested that the responses to microgravity and space flight are actually an accelerated aging scenario [34]-[36]. If the analogy is correct, then finding solutions to responses to microgravity may have application for aging earth-bound populations as well.

Interestingly, with prolonged exposure to micro-gravity, there is a reported "fatty or lipid infiltration" of the muscle (reviewed in [37]). Where such fatty infiltrates come from, or why they appear has not been addressed,

the phenomenon merely described. The recovery from such fatty infiltrates after a return to a 1G environment is apparently slow, but has not been described well in the literature (but likely could using improved ultrasound methodology or others). With six team members on the International Space Station presently for ~6 month stays, the number of individuals who could be monitored for onset and recovery is quite large by space-study standards. Similarly, one could perform a limited number of muscle biopsies to evaluate fat-associated MSC in such astronauts.

A surrogate for exposure to micro-gravity is "head down-tilt bed rest" (reviewed in [38] [39]; and many others). Individuals subjected to this type of bed rest develop rapid bone loss indicators [40], and subsequently muscle atrophy, overt bone loss, and other characteristics of space flight (reviewed in [31] [38] [39]; and many others). Over time, individuals exposed to this environment also develop "fatty infiltrates" in their skeletal muscle and other tissues. And again, the condition has been described, but the source and causes have not been addressed. While this surrogate has been studied in relation to micro-gravity aspects, it is also clear that with the aging population, many of whom are confined to prolonged bed rest conditions, there is also risk for atrophy and other complications, including "fatty infiltration" of their skeletal muscles and other tissues [41], and some counter measures prevent such accumulation [42].

Therefore, in the Botulinum toxin studies, the space flight/microgravity studies, and the bed rest studies, as well as other animal studies of weightlessness (e.g. hind limb elevation to mimic loss of ground reaction forces) (reviewed in [37] [43]), an accumulation of fat/lipid infiltrates occurs during prolonged exposure to the altered environment. It is clear that it happens, but the why and how it happens still remains to be determined. One possibility that could explain at least some of this response to weightlessness or loss of mechanical integrity is that mesenchymal stem cells in muscle and bone marrow are maintained in a meta-stable state by mechanical loading of the tissues, directly (via the MSC themselves) or indirectly via loading effects on the neuronal state of the tissues which when compromised, leads to a loss of the maintenance of the MSC in such a meta-stable state. Thus, alterations in these influences on MSC regulation may lead to the preferential differentiation of the MSC towards the adipose lineage (the default response). This may be due to an enhanced responsiveness to lineage-specific signals in the environment, or to an intrinsic loss of stability in the MSC.

While there is not much in the literature regarding this possibility, certainly the report of Meyers *et al*. [44] indicates that in a model of microgravity, there is enhanced adipogenesis and reduced osteoblastogenesis by human MSC. Interestingly, recent studies from the author's laboratory have indicated that a subset of cloned MSC/MPC from porcine synovial fluid are restricted to differentiating towards either the osteogenic or adipogenic lineages ([3]; JJ Kutcher, MSc Thesis, University of Calgary, 2012; Kutcher *et al*., in preparation). While not the dominant phenotype of MSC from porcine synovial fluid, the prevalence of this unique subset of MSC in other compartments such as muscle, bone marrow, and adipose tissue (e.g. abdominal fat, subcutaneous fat, Hoffa's fat pad of the knee, brown fat) is unknown. Relevant to this point is also the fact that the studies by Meyers *et al*. [44] used mixed populations of human MSC, so it was not possible to make conclusions regarding whether only a subpopulation of MSC were affected by the microgravity conditions employed. Thus, it would be relevant to repeat those studies with well characterized cloned cells to allow stronger conclusions to be made. Such studies are currently underway (unpublished).

While the above question has not been answered directly, there are reports that mechanical loading can influence MSC lineage specificity even when the subjects are in the 1G environment of earth [45] [46]. Thus, low magnitude mechanical stimulation of C57BL/6 male mice promoted bone formation and depressed/inhibited diet-induced obesity. There were also increases in bone marrow MSC numbers following such stimulation. A pilot study with 48 young females also indicated that this low magnitude mechanical stimulation enhanced bone formation and kept visceral fat at baseline levels. Thus, unique mechanical loading regimens in a 1G environment may also be a methodological approach to interfere with obesity development [45] [46]. This concept has also been postulated recently by Gutin [47] who hypothesized that vigorous physical exercise may contribute to MSC differentiation preference.

4. MSC in Adipose Tissue: Influence of Obesity, Inflammation and Culture

MSC have been derived from a variety of human and preclinical model adipose tissues (e.g. subcutaneous fat, fat pads in the knee [e.g. Hoffa's fat pad, infrapatellar fat pad], brown fat, and abdominal fat) (reviewed in [8] [48]-[50]; and many others). In particular, MSC from subcutaneous fat via "liposuction" have been promoted as

a ready source of cells for cartilage repair (reviewed in [8] [51]), and a number of clinical trials are on-going to evaluate their efficacy.

Interestingly, MSC derived from obese mice are reported to be altered in their differentiation potential and they are altered irrespective of tissue source (bone marrow, infrapatellar fat pad, subcutaneous fat), and the alterations appeared to be lineage-specific with the chondrogenic lineage specifically affected (discussed in [55]). Certainly obesity can lead to a metabolic disease or syndrome (reviewed in [52]-[54]; and others), so this is finding is not surprising at some levels. This metabolic syndrome also likely contributes to the altered inflammatory state observed in obesity, directly via adipokine secretion ([53] [54]; and others) or via subsequent amplification at systemic sites (discussed in [55]-[57]; and others). Obesity may also influence the proliferative potential of adipose-derived MSC [58], and therefore could alter regulation of these stem cells.

Acute joint inflammation due to an injury can also lead to alterations in the infrapatellar fat pad of the knee in ovine models [59], and transient alterations in adipokine expression [59] and MSC levels (Solbak *et al*., in preparation). As yet, it is not yet clear as to whether such inflammation leads to altered lineage differentiation.

Finally, merely culturing adipose-derived MSC in monolayer culture is also reported to alter lineage-specific differentiation [60], so care must be taken when making conclusions regarding adipose-tissue derived cells. Certainly culturing cells on plastic, in the presence of specific growth mediators, or serum has the potential to impact the MSC and influence the ability to translate *in vitro* findings to the *in vivo* condition.

These findings with MSC and other cells in adipose tissues are likely also relevant to the above discussion on the "fatty infiltration" of muscle, and bone marrow in response to an altered environment of muscle weakness, microgravity and prolonged bed rest. That is, these MSC populations are dynamic and respond to altered environments (e.g. loss of mechanical integrity, diet-induced obesity). In the case of diet-induced adiposity, it is clear there are still a large number of MSC in the tissues. Whether the MSC are contributing to the obesity (differentiation to adipocytes), as well as maintaining proliferation of the MSC compartment is possible, but not proven at this point. What is also unknown is whether the MSC compartment in muscle and bone marrow is also maintained in the tissues as the fatty infiltrate occurs. Thus, this should be a focus for future studies. The findings from such studies should either support or refute the premise that the adipose lineage for MSC differentiation is a "default" lineage when the mechanical environment is compromised.

5. Why Are There So Many MSC in Adipose Tissues, and Could They Play a Role in Obesity When Regulation Is Compromised/Dysregulated?

As discussed above, it is clear that MSC/MPC are present in a variety adipose tissues. The numbers decline with age, and obesity can apparently influence the functionality of such cells. Also with age, the incidence of lipid deposition in various compartments (e.g. subcutaneous fat vs abdominal fat in males vs females with age) increases. All of the above beg the question, why are there so many MSC/MPC in adipose tissues, and could their dysregulation be a contributing factor in obesity development? If so, then MSC/MPC leading to the adipose lineage may be a potential target for interventions (e.g. new drug therapies) to regulate MSC/MPC in both prolonged space flight, prolonged bed rest, and in some forms of obesity (discussed above and in [45]).

This concept is not entirely new as a number of reports have indicated that perhaps interfering with MSC differentiation towards the adipocyte lineage [61]-[64], or even replacement of neural MSC in the hypothalamus [56] [65]-[67] could modulate obesity-related events. Some reports have even postulated certain molecules could be potential molecular targets for limiting such pathways [61]-[64] [68] [69].

While performed prior to the onset of most information on MSC, a set of studies from the author's laboratory using (NZB x NZW) F1 mice (referred to as NZB/W mice) may also provide some clues in this regard. This strain of mice develop large deposits of subcutaneous and intra-abdominal fat as they mature and age, reaching weights that are nearly double that of parental strain animals or younger animals. Treatment of females of the NZB/W strain with daily injections of lithium chloride (LiCl) leads to a nearly complete inhibition of the development of fat deposits over months, and there was a "re-activation" of fat deposition after treatment was discontinued [70]-[72]. While MSC in these animals were not assessed, more recent studies have indicated that LiCl treatment leads to inhibition of expression of adipocyte lineage genes *in vitro* with human bone-morrow-derived MSC [73]. Therefore, as lithium ion is a known inhibitor of GSK-3 and a modulator of WNT/beta-catenin pathway components, such studies should be revisited to ascertain whether the outcomes were due to impact on MSC stability and differentiation. Furthermore, as lithium ion treatment of aged MSC restores some of their functionality [74], possibly such treatment could allow for re-establishment of MSC regulation following compromise

as discussed above. However, as lithium ions do have potential side-effects, studies in mice to follow up the above discussed studies may lead to identification of specific targets that can be modulated using new drugs and therapeutic modalities for application in human populations.

5.1. Implications of Such Alterations in the MSC Compartment for Tissue Homeostasis and Repair

The infiltration of tissues by lipids and fat deposits could not only affect tissue function, but also the ability to repair if indeed the move towards such fatty infiltrates is due to loss of regulatory control of the MSC with differentiation towards the adipocyte lineage. If the extent of such infiltrates/deposition becomes excessive, loss of normal function would likely be expected if the condition persisted. In bone marrow, which is also responsible for blood cell formation from other stem cells, crowding out the normal environment with fat could lead to impaired blood cell formation. This concept has already been postulated by Payne *et al*. [75]. Similarly, in muscle the infiltrates could impair muscle contractions and responsiveness to stimuli. Depending on the breadth of different tissue involvement, and the extent and duration of these changes, one may also see impaired function in the liver, heart, and potentially the brain. As discussed earlier, some of the changes associated with micro-gravity and prolonged bed rest exposure may be a form of accelerated aging, so identifying suitable targets to prevent such changes from happening in younger, otherwise healthy individuals or in model systems, may also assist in understanding the normal aging process.

Finally, the observations discussed above regarding what may be the result of MSC dysregulation could also have implications beyond the microgravity/surrogate domain. That is, the focus has been on altered regulation due to altered mechanical environments, but regulation compromised by genetic, epigenetic and other biological factors could also have relevance to development and progression of obesity. That is, the MSC may play a direct participatory role in obesity development and progression in some people (discussed in [76]-[78]; and others). Such a role has prompted search for anti-obesity drugs that target MSC (for example, [79]). However, this challenge is complicated by the heterogeneity of MSC [2], even those within adipose tissues (discussed in [80]).

5.2. Implications of Such Alterations in the MSC Compartment Regarding Tissue Engineering Potential

As mentioned earlier, a number of clinical trials are on-going with adipose tissue derived MSC and such cells have been proposed to be used for cartilage tissue engineering ([51]; and others). Furthermore, a number of centers are using MSC from various tissues to inject into joints of patients with osteoarthritis or sports injuries in order to decrease symptoms (e.g. pain) or improve tissue repair.

Other centers are using MSC to generate **T**issue **E**ngineered **C**onstructs (TEC) in attempts to repair cartilage defects, as well as other injured tissues. Thus, attempts to use MSC from tissues of individuals compromised by prolonged bed rest, or other “weightlessness” environments may lead to less than ideal outcomes, either at the generation stage, or the outcome stage after implantation as the TEC adapts to the *in vivo* environment.

Finally, most studies report proliferation and differentiation of MSC *in vitro* in the absence of mechanical loading. The above discussion certainly indicates that a lack of loading can influence MSC outcomes, and therefore, studies should likely be performed in the presence of mechanical loading that is appropriate for generating TEC for specific applications (e.g. tensile loading, shear loading, compressive loading, etc.). Optimizing loading for specific TEC applications may minimize some of the heterogeneity observed in mixed populations and yield better outcomes.

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