Pathology International

*Pathology International* 2014; **64**: 1–9 doi:10.1111/pin.12129

Review Article

# Muse cells, newly found non-tumorigenic pluripotent stem cells, reside in human mesenchymal tissues

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**Mesenchymal stem cells (MSCs) have been presumed to include a subpopulation of pluripotent-like cells as they differentiate not only into the same mesodermal-lineage cells but also into ectodermal- and endodermal-lineage cells and exert tissue regenerative effects in a wide variety of tissues. A novel type of pluripotent stem cell, Multilineage-differentiating stress enduring (Muse) cells, was recently discovered in mesenchymal tissues such as the bone marrow, adipose tissue, dermis and connective tissue of organs, as well as in cultured fibroblasts and bone marrow-MSCs. Muse cells are able to differentiate into all three germ layers from a single cell and to self-renew, and yet exhibit non-tumorigenic and low telomerase activities. They can migrate to and target damaged sites *in vivo*, spontaneously differentiate into cells compatible with the targeted tissue, and contribute to tissue repair. Thus, Muse cells may account for the wide variety of differentiation abilities and tissue repair effects that have been observed in MSCs. Muse cells are unique in that they are pluripotent stem cells that belong in the living body, and are thus assumed to play an important role in 'regenerative homeostasis' *in vivo*.**

**Key words:** cell transplantation, mesenchymal stem cells, pluripotent stem cells, regenerative medicine, telomerase

## CURRENT STATE OF MESENCHYMAL STEM CELL (MSC) RESEARCH

Nearly 400 clinical studies of mesenchymal stem cell (MSC) transplantation have been performed around the world, targeting various diseases, such as Parkinson's disease, Crohn's disease, pulmonary fibrosis, and diabetes mellitus.[1–4] Sources of MSCs vary, with the bone marrow, adipose tissue, and umbilical cord currently being the most common. These sources are easily accessible and avoid the ethical problems associated with the use of fertilized eggs and fetal tissue. Tissue banks are available for bone marrow and umbilical cord tissues. Human MSCs have high proliferative activity and therefore large numbers of harvested MSCs can be obtained for clinical use.[5,6]

The most important requirement for clinical application is safety. To date, there have been no reports of tumorigenesis related to MSCs. They are not artificially induced or manipulated, but are naturally existing stem cells, and are thus considered non-tumorigenic. Although MSCs have great advantages for clinical use, they are not superior in all aspects, and the effects of MSCs on tissue regeneration and functional recovery are controversial.

While MSCs are referred to as 'stem cells', the rigorous methods of stem cell biology that are applied to hematopoietic and neural stem cells have not been applied in most of the studies performed using MSCs. Mesenchymal stem cells are usually collected just as adherent cells from the bone marrow and other mesenchymal tissues. While the morphology of collected adherent cells is similar to that of fibroblasts, they are not the same as fibroblasts. Some basic information about MSCs remains obscure, such as how many cells in the MSC population critically meet the criteria of stem cells, how many types of cells comprise MSCs, or the ratio of each cell type. The MSCs are a crude population and may include cells other than stem cells,

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Conflicts of interest: Nothing to declare in terms of conflict of interest and funding.

Received 28 November 2013. Accepted for publication 13 December 2013.

such as fibroblasts and endothelial cells, which are normally found in mesenchymal tissue.

Mesenchymal stem cells are heterogeneous, and their actions are pleiotropic. They produce humoral factors that exert trophic and anti-inflammatory effects and modulate immunologic reactions.[1,7] In addition to these humoral effects, MSCs exhibit a broad spectrum of differentiation abilities that cross the boundaries from mesodermal- to ectodermal- or to endodermal-lineage cells, suggesting that MSCs have an aspect of pluripotency.[8] Although at very low frequency, transplanted MSCs show triploblastic differentiation ability. In animal models transplanted with naive MSCs, the integration of a very small number of MSCs into damaged liver, brain, or heart, and differentiation into hepatocyte-, neural-, or cardiomyocyte-marker expressing cells in each organ were observed, suggesting the involvement of MSCs in tissue repair.[9–11] These tissue repair effects of MSCs, however, have not yet been clearly demonstrated in humans. Trophic effects are the most obvious effects of MSC transplantation, while tissue repair effects are considered to be minor and with a low frequency. Although MSCs are safe and feasible for clinical use, the low frequency of tissue repair effects limits the effectiveness of MSCs for regenerative medicine.

Nevertheless, MSCs are suggested to include a small population of stem cells that have the ability to differentiate into any cell type, much like pluripotent stem cells, and participate in tissue repair. Isolation of such stem cells from MSCs could have a critical impact in the fields of regenerative medicine and cell-based therapy. What kinds of cells might these be?

## DISCOVERY OF MUSE CELLS

Pluripotent stem cells that account for one to several percent of MSCs, Muse cells, were first reported in 2010.[12] Muse cells are found in adult mesenchymal tissues such as the bone marrow, adipose tissue and dermis, but are generally distributed sparsely in organ connective tissue.[13] Muse cells can be conveniently obtained from commercially available mesenchymal cultured cells such as bone marrow- and adipose tissue-MSCs, as well as from fibroblasts, one of the most generally used cultured cells in the world, as several percentage of total MSCs. (Fig. 1)[12,14,15] Muse are pluripotent but non-tumorigenic, thus early realization of their application to regenerative medicine is highly anticipated.

The discovery of Muse cells is important in several aspects. First, the pluripotency of Muse cells and their small proportion of total MSCs are consistent with the previously reported low frequency of trans-differentiation of MSCs across triploblastic lineages. Second, the pleiotropic actions of MSCs are clarified by the division of the roles played by Muse cells and cells other than Muse cells, namely non-Muse cells. That is, Muse cells are responsible for the triploblastic differentiation and tissue repair effects, while non-Muse cells are deeply involved in trophic and immunosuppressive effects.[12,16]

Muse cells were initially identified as stress-tolerant cells. When bone marrow-MSCs (BM-MSCs) or fibroblasts are cultured for longer than overnight under stress-inducing conditions, e.g., incubated in trypsin or under low nutrition conditions, the vast majority of MSCs die and only a small number of cells, containing a high ratio of Muse cells, survive.[12] Somatic stem cells that normally reside in tissue are dormant and not usually active, but once the tissue is damaged or exposed to stress, they become activated and begin to proliferate, differentiate, and contribute to tissue restoration. In contrast to these stem cells, functioning differentiated cells tend to die after damage or stress. For example, neural stem cells that are located in the brain are normally inactive, but following stroke, these stem cells enter into the cell cycle and begin to generate neuronal and glial cells whereas mature neuronal cells tend to die.[17] Recently, Shigemoto *et al.* succeeded in efficiently collecting muscle stem cells, namely satellite cells, from adult skeletal muscle tissue by taking advantage of their stress tolerance properties.[18] In the same manner, Muse cells are stem cells that reside in mesenchymal tissues, and are tolerant to stress. In contrast to other somatic stem cells, however, such as neural and muscle stem cells, their actions are not confined to the tissue where they are located but they expand their field of activities, perhaps via the peripheral blood stream, and participate in extensive tissue repair, as described below.

## CHARACTERISTICS OF MUSE CELLS

Muse cells have remarkable characteristics, including:

1. Muse cells are pluripotent stem cells that are able to differentiate into mesodermal-, ectodermal-, and endodermal-lineage cells from a single cell and can be directly collected from human tissues (Fig. 1).[12]
2. Muse cells can be obtained from easily accessed tissues, such as the bone marrow, adipose tissue, and dermis, as well as from commercially available cultured fibroblasts and BM-MSCs (Fig. 1).[12,13,15,16]
3. Muse cells have low telomerase activity and are non-turmorigenic.[14]
4. Muse cells comprise 0.03% of bone marrow mononucleated cells, and several percentage of cultured fibroblasts and BM-MSCs.[12]
5. Muse cells also comprise a part of MSCs, which are already used in clinical studies; thus, Muse cells are highly expected to be safe for clinical use.

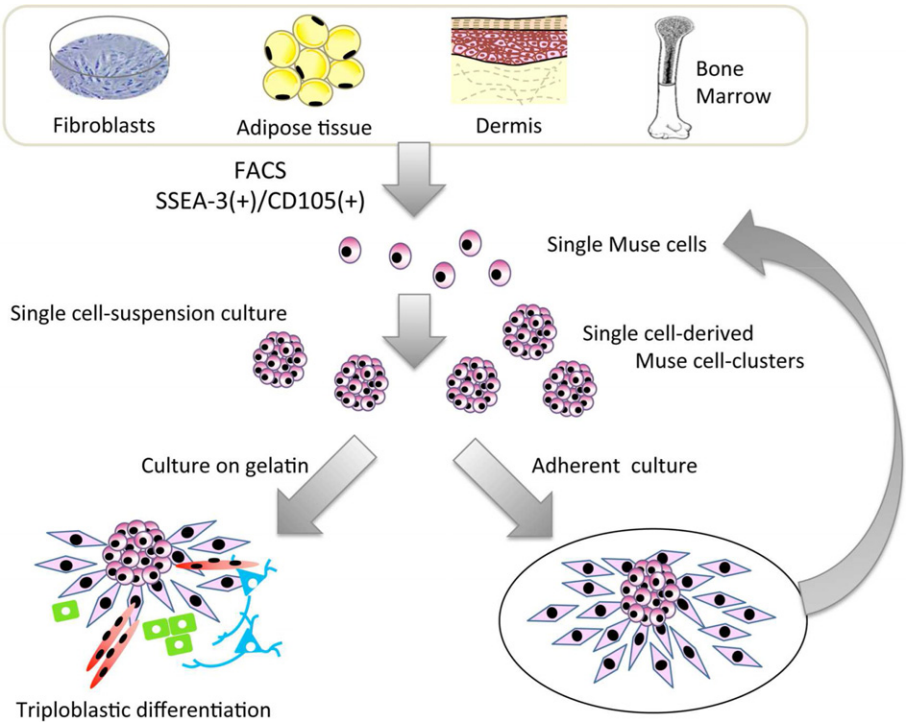


**Figure 1** Pluripotency of Muse cells.
Muse cells can be collected from cultured mesenchymal cells (for example, fibroblasts and bone marrow mesenchymal stem cells (BM-MSCs)) and mesenchymal tissues (adipose tissue, dermis and bone marrow) as cells double-positive for SSEA-3 and CD105. After isolating Muse cells by fluorescence-activated cell sorting, single Muse cells cultured in suspension (single cell-suspension culture) generate characteristic clusters that are very similar to the embryoid bodies formed by human embryonic stem (ES) cells. When the cell clusters are transferred onto gelatin culture and spontaneous differentiation is induced, cells with endodermal- (i.e., hepatocytes), ectodermal- (neuronal cells), and mesodermal- (skeletal muscle cells) lineage are observed. The rest of the clusters were individually transferred to adherent culture and allowed to proliferate for 7 to 10 days, after which they underwent a second round of single cell-suspension in culture to generate second generation clusters. This experimental cycle was repeated three times, demonstrating that Muse cells maintain self-renewal, as well as triploblastic differentiation ability up to the third generation.

6 Muse cells have a proliferation rate of ~1.3 day/cell division, slightly slower than that of fibroblasts in adherent culture, so a large number of Muse cells can be prepared.[14]
7 Muse cells act as repair cells *in vivo*.[12]

## Muse cells have dual aspects

Muse cells belong to MSCs. Therefore, they have nearly all of the properties of MSCs. Unlike general mesenchymal cells, however, Muse cells are pluripotent. These dual aspects of Muse cells are reflected by their expression of cell surface markers; they are positive for both mesenchymal (CD105, CD90 and CD29) and pluripotency (SSEA-3) markers (Fig. 1).[12]

Muse cells are unique, not only in their surface marker expression profile, but also in their behavior and other properties. In adherent culture, they appear similar to fibroblasts, but when they are transferred to a single cell-suspension culture, they can survive and begin to proliferate to form cell clusters that resemble embryonic stem (ES) cell-derived embryoid bodies formed in suspension. Such single cell-derived Muse cell clusters are similar to ES cells in their appearance, and positive for alkaline phosphatase as well as for the pluripotency markers Nanog, Oct3/4, and Sox2. Consistent with the expression of pluripotency markers, cells derived from Muse cell clusters are able to differentiate into endodermal-, ectodermal-, and mesodermal-lineage cells when transferred to gelatin cultures, proving that single Muse cells are able to generate cells representative of all three germ layers.[12,14] Importantly, non-Muse cells in MSCs have only mesenchymal aspects; that is, they do not express pluripotency markers, nor do they survive, proliferate, or form clusters in suspension.[12,14]

## Triploblastic differentiation and self-renewal abilities of Muse cells

Muse cells are pluripotent stem cells because they can generate endodermal-, mesodermal- and ectodermal-lineage cells

from a single cell and to self-renew (Fig. 1). The markers of each lineage into which Muse cells are able to differentiate are: ectodermal- (neural markers such as nestin, NeuroD, Musashi, neurofilament, microtubule associated protein-2, and markers for melanocytes such as tyrosinase, microphthalmia-associated transcription factor, gf100, tyrosinase-related protein 1, and dopachrome tautomerase[13,19]), mesodermal- (brachyury, Nkx2.5, smooth muscle actin, osteocalcin, oil red-(+) lipid droplets, and desmin[12,13]), and endodermal-lineages (GATA-6, $\alpha$-fetoprotein, cytokeratin-7, and albumin[12,13]). Expression of these markers is recognized under both spontaneous differentiation on gelatin and cytokine induction systems.

With regard to ectodermal differentiation, Tsuchiyama *et al*. recently demonstrated that human dermal fibroblast-Muse cells could cross the boundary between mesodermal and ectodermal-lineages and efficiently differentiate into functional melanin-producing melanocytes by applying a cocktail of cytokines, including Wnt3a, stem cell factor, endothelin-3, and basic fibroblast growth factor, while the remainder of the fibroblasts, non-Muse cells, could not differentiate into melanocytes at all.[19] Muse cell-derived melanocytes expressed the melanocyte markers tyrosinase and microphthalmia-associated transcription factor, were positive for 3,4-dihydroxy-L-phenylalanine, an indicator of melanin production, and maintained their melanin-producing activity in the basal layer of the epidermis when transplanted into the skin. Together, these results demonstrated the absolute superiority of Muse cells over non-Muse cells in terms of pluripotency.

Although Muse cells are pluripotent, they tend to differentiate more frequently into their background lineage; they spontaneously differentiate into mesodermal-lineage cells with a higher percentage (10–15%) than into ectodermal (3–4%) or endodermal (3–4%)-lineage cells.[12]

The ratio of spontaneous differentiation of Muse cells is not very high, but an induction system with a certain combination of cytokines and trophic factors directs their differentiation more efficiently. For example, when Muse cells are treated with hepatocyte growth factor, fibroblast growth factor-4, and dexamethasone in insulin-transferrin-selenite medium, more than 90% of the cells become hepatocyte-like cells that express alpha-fetoprotein and human albumin[13] Muse cells treated with Neurobasal medium supplemented with B-27, basic fibroblast growth factor, and brain-derived neurotrophic factor differentiate into neuronal cells that are positive for MAP-2 and neurofilament.[13] In osteocyte or adipocyte induction medium, nearly 98% of Muse cells differentiate into cells positive for osteocalcin or oil-red, respectively.[13] In this manner, mesodermal-, ectodermal-, or endodermal-lineage cells can be more efficiently obtained from Muse cells, depending on the induction system. More importantly, none of the above differentiations requires the introduction of exogenous genes, and thus Muse cells produce the desired cells with lower risk.

Muse cells are self-renewable. When half of the first-generation clusters formed from Muse cells in single cell-suspension culture were transferred individually onto a gelatin culture and expanded, the expression of endodermal (alpha-fetoprotein, GATA-6), mesodermal (Nkx2.5), and ectodermal markers (MAP-2) was observed. The remaining clusters were individually transferred to an adherent culture and allowed to proliferate, after which they underwent a second round of single cell-suspension in culture to generate second generation clusters (Fig. 1). This experimental cycle was repeated up to three times and clusters from each step were analyzed. Expression of the above genes was detected in first, second, and third generation clusters, demonstrating that Muse cells maintain the gene expression profile required for self-renewal, as well as triploblastic differentiation ability.[16]

## Non-tumorigenicity of Muse cells

When Muse cells are compared with tumoirgenic pluripotent stem cells such as ES and induced pluripotent stem (iPS) cells, the repertoire of the genes related to pluripotency, including Nanog, Oct3/4, and Sox2, expressed in Muse cells is similar to that of ES and iPS cells, while the expression level of those factors in Muse cells is lower compared to ES and iPS cells. Compatible with their tumorigenic activity, ES and iPS cells have high levels of telomerase activity as well as high expression levels of genes related to cell-cycle progression compared with Muse cells, which have the same low levels as naive fibroblasts.[13]

In sharp contrast with Muse cells, non-Muse cells do not originally express pluripotency genes. Expression levels of genes related to cell-cycle progression are similar between Muse and naive fibroblasts.[13]

Embryonic stem and iPS cells are known to form teratomas when transplanted *in vivo*. In fact, teratomas form when those cells are transplanted into the testes of immunodeficient mice.[12,20] In contrast, Muse cells do not develop into teratomas *in vivo*. Even after 6 months, none of the Muse cell-transplanted immunodeficient mouse testes formed teratomas (Fig. 2).[12,20] Together these results support that Muse cells are pluripotent but with non-tumorigenic and low telomerase activities. The non-tumorigenicity of Muse cells is considered to be consistent with the fact that they reside in normal adult mesenchymal tissue.

## Ability of Muse cells to spontaneously repair damaged tissues *in vivo*

For application of ES and iPS cells to regenerative medicine in humans, two major conditions are required: (i) the cells must be differentiated into objective cells in a cell processing

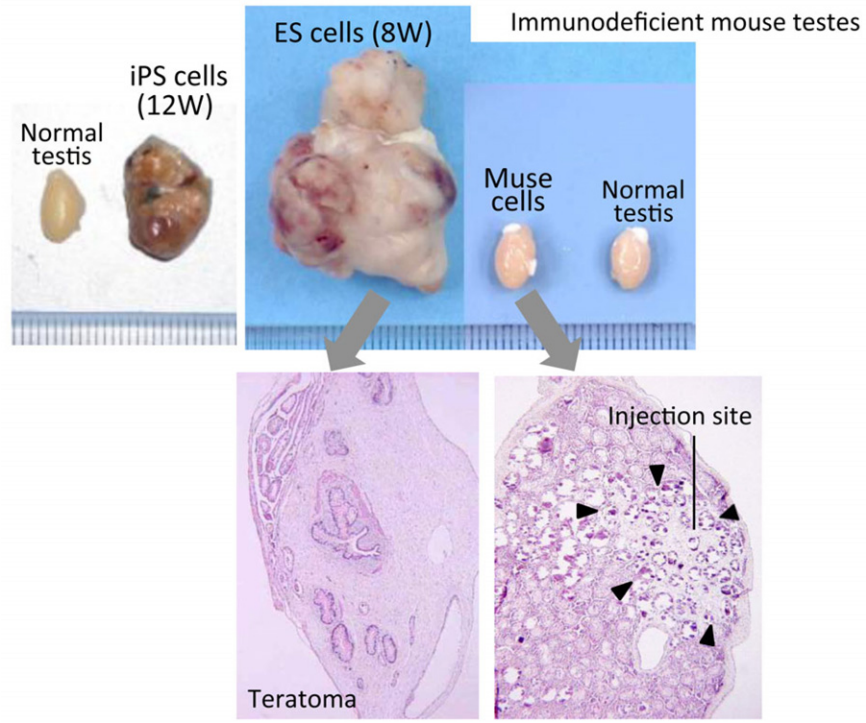


**Figure 2** Muse cells are non-tumorigenic. When embryonic stem (ES) or induced pluripotent stem (iPS) cells were infused into immunodeficient mice (SCID mice) testes, they formed teratomas within 8 to 12 weeks while none of the Muse cell-transplanted testes generated teratomas and instead maintained normal tissue structure. (pictures reproduced from *Proc Natl Acad Sci USA* 2010; **107**: 8639–43 and *Proc Natl Acad Sci USA* 2011; **108**: 9875–80) (12,13).

center; and (ii) undifferentiated cells must be eliminated from the differentiated population before transplantation. These prerequisite conditions are based on the fact that undifferentiated ES and iPS cells have tumorigenic activity. As mentioned above, directly transplanted undifferentiated ES or iPS cells may form tumors *in vivo*. Furthermore, even if differentiation induction with high efficiency could be realized, some undifferentiated cells will remain.

For Muse cells, however, the above two conditions are not required. One possible scheme is that naive Muse cells can be applied directly to patients. Muse cells have the ability to migrate and integrate into the site of damage and then spontaneously differentiate into cells compatible with the tissue they target (Fig. 3). Such differentiation is observed in mesodermal, ectodermal, and endodermal tissues, and the Muse cells can act as 'repairing cells' in a wide spectrum of tissues and organs as described below.[12] Because differentiation and repair are induced spontaneously by Muse cells themselves, there is no need to control their differentiation prior to transplantation. Furthermore, as Muse cells are inherently non-tumorigenic and have low telomerase activity, it is not necessary to eliminate undifferentiated naive Muse cells. Ultimately, a cell processing center and complex systems are not necessary for Muse cell therapy.

The repairing effect of naive Muse cells is most striking in acute damage models. This was demonstrated by the infusion of green fluorescent protein-labeled naive human Muse cells into immunodeficient mouse (SCID mouse) models with fulminant hepatitis, skeletal muscle degeneration, spinal cord injury and skin injury. (Fig. 4)[12,21] Naive human Muse cells infused into the bloodstream of mouse models targeted damaged sites and differentiated into hepatocytes (positive for human albumin), skeletal muscle cells (human dystrophin), neuronal cells (neurofilament), and keratinocytes (cytokeratin 14), respectively (Fig. 4). The findings revealed that Muse cells can differentiate into ectodermal- (neuronal cell, keratinocytes), endodermal- (hepatocytes), and mesodermal-lineage cells (skeletal muscle cells) that are compatible with the targeted tissue and contribute directly to tissue repair.

While some infused Muse cells were trapped in the lung, the majority integrated into damaged tissues but not into intact tissues.[12] This suggests that disruption of blood vessels and destruction of tissues in damaged tissue are required for naive Muse cells to migrate and target, and thus Muse cells are able to perceive damage signals produced by damaged tissues. After integration, Muse cells differentiate into tissue-specific cells, but the factors that define the microenvironment of the site, which instruct the Muse cells how to differentiate correctly, remain unclear. Further elucidation of signals responsible for Muse cell migration and differentiation is needed.

## DIFFERENT ROLES OF MUSE CELLS AND NON-MUSE CELLS IN MSCS

Although the action of MSCs is considered pleiotropic, recent findings of Muse cells are expected to elucidate the various

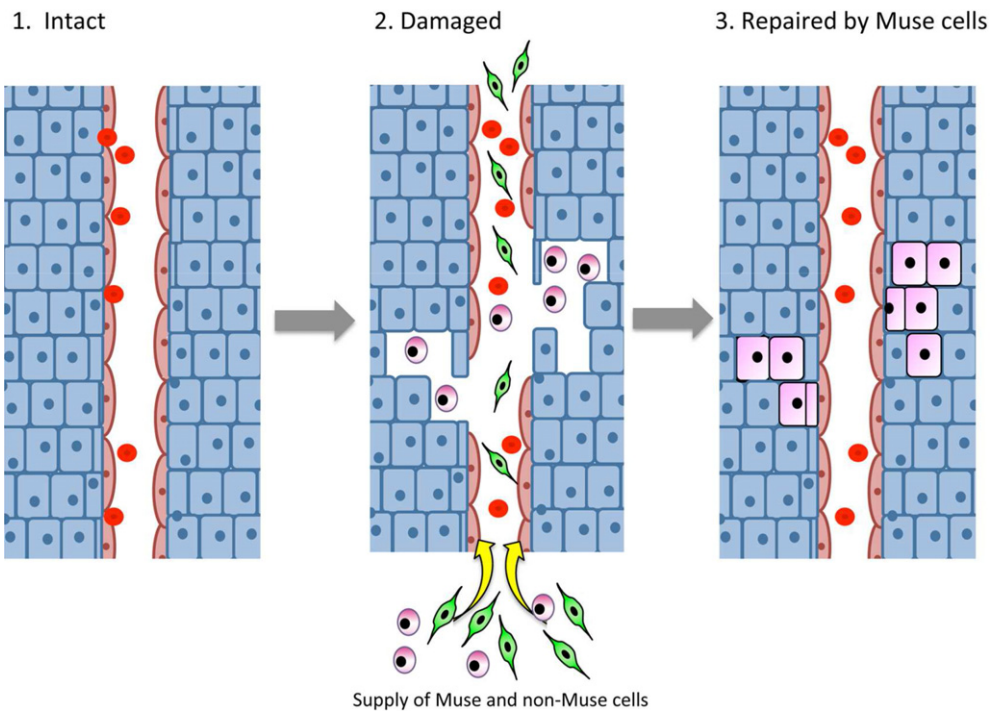


**Figure 3** Tissue repair effect delivered by Muse cells. ●, red blood; ◉, Muse cells; 👁, non-Muse cells.
When Muse and non-Muse cells were supplied to the blood stream, only Muse cells integrate into the damaged site, differentiate, and repaired the tissue, while non-Muse cells do not remain in the damaged tissue nor do they participate in tissue repair.

functions of the MSC components. Although Muse cells account for only several percent of the total MSCs, they play an exclusive role in triploblastic differentiation and tissue repair, while non-Muse cells do not directly participate in these events and rather have major roles in trophic and immunosuppressive effects. There are remarkable differences between Muse and non-Muse cells. First, non-Muse cells do not form clusters in suspension like single Muse cells.[12] Assuming that non-Muse cells are just like general mesenchymal cells, such as fibroblasts, they are essentially adherent cells and thus do not inherently survive and function in suspension.

Second, pluripotency genes that are expressed in Muse cells are not expressed in non-Muse cells and thus non-Muse cells are not pluripotent. Although they have lower efficiency than Muse cells, non-Muse cells do have the ability to differentiate into osteocytes, chondrocytes, and adipocytes. They are, however, unable to differentiate into neuronal cells (ectodermal), hepatocytes (endodermal), or even into the same mesodermal lineage skeletal muscles.[16] Thus, they are not pluripotent. Consistently, as shown in melanocyte induction, Muse cells from dermal fibroblasts can differentiate into functional melanocytes that produce melanin pigment following induction with cytokine cocktails while fibroblast-derived non-Muse cells fail to differentiate.[19] Gene expression patterns in non-Muse cells during melanocyte induction are interesting to observe; they respond partially to the induction stimulation and indeed some melanocyte markers are newly expressed in an earlier period of induction, but those markers disappear later and the gene expression pattern returns back to the original state of fibroblasts at the later stage.[19]

The partial responsiveness of non-Muse cells is also observed in iPS cell generation. Muse cells that are already pluripotent express pluripotency genes and lack tumorigenic activity, readily become iPS cells when treated with the four Yamanaka factors, and newly acquire tumorigenicity, whereas non-Muse cells do not show an increase in major pluripotency genes, including Nanog and Sox2, even after receiving the four Yamanaka factors.[13,22] Their responsiveness to the four Yamanaka factors is only partial, however, and thus non-Muse cells fail to generate iPS cells.

Third, non-Muse cells, unlike Muse cells, do not integrate nor differentiate into functional cells in damaged tissues.[12,21] Previous reports demonstrated that the large majority of MSCs do not remain in the transplanted tissue, but rather exert trophic effects that occasionally lead to some degree of functional recovery. As the majority of MSCs are non-Muse cells, the major role of non-Muse cells after transplantation might be a trophic effect.

## LOCALIZATION OF MUSE CELLS *IN VIVO*

Mesenchymal tissues, such as the bone marrow, adipose tissue, and dermis, are the main reserve of Muse cells *in vivo*.

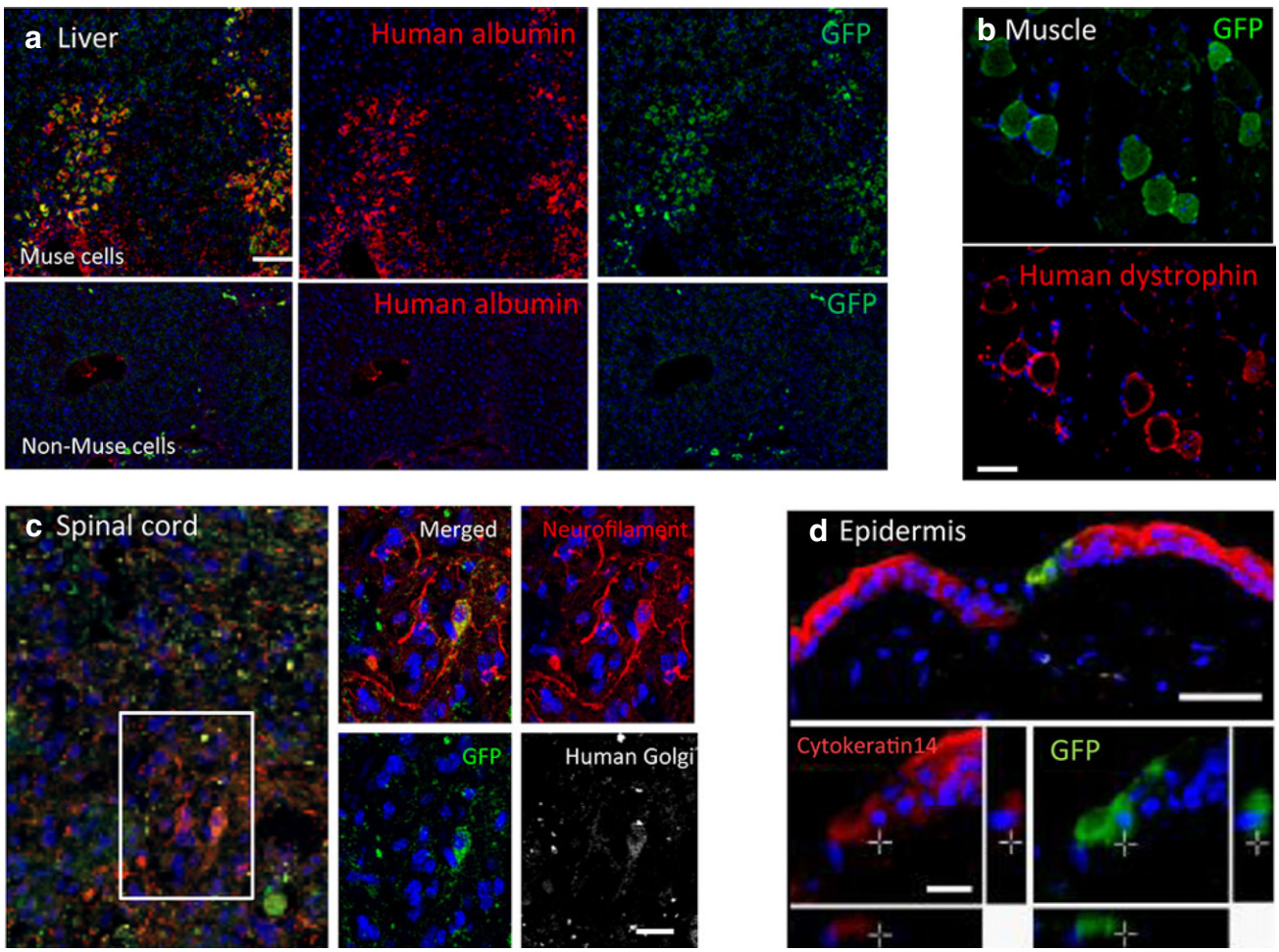


**Figure 4** Tissue repair effect of Muse cells.
Green fluorescent protein (GFP)-positive human Muse cells integrated into (**a**) fulminant hepatitis, (**b**) muscle degeneration, (**c**) spinal cord injury (made by crush injury), and (**d**) skin injury models, and became (**a**) human albumin-, (**b**) human dystrophin-, (**c**) neurofilament- (cells were also positive for the human cell marker, anti-human Golgi complex, confirming that the positive cells were of human origin), and (**d**) cytokeratin 14- positive cells 4 weeks after injection. When non-Muse cells were infused into fulminant hepatitis, cells did not differentiate into albumin-positive cells. Scale bars; **a, b** = 100 μm, **c, d** = 50 μm. (Pictures reproduced from *Proc Natl Acad Sci USA* 2010; **107**: 8639–43, and *Cells* 2012; **1**: 1045–60, 2012).[12,21]

In the human dermis and adipose tissue, Muse cells detected as SSEA-3-positive cells locate sparsely in the connective tissues of the dermis and hypodermis, and do not associate with particular structures such as blood vessels or dermal papilla (Fig. 5)[13]. Similarly, they distribute in the connective tissue of many organs in the same manner as seen in the dermis and adipose tissue (unpublished data). Because tissue stem cells are generally confined to the tissue where the stem cells belong, i.e., neural stem cells in the brain, hematopoietic stem cells in the bone marrow, Muse cells are unique in that they are distributed throughout the body and are not confined to a specific organ or tissue.

Organ-derived Muse cells, however, might not be a practical source for clinical use. Rather, easily accessible mesenchymal tissues are realistic and feasible sources for obtaining Muse cells for clinical use. In the case of human bone marrow aspirate, SSEA-3/CD105 double-positive Muse cells were identified at a ratio of 0.03%, namely, 1 in 3000 mononucleated cells.[12] The proliferation speed of Muse cells is ~1.3 day/cell division, so that 10 ml of fresh bone marrow aspirate may yield nearly 1 million Muse cells within 10 days.[12]

Commercially available cultured mesenchymal cells, such as human dermal fibroblasts and BM-MSCs, are another potential source for Muse cells. While the ratio and quality of Muse cells may be altered by handling and depend the number of subcultures, fibroblasts and BM-MSCs contain Muse cells at levels ranging from 1% to 5–6%.[13]

## MUSE CELLS AND REGENERATIVE HOMEOSTASIS

The fact that Muse cells reside in connective tissue and bone marrow suggests that they are widely distributed in the body.

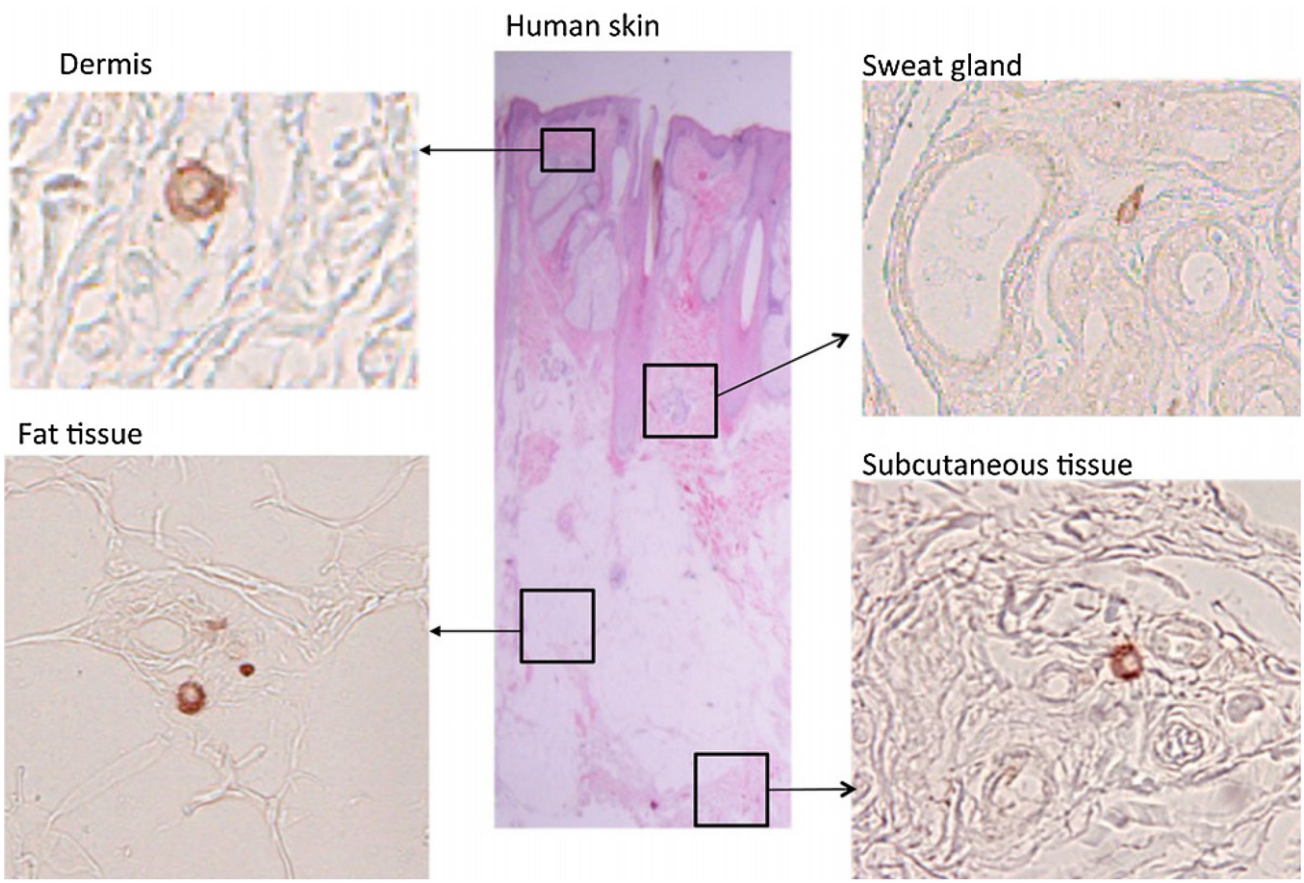


**Figure 5** Muse cells sparsely locate in the adult human skin connective tissue.
Muse cells labeled by SSEA-3 are sparsely detected in the connective tissue of the dermis, sweat glands, adipose tissue and hypodermis. (pictures reproduced from *Proc Natl Acad Sci USA* 2011; **108**: 9875–80).[13]

If so, what kind of systems do Muse cells maintain *in vivo*? Because the bone marrow is directly connected to the peripheral bloodstream, the marrow is thought to be the hub of the Muse cell system in the body where the Muse cells are reserved and maintained in the normal state. Muse cells might be mobilized very slowly to the peripheral blood from the bone marrow in the normal state and distributed to the connective tissue of peripheral organs, including mesenchymal tissues such as adipose tissue and the dermis.

Comparison of the gene expression levels of Muse cells from bone marrow, adipose tissue, and dermis reveals that bone marrow-Muse express higher levels of genes related to ectodermal and endodermal-lineages than adipose- and dermal-Muse cells, suggesting that bone marrow-Muse cells have higher pluripotency than the other two types of Muse cells.[16] Bone marrow Muse cells are also unique in that they are highly dormant and more stress tolerant than adipose- and dermal-Muse cells.

Assuming that Muse cells build up a system *in vivo*, what is the function of Muse cells in the connective tissue of each organ? Because Muse cells are pluripotent, they can repair tissues that span endodermal-, mesodermal- and ectodermal-lineages. Connective tissue is very common and generally distributed in each organ, so that Muse cells residing in connective tissue can easily access small areas of damage that occur every day and replenish cells that are compatible with the tissue in the nearest parenchyma. It is conceivable that each organ is exposed to daily stress and minute damage that may cause cell degeneration. Our bodies are able to maintain function because of 'regenerative homeostasis' due to these small maintenance systems. The true mechanisms of regenerative homeostasis are still not clear, but the Muse cell system may have an important function. Further studies are needed to elucidate how Muse cells relate directly to regenerative homeostasis.

## ACKNOWLEDGMENTS

This study was supported by grants from the New Energy and Industrial Technology Development Organization (NEDO) and from the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO).

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