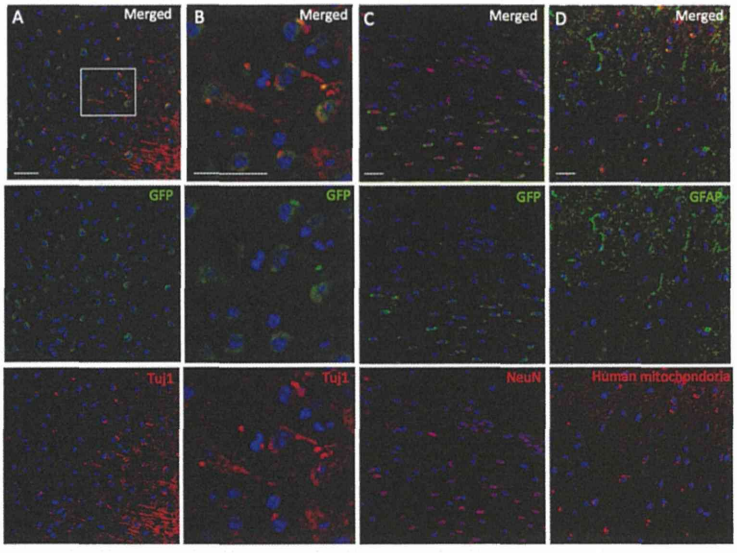

Fig 5. Double immunohistochemistry of GFP-Tuj-1 (A and B), GFP-neuronal nuclear antigen (NeuN; C) and human mitochondria-GFAP (D) in ipsilateral cortex of Muse cell-group (42 days after transplantation). The white square in panel A represents the location of Panel B. Scale bars = 50 μm.

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Discussion

Although further analysis would be necessary, the present data provide important information on the mechanisms through which the engrafted BMSCs promote functional recovery after ischemic stroke. This study clearly demonstrates that at least two subpopulations in BMSCs,

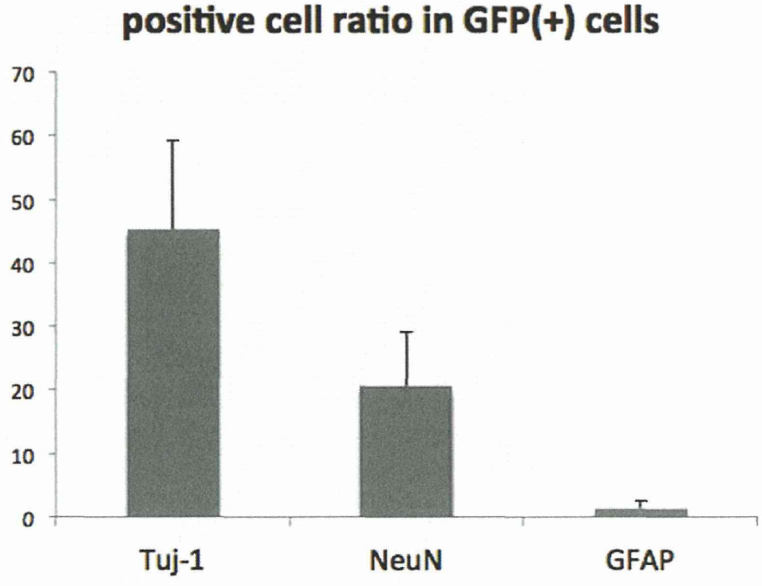

Fig 6. Column graph shows the percentages of Tuj1-, NeuN- and GFAP-positive cells in GFP(+) cells in Muse group (42 days after transplantation).

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Muse and non-Muse cells, may contribute differently to functional recovery when they are directly transplanted into the infarct brain. Non-Muse cells are considered to play a key role in early phase recovery of motor function after transplantation, because the non-Muse cell-transplanted animals started to recover motor function as early as 21 days after transplantation, while their therapeutic effects were limited and did not further promote functional recovery over 42 days. Histological analysis supports the findings. Immunostaining against human mitochondria detects negligible number of non-Muse cells, suggesting that they do not effectively remain or are not integrated into the host brain or even though they are once integrated, they might have disappeared by 42 days after transplantation.

On the other hand, Muse cell-treated animals did not show significant functional recovery during 28 days after transplantation, but started to recover motor function at 35 days post-transplantation, which was later than non-Muse cell-treated animals. On immunohistochemistry, however, a large number of human mitochondria- or GFP-positive cells were shown to engrafted and integrated into the peri-infarct area of ipsilateral hemisphere. There is possibility that some injected Muse cells were phagocytosed, while comparing remaining cell numbers in Muse, BMSC and non-Muse groups, Muse group showed substantial number of remaining cells with significant statistical difference to other two groups. Since cells were directly injected into the striatum by single shot, these integrated Muse cells might have migrated from the striatum into the peri-infarct area. Previous studies have shown that the transplanted cells aggressively migrate towards the lesion through the system of chemokines such as stromal cell-derived factor (SDF)-1 [24]. Majority of integrated Muse cells in the peri-infarct area expressed neuron-specific markers, Tuj-1 and NeuN at 42 days post-transplantation. Nearly 45% of GFP-labeled human Muse cells expressed Tuj-1, and about 20% of them were positive for NeuN, while the Muse cells doubly positive for GFAP was only ~1%, suggesting that Muse cells may preferentially differentiate into neuronal-lineage cells. We have previously shown that Muse cells express nestin, Musashi-1, NeuroD, and MAP-2 after neural induction, which correlates well with present results [14,29]. Longer evaluation of motor function is expected to find further improvement of motor function in Muse cell group.

Because of the sharp contrast between Muse cells and non-Muse cells in functional and histological analysis, it is most likely that these two BMSC subpopulations play biologically different roles in the infarct brain and contribute to post-stroke recovery of motor function and tissue repair by different ways and different timings. Since non-Muse cells did not remain in the host brain, early phase recovery observed in non-Muse cell group may result from the trophic effect exerted by transplanted non-Muse cells rather than cell replacement. In contrast, Muse cells are more responsible for replacement of the lost neuronal cells by integration into the host brain and spontaneous differentiation into neuronal lineage cells.

BMSCs that is consisted of several percentage of Muse cells plus vast majority of non-Muse cells significantly improved motor function by 21 days post-transplantation and yielded better therapeutic effects at 42 days than Muse cells and non-Muse cells. However, the number of integrated BMSCs was very small compared to Muse cells (Fig. 4). At least in short-term evaluation up to 49 days in functional recovery, the BMSCs look as the best to transplant because the mixture of biologically various subpopulations of cells may exert maximal therapeutic effects against ischemic stroke. Nevertheless, longer period observation would be necessary to evaluate therapeutic effects of Muse cells on motor function, because the number of BMSCs integrated into the brain was very small.

Very recent report has provided interesting information on the behaviors of BMSCs engrafted into the infarct brain. Fluorescence in situ hybridization (FISH) studies showed that about half of the engrafted BMSCs express mRNA for brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) at 14 days after transplantation into the infarct brain;

however, their percentages rapidly decreased thereafter. Instead, the percentage of microtubule-associated protein (MAP) 2–positive BMSCs gradually increased during 28 days after transplantation. These findings strongly support our results suggesting that the BMSCs may exhibit the trophic effect in the early (~2 weeks) stage of cell therapy and the phenotypic change toward neural cells thereafter, when transplanted into the infarct brain [23]. Previous reports have suggested that a certain subpopulation of transplanted BMSCs are integrated in the brain of rodent stroke model [2]. Since Muse cells have the ability to differentiate into neuronal cells both *in vitro* and *in vivo* while non-Muse cells do not show such neuronal differentiations[15], it is plausible small number of neuronal differentiation observed in BMSCs transplantation might be due to Muse cells.

Pathophysiological mechanism of post-stroke cognitive impairment is quite complicated [30]. However, recent studies have shown that the engrafted BMSCs may contribute to improve cognitive function in rodent models of chronic cerebral ischemia, although the underlying mechanisms are still unclear [10]. Indeed, both the BMSCs and non-Muse cells significantly increase the correct choice and decrease the error choice at 28 days after transplantation. Muse cells also decrease the error choice. Likewise the results in Rotarod test, longer evaluation of spatial memory may detect further improvement in the Muse cell-treated animals. It would be quite valuable to assess the mechanisms through which the donor cells recover cognitive function in animal models of CNS disorders. However, it should be reminded that the outcome measurements in animal experiments, including Rotarod and eight-arm radial maze test, may require further development. As recently pointed out by Balkaya et al. [31], the rotarod is a relatively simple and well-evaluated test for short-term (~4 weeks) evaluation of deficits after proximal MCA occlusion in a variety of mouse strains, but may depend on the other factors such as training protocol and motivation. Post-stroke cognitive function may also require several testing to determine the beneficial effects of cell therapy because of its complicated mechanisms.

As aforementioned, the Muse cells have high potential to become to neuronal cells because they differentiate into neuronal cells with very high ratio of ~90% under the presence of cytokine stimulation [14]. They have low telomerase activity and are non-tumorigenic. They comprise 0.03% of bone marrow mononucleated cells in bone marrow aspirate and several percentages of cultured BMSCs [32]. Wakao et al. (2014) have very recently suggested that Muse cells pay an exclusive role in trioblastic differentiation and tissue repair, while non-Muse cells do not directly participate in these events but rather have major roles in trophic and immunosuppressive effects [32]. These findings correlate well with the present histological data. However, the fact that the BMSCs include only several percentages of Muse cells may limit the beneficial effects of BMSC transplantation into the infarct brain. In other words, the therapeutic effects would be enhanced if the Muse cells are isolated or enriched and then transplanted into the infarct brain. Muse cells are quite attractive, because non-tumorigenic stem cells with the ability to generate the multiple cell type of the three germ layers can be obtained through easily accessible BMSCs without introducing exogenous genes [12,13,32]. Further studies are warranted to evaluate whether the therapeutic effect of BMSC transplantation can substantially be improved when Muse and non-Muse cells are combined at a certain best ratio.

Conclusions

This study demonstrates that among BMSCs, Muse cells and cells other than Muse cells, namely non-Muse cells, may contribute differently to tissue regeneration and functional recovery when they are directly transplanted into the infarct brain. Substantial number of Muse cells remained in the host brain for up to 42 days and expressed neuronal markers Tuj-1 and NeuN,

suggesting they replaced the lost neurons, while only negligible number of BMSCs and non-Muse cells remained in the brain at the same time point. Muse and non-Muse groups did not show significant statistical difference in functional recovery, but Muse group was less potential than in BMSC group. Non-Muse cells, however, showed functional recovery at ~2 weeks, and thus they are speculated to exhibit trophic effects to improve the microenvironments of damaged brain at early stage. Since the proportion of Muse cells in BMSCs is only several percentage, tissue repair effect of BMSC transplantation would be enhanced when the ratio of Muse cells are increased, while trophic effect of non-Muse cells might also enhance therapeutic effect synergistically when combined with Muse cells with the best ratio.

Author Contributions

Conceived and designed the experiments: MD SK. Performed the experiments: TY YK TM. Analyzed the data: TY YK TM SK. Contributed reagents/materials/analysis tools: TY YK TM HS KH. Wrote the paper: MD SK.

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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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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.

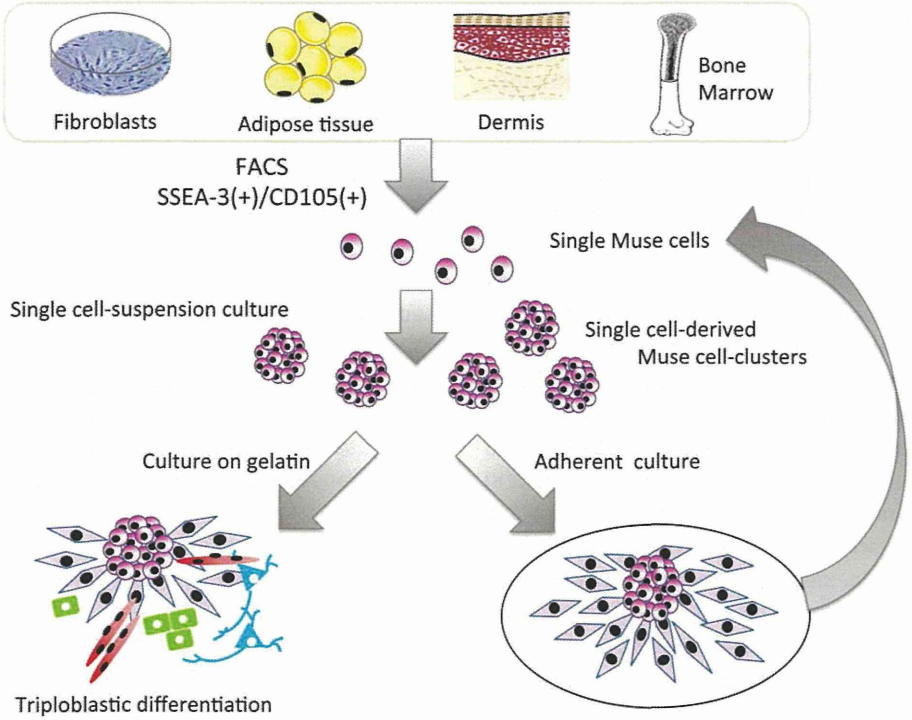

Figure 1 Pluripotency of Muse cells.
Muse cells can be collected from cultured mesenchymal cells (for example, fibroblasts and bone marrow mesenchymal stem cells (BM-MSCs)) and mesenchymal tissues (adipose tissue, dermis and bone marrow) as cells double-positive for SSEA-3 and CD105. After isolating 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, α-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

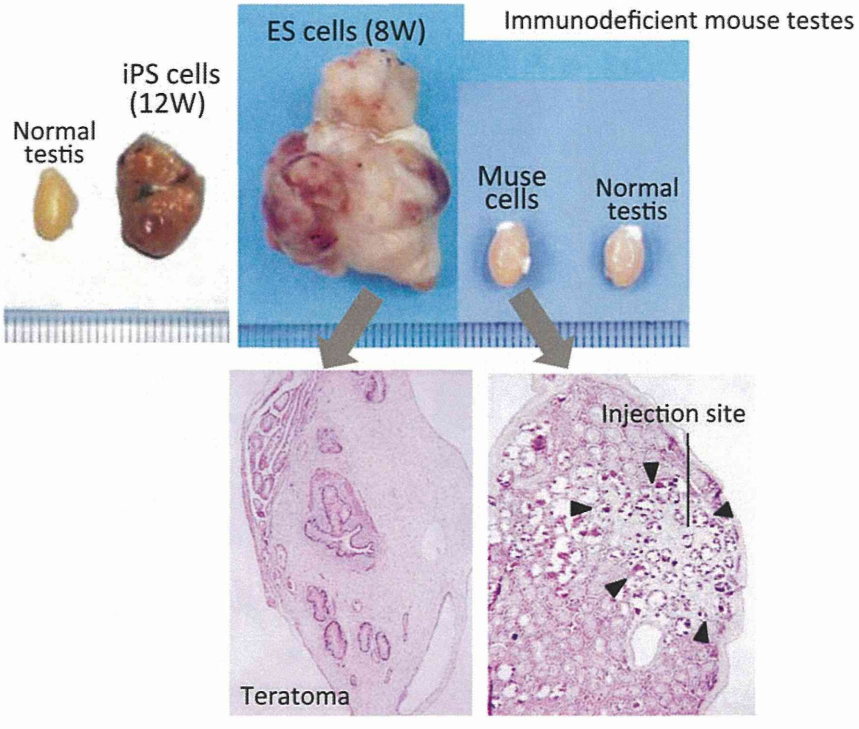

Figure 2 Muse cells are non-tumorigenic. When embryonic stem (ES) or induced pluripotent stem (iPS) cells were infused into immunodeficient mice (SCID mice) testes, they formed teratomas within 8 to 12 weeks while none of the Muse cell-transplanted testes generated teratomas and instead maintained normal 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