



Review

Regenerative potential of pluripotent nontumorigenic stem cells: Multilineage differentiating stress enduring cells (Muse cells)

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ABSTRACT

Multilineage differentiating stress enduring cells (Muse cells), double positive for SSEA-3 and CD105, can be isolated by fluorescence-activated cell sorting (FACS) or severe cellular conditions from dermal fibroblasts, bone marrow stem cells (BMSCs), adipose tissue derived stem cells (ADSCs), fresh bone marrow and liposuction fat. When cultured in a single-cell suspension, Muse cells can grow into characteristic cell clusters. Muse cells maintain pluripotency as evidenced by pluripotent markers in vitro. Besides, Muse cells have no tumorigenesis up to 6 months in SCID mice. Muse cells differentiate into cells representative of all three germ layers both spontaneously and under specific induction. In comparison to mesenchymal stem cells (MSCs), Muse cells show higher homing and migration capabilities to damaged sites which is predominantly attributed to S1P–S1PR2 axis. The regenerative effects of Muse cells have been demonstrated by many models in vivo or in vitro, including stroke, intracerebral hemorrhage, myocardial infarction, aortic aneurysm, lung injuries, liver fibrosis, focal segmental glomerulosclerosis, osteochondral defects and skin ulcer. In general, migration, differentiation and paracrine play a pivotal role in the regeneration capability. Here we review the isolation, core properties, preclinical studies as well as the underlying molecular and cellular details to highlight their regenerative potential. © 2020, The Japanese Society for Regenerative Medicine. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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1. Introduction

In 2010, a research team of the Tohoku University firstly isolated a new subpopulation of MSCs, termed Muse cells. Muse cells, positive for the pluripotent marker stage-specific embryonic antigen - 3 (SSEA-3), were discovered as stress-tolerant pluripotent stem cells [1]. Since then, Muse cells have drawn many researchers' attention due to their pluripotent gene expression, tri-lineage differentiation and nontumorigenic capabilities at the single-cell level.

2. Sources and isolation

So far, Muse cells are successfully isolated from human skin fibroblasts (~5%) [2], human adipose-derived stem cells (~2–10%) [3,4], human bone marrow stem cells (~1–2%) [1,5] or fresh bone marrow samples [6]. Besides, abdominal subcutaneous lipoaspirate materials can yield about 60–90% Muse cells via being treated by severe cellular stress [7,8]. Particularly, adipose tissue is a source of multipotent stem cells with minimally invasive procedures [9]. The number of Muse-AT cells (Muse cells from adipose tissue) was negatively correlated with donor age, but pluripotency of the cells were observed equally regardless age of donors [4].

Muse cells are chiefly isolated by fluorescence-activated cell sorting (FACS): double positive for SSEA-3 (a marker of human ES cells) and CD105 (a marker of MSCs) [3,4,10]. As almost all of the MSCs are positive for CD105, single application of SSEA-3 will be sufficient to purify Muse cells from mesenchymal cells. When FACS is performed to isolate Muse cells, the original mesenchymal cells were separated into Muse cells (SSEA-3⁺) and non-Muse cells (SSEA-3⁻). Then Muse cells will gradually generate non-Muse cells, leading to a decrease of proportion of Muse cells. The final percentage corresponds to that of Muse cells in general MSCs. Considering the heterogeneity of MSCs, some scholars suppose that Muse cells are the early stage of MSCs [11].

Additionally, Muse cells can be isolated by severe cellular stress conditions, including long-term exposure to the proteolytic enzyme collagenase, serum deprivation, low temperatures and hypoxia [7,8]. It is reported that severe conditions can activate quiescent stem cells from their dormant state and approximately 90% of isolated cells were positive for SSEA-3 [8]. Muse cells isolation becomes simple yet highly efficient through this method. It is estimated that about 250,000–500,000 cells can be obtained from per gram lipoaspirate material [7,8], thus cell expansion is unnecessary.

3. Biological characteristics

3.1. Clusters formation

Apart from adherent environments, Muse cells also can survive and proliferate in suspension. When Muse cells are cultured in a single-cell suspension, they begin form characteristic cell clusters, termed "M clusters" [1], resembling to Embryoid bodies formed

from ESCs and iPS cells [12]. The percentage of cluster formation is about 40–60%, varying from origins of Muse cells [1,5].

M clusters extensively express higher levels of pluripotent stem cell markers, such as Oct3/4, Sox2, and Nanog, up to 50–100 times higher than those in adherent Muse cells [11]. Even though a suspension environment enhances pluripotency of Muse cells, their proliferative activity is restricted. When M clusters reach 70–150 μm in diameter, their growth slows down. Once M clusters are transferred into an adherent culture, the cells gradually grow out of the clusters [1].

3.2. Pluripotency

M clusters express pluripotent markers such as Nanog, Oct3/4, Par-4, Sox2, and TRA1-60 spontaneously [7,13]. Compared to general MSCs, Muse cells break down the germ layers boundaries and display potent potential for triploblastic differentiation from a single cell, which is a core characteristic of Muse [5]. A huge amount of researches prove Muse cells are positive for markers of endodermal (cytokeratin 7, α -fetoprotein), mesodermal (desmin, smooth muscle actin), and ectodermal (neurofilament) cells under both spontaneous and induced conditions [3,4,11,14,15].

Muse cells can spontaneously differentiate into endodermal and ectodermal lineages (both about several percent) and mesodermal cells (10–15%) [6]. When specific induction is supplied they show a higher rate (80–95%) into hepatocyte, neural lineage cells, adipocytes as well as melanocytes [3,5].

Additionally, differentiation potentials of Muse cells differ according to their derived tissues. Osteogenic, adipogenic, and myogenic genes were generally higher in Muse-AT rather than BM-Muse or dermal-Muse cells. Moreover, some of the factors, such as SP7, osteogenic factor, and Pax7 muscle stem cell marker, were only detected in Muse-AT cells [3].

3.3. Stress endurance

Muse cells are inherently stress-resisted. When isolated by severe conditions, only Muse cells can survival [8,16]. A high-stress conditions is crucial to induce Muse cell activation from their quiescent state [1,8].

Several factors are responsible for stress endurance capabilities. First, overexpression of CXCL2 in cancer cells confers them survival and malignancy [16]. In Muse-AT cells, CXCL2 is 770 folds higher as compared with ADSCs. Therefore high expression of CXCL2 may be one reason for the stress-resistance potential of Muse-AT cells [8]. Besides, ALDH1A2 (aldehyde dehydrogenase 1 family member A2) is an enzyme that are critical for certain life processes and detoxification. Antioxidative and antiapoptotic roles are attributed to overexpression of ALDH1A2 (47-fold change versus ADSCs) [16,17] and SOD2 (41-fold change versus ASCs) [18] at least partially. In addition, upregulation of DNA repair genes modulated by harsh stress bestow Muse-AT cells upon the ability to resist DNA damage [8].

3.4. Nontumorigenic activity

Pluripotent stem cells, such as ES and iPS cells, typically form teratoma during self-renewal and triploblastic differentiation. But a 6-month observation reveal that Muse cells do not show teratoma formation after being injected into the testis of NODscid mice, conferring them a much more attractive candidate than iPS and ES cells for clinical application [3,7,19].

Pluripotency and tumorigenesis have commonly been considered two sides of the same coin, which leads to the question-what keeps Muse cells away from tumor formation while maintaining triploblastic differentiation potential. A stable karyotype in culture, at least partially account for this question [7]. Furthermore, Muse cells display low telomerase activity [8]. Though telomerase activity is high in HeLa cells (tumor cells), that of Muse cells is nearly as low as somatic cells [3].

In comparison to iPS, increased epigenetic modifications in Muse cells might explain low level of pluripotent markers and suppression of teratogenic activity [5]. An increased methylation in genes encoding for Nanog and Oct3/4 is observed in Muse cells compared to iPS cells.

The ratio of Lin28/Let-7 matters in Muse. Lin28, a RNA-binding protein gene, has been proved to keep both pluripotency and tumorigenesis in ES and iPS cells. While Let-7, a microRNA, regulates embryonic development, cell differentiation and tumor suppression. They function the opposite effects in Muse cells [20]. Lin28 gradually declines, meanwhile Let-7 expression increases throughout embryonic development. ES and iPS cells have a very high Lin28/Let7 ratio, which is supposed to lead these cells to tumorigenic proliferation [20]. In contrast, Muse cells show a low Lin28/Let7 ratio [1,5,21]. We can infer that over-expression of Let-7 in combination with Lin28 low-expression may likewise be responsible for suppression of tumorigenic formation.

CDKN2A (cyclin-dependent kinase inhibitor 2A) is also a key tumor suppressing factor found in Lin28-negative Muse cells [5]. CDKN2A, together with p16INK4a, make up a tumor suppressing complex [22]. Suppression of this complex can enhance possibilities of tumor formation [23].

3.5. Paracrine effects

As a subpopulation of MSCs which show paracrine effects, Muse cells are demonstrated to have immunomodulatory capacity. Muse cells express MMP-2 (matrix metalloproteinase-2) and MMP-9, which exert antifibrosis/fibrinolytic effects [24]. Additionally, HGF (hepatocyte growth factor) and VEGF (vascular endothelial growth factor) released by Muse cells play a role in antiapoptosis, neo-vascularization and stimulation endogenous cardiomyocyte progenitors [25].

Muse cells display immunosuppressive effects by activating regulatory T cells, suppressing dendritic cell differentiation and expressing HLA-G [26]. HLA-G has been shown to promote graft tolerance in heart transplantation [27], which may protect Muse cell from immunologic attack in the early phase of integration.

Gimeno et al. [7] and Alessio et al. [28] report that Muse cells decrease proinflammatory TNF- α in a mouse macrophage like cell line. Moreover, Muse cell conditioned media have anti-inflammatory effects via downregulating the secretion of IFN- γ and TNF- α , and upregulating the secretion of IL-10, an anti-inflammatory cytokine. Similar trend is proved by another experiment that coculture of Muse cells-T cells display an upregulation of regulatory T cell factors, IL-10 and CD25 [26]. In addition, Muse cells can spontaneously gain TGF- β 1 expression which play pivotal role in their immunoregulatory capabilities [7]. Collectively, these studies demonstrate the feasibility of Muse cells. Thus Muse cells

can potentially be harnessed as immunoregulators to treat immune-related disorders.

3.6. High homing capacity

In contrast to MSCs, human Muse cells homed more efficiently to the injured site which is evidenced by a higher engraftment ratio into AMI heart ($\approx 14.5\%$) at 3 days than that of MSCs (a few or no) [29,30].

It has been reported that S1P (sphingosine monophosphate) could be released by ischemic myocardium in an animal model [31]. Data showed that Muse cell numbers positively correlated with plasma S1P levels [32]. When a selective antagonist (JTE-013) or gene silencing for S1PR2 is used, the migration and homing effects of Muse cells are remarkably attenuated [26]. These studies confirmed the S1P–S1PR2 (S1P receptor 2) axis mediated the migration of Muse cells to the infarct and the border areas [26].

Although homing of MSCs is mediated by SDF-1 (stromal cell-derived factor 1) - CXCR4 (CXC chemokine receptor type 4) system [33], migration of Muse cells toward damaged liver could only partially be suppressed when CXCR4 is blocked [34], suggesting that the contribution of the SDF-1–CXCR4 system is smaller than that of the S1P–S1PR2 axis.

4. Preclinical studies

During the last 9 years, many preclinical studies have revealed the potential of Muse cells as a therapeutic policy in tissue repair and regenerative medicine. In short, Muse cells migrate to the damage after implantation and differentiate spontaneously into new functional cells to replace the injured ones.

4.1. Nervous system

Endogenous Muse cells can be successfully mobilized into peripheral blood in the acute stage of ischemic stroke [35]. When exogenously administrated, Muse cells could replenish the lost neurons by integration into peri-infarct cortex and differentiation into cells positive for Tuj-1 ($45.3 \pm 13.9\%$) and NeuN ($20.5 \pm 8.7\%$), whereas non-Muse cells showed trophic effects rather than cell replacement [36]. After integration, transferred Muse cells extended their neurites into the contralateral site and reached at least the upper cervical neuron spinal cord [37]. Besides, a mouse intracerebral hemorrhage model showed that Muse cells could integrate into areas of cerebrovascular damage [38]. In comparison to non-Muse groups, Muse cells exhibited faster recovery of motor skills as demonstrated by the Morris water maze and motor function test. Moreover, electrophysiological improvement was found in Muse group.

4.2. Cardiovascular system

Researchers verified that the number of endogenous peripheral blood Muse cells was significantly higher in AMI (276 ± 137 cells/100 μ L) than in normal subjects (164 ± 125 cells/100 μ L) groups. Muse cells are mobilized to repair the infarcted myocardium and improve cardiac function. Hence, the number of Muse cells is regarded as a predictor of prognosis in patients with AMI [32].

Both exogenous and endogenous Muse cells can reduce infarct size, improve left ventricular function, and suppress fibrotic scar formation in AMI [26,32]. The multiple effects are mediated by various paracrine factors. In vivo, Muse cells spontaneously differentiated into cardiac and vascular lineages evidenced by cardiac markers [26]. The potential of Muse cells committing to cardiac lineage cells was also reported by Mohamed and his colleagues

[39]. In vitro, treated by 5'-azacytidine and a series of cardiac differentiation factors, Muse cells were converted into cardiomyocyte-like cells expressing α -actinin and troponin-I with a striation-like pattern. Moreover, expressions of MLC2a (myosin light chain 2a) and MLC2v (myosin light chain 2v) indicated the ability of Muse cells differentiation into atrial and ventricular cardiomyocytes [39].

Recently, a research team has proved the therapeutic efficacy of Muse cells by a murine aortic aneurysm model [40]. Except for a small amount of cells in the lung and spleen, infused Muse cells selectively migrated and integration into aneurysmal tissue. After integration, Muse cells spontaneously differentiated into vascular component cells (endothelial cells and vascular smooth muscle cells). Compared with non-Muse, Muse cells significantly decreased the diameters of aneurysm.

4.3. Liver disease

BM-Muse cells can positively differentiate into hepatocytes. When intravenously injected in a damaged liver model, Muse cells can express liver progenitor markers (CK19, DLK, OV-6, and AFP) during the early phase and then differentiate spontaneously into major liver components, including hepatocytes (~74.3%), cholangiocytes (~17.7%), sinusoidal endothelial cells (~2.0%), and Kupffer cells (~6.0%). Although Muse cells differentiated into different cells with distinct efficiency, a vast amount of Muse cells ($1.89 \pm 0.65\%$ of total cells/mm²) positive for human Golgi-complex was detected [34]. Data showed the cellular integration of Muse cells was 48-fold higher than that of non-Muse cells. Fluorescent labeling rules out the possibility of Muse cell fusion with previously established hepatocytes [10], which confirmed the regeneration capacity of Muse cells.

In addition to structural regeneration, functional improvement has also been proved by a decrease in bilirubin production and fibrotic tissues whereas an increase in albumin levels in Muse groups [34]. 97% of Muse cells that incorporate in the liver can differentiate into hepatocyte marker-positive cells, showing that preimplantation induction is not necessary due to their spontaneous differentiation in vivo. These findings suggest that Muse cells are the feasible stem cell type for the treatment of liver disease.

4.4. Lung injuries

Muse cells efficiently ameliorated lung ischemia-reperfusion injury via pleiotropic effects in a rat model [41]. In this study, functional parameters (arterial oxygen partial pressure to fractional inspired oxygen ratio, alveolar-arterial oxygen gradient, left lung compliance) and histological injury scores were significantly better in the Muse group relative to the BMSCs groups. Besides, Muse cells suppressed apoptosis and stimulated proliferation of host type II alveolar epithelial cells. Human Muse cells also secreted beneficial substances (KGF, HGF, Ang-1, and PGE2) in vitro, and these protective factors together contributed to tissue repair, apoptosis prevention, and alveolar fluid clearance.

4.5. Kidney disease

Scientists also proved Muse cells contributed to structural and functional regeneration in mouse kidney disease models induced by adriamycin [14]. Researchers intravenously injected Muse cells without concurrent administration of immunosuppressants to severe combined immune-deficient and BALB/c mouse FSGS (focal segmental glomerulosclerosis) models. In both models, injected Muse cells preferentially integrated into damaged glomeruli while non-Muse cells mainly remained in spleens and lungs. Similar with the findings of Katagiri et al. [10], no cell fusion between Muse and

host cells was found in situ hybridization. Muse cells attenuated glomerular sclerosis and interstitial fibrosis by differentiation into cells expressing markers of podocytes (podocin, 31%), mesangial cells (megsin, 13%), and endothelial cells (CD31, 41%). Apart from the structural repair, functional improvement in Muse group was confirmed by improved urine protein, plasma creatinine levels, and creatinine clearance at 5 weeks [14].

4.6. Osteochondral defects

Muse cells exerted therapeutic potential for osteochondral repair [42]. An osteochondral defect was generated in the patellar groove of immunodeficient rats and both Muse cells and non-Muse cells were intra-articular injected. At 12 weeks, the defects in the non-Muse group were filled with brown tissue positive for collagen type I. However, in the Muse group, the defects were completely filled with smooth homogenous tissue, which makes it hard to clearly recognize the defect margins. Even though the repaired tissue in Muse group was negative for collagen type II, suggesting that cartilage repair was not satisfactory, histological scores displayed better subchondral bone repair at the cartilage defect sites. Extensive researches into the chondrogenic potential of Muse cells are needed.

4.7. Skin regeneration

Muse cells derived from dermal fibroblasts can be induced to express melanocyte specific markers [2,43]. And the morphology of the induced cells was similar to that of human melanocytes [43], which is consistent to another study suggesting Muse-AT cells can express melanocyte-related genes at a comparable level to melanocytes [4]. Functionally, induced melanocytes could produce melanin in vivo and in vitro [4,43].

Recently, a research team managed to induce Muse-AT cells into fibroblasts, keratinocytes and melanocytes [44]. Then they reconstituted skin sheets by these differentiated cells and collagen gel layers. It was amazing that the reconstituted pigmented skins formed an epidermis-like structure. Moreover, immunofluorescence staining showed markers of keratinocytes, melanocytes and fibroblasts.

Apart from epidermal cells, Muse-AT cells also successfully differentiate into dermal cells and promote wound healing. In a diabetic skin ulcers model [45], Muse-AT cells differentiate into dermis and vascular endothelial cells in the upper dermis and into other cell types in the lower and middle dermis. The studies demonstrate the prospects of Muse cells as a promising method for skin regeneration.

5. Conclusions

Muse cells offer a new possible source of pluripotent stem cells naturally present within mesenchymal tissues. Since Muse cells are successfully isolated, they have drawn much attention due to their pluripotency and nontumorigenesis abilities. Muse cells are able to differentiate into three germ layers from a single cell and yet exhibit non-tumorigenic activities. They contribute to tissue repair by efficient migration to damaged sites, spontaneous differentiation into cells compatible with the targeted tissue, and secretion factors to modulate the microenvironment. Such a repairing activity of Muse cells via exogenous administration is confirmed in various tissues. The impressive regenerative performance of Muse cells may provide a simple, feasible strategy for treating a variety of diseases. It is imperative to further investigate and exploit Muse cells' unique qualities and vast potentials in tissue regeneration and stem cell therapy.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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