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Muse cells and Neurorestoratology

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ABSTRACT
Multilineage-differentiating stress-enduring (Muse) cells were discovered in 2010 as a subpopulation of mesenchymal stroma cells (MSCs). Muse cells can self-renew and tolerate severe culturing conditions. These cells can differentiate into three lineage cells spontaneously or in induced medium but do not form teratoma in vitro or in vivo. Central nervous system (CNS) diseases, such as intracerebral hemorrhage (ICH), cerebral infarction, and spinal cord injury are normally disastrous. Despite numerous therapy strategies, CNS diseases are difficult to recover. As a novel kind of pluripotent stem cells, Muse cells have shown great regeneration capacity in many animal models, including acute myocardial infarction, hepatectomy, and acute cerebral ischemia (ACI). After injection into injury sites, Muse cells survived, migrated, and differentiated into functional neurons with synaptic junctions to local neurons and contributed to recovery of function. Furthermore, Muse cell differentiation did not need to be induced pre-transplantation and no tumors were observed post-transplantation. The Muse cell population is promising and may lead to a revolution in regenerative medicine. This review focuses on recent advances regarding the Muse cells therapies in Neurorestoratology and discusses future perspectives in this field.

1 Introduction
Multilineage-differentiating stress-enduring (Muse) cells are a novel pluripotent stem cell population first described by Professor Mari Dezawa et al. in 2010 [1]. They are a subtype of mesenchymal stromal cells (MSCs) that express the state-specific embryonic antigen 3 (SSEA3). Muse cells have the characteristics of stem cells. Firstly, Muse cells can self-renew. Through limiting dilution, Kuroda et al. [2] and Wakao et al. [3] seeded 50 single Muse cells into a 96-well plate coated by poly-HEMA. Muse cell clusters formed after 7–10 days and were stained SSEA3+. After that, the clusters were transferred into an uncoated plate. The cells attached to the bottom of the plate and extended. The single cluster culturing cycle was repeatable. To keep the stemness, the culturing medium consisted of DMEM-low glucose (Gibco, 10567-014), 10% FBS (HyClone, vol/vol), 2 mM GlutaMAX (Gibco), 1 ng/ml bFGF, 0.1 mg/ml kanamycin [2]. Secondly, Muse cells can differentiate into endodermal-, ectodermal- and mesodermal-lineage cells spontaneously in vitro or through induction [1, 4, 5]. After 10–14 days of culturing in gelatin-coated dishes, Muse cell clusters were NF+ and MAP2+ (ectoderm marker), SMA+ and Desmin+ (mesoderm markers), and CK7+ and α-FP+ (endoderm markers) [2]. Another study showed that the rates of spontaneous Muse cell differentiation to endodermal, ectodermal, and mesodermal cells were 20%, 22% and 23%, respectively [6]. When Muse cells received
induction factors in different specific media in vitro, 98% of cells were Osteocalcin+ (osteocyte, mesoderm) [2], 98% were red oil+ (adipocyte, mesoderm) [2], 90% were α-FP+ (hepatocyte, entoderm) [2], 90% were MAP-2+ (Neuron, ectoderm) [2], and 43.1 ± 17.1% were GP100+ (melanocyte, ectoderm) [7, 8]. When induced Muse cell cultures were transplanted in vivo, cells differentiated into adipocytes, skeletal muscle cells, myocardial cells, biliary epithelial cells, glomerular cells, glomerular endothelial cells, melanocytes and neurons [8–16].

2 Characteristics of Muse cells

Muse cells own five special characteristics beyond their basic stem cell qualities.

Firstly, Muse cells are stress enduring. Muse cells survived in 0.5% trypsin for up to 16 hours [1]. They also survived in a severe hypoxic environment without fetal calf serum at 4 °C [17]. Researchers have used severe culturing conditions to isolate and purify Muse cells. Further gene analysis showed that the expressions of CXCL2, ALDH1A2, and SOD2 were higher in Muse cells than in non-Muse cells [6, 18, 19].

Secondly, Muse cells do not form teratomas in vivo after the transplantation. The telomerase activity of Muse cells is as low as zero [10]. Gene analysis showed that CDH1 was overexpressed and alpha-6 integrin (ITGA6) and Lin28 were down-regulated in Muse cells [4, 6, 20, 21]. Animal studies in the testis of severe combined immunodeficient mice (SCID) and myocardial infarction sites of rabbits demonstrated that injected Muse cells did not form tumors 6 months post-injection [1, 4, 22]. Moreover, Muse cells are a subpopulation of MSCs, which have been safely transplanted in basic and clinical studies [23, 24].

Thirdly, Muse cells can autonomously home to injured tissue. In the acute injury period, the level of sphingosine monophosphate (S1P) was significantly higher. But once the S1PR2 receptor antagonist, JET-013, was administrated, the S1P-S1PR2 axis was blocked. In this case, Muse cells did not show the homing function. However, non-Muse cells still migrated slightly as before. Furthermore, if the S1PR2 gene was knocked out in Muse cells, they would lose the homing feature. These results suggest that the S1P-S1PR2 axis is the mechanism for Muse cell homing to lesion areas [22].

Fourthly, Muse cells can be transplanted via allograft without rejection from the host organism. Although autologous cell transplantation is the safest method, it takes 3–4 weeks to prepare cells, such as mesenchymal stromal cells isolated and cultured from adipose tissue. For strokes, spinal cord injuries and other acute central nervous system injuries, cell therapies are best if carried out before formation of glial scars. Muse cells make this timeline possible [22].

Fifthly, Muse cells do not need to receive any gene editing before transplantation, unlike induced pluripotent stem cells (iPSCs), which need to be induced by the transcription of factors Oct3/4, Sox2, Klf4 and c-Myc [25]. However, the iPSCs would form teratoma at 8–12 weeks after injected in vivo, while Muse cells would not [4, 5, 26].

Distribution of Muse cells in the body

Muse cells were first discovered in the bone marrow, and subsequent researches showed the presence of Muse cells in the adipose tissue, dermis, and the umbilical cord [3–5]. Because Muse cells were initially considered to reside in mesenchymal tissues, the percentage of Muse cells in the human bone marrow is assumed to be around 0.01%–0.03% of the mononucleated cell fraction [1]. Muse cells also exist in the peripheral blood at the proportion of 0.01%–0.2% of the mononuclear fraction [27]. But the bone marrow is directly connected to the peripheral blood, so Muse cells in blood should be from the bone marrow. Moreover, Muse cells also distribute to the connective tissue of every organ, which has been demonstrated in the dermis, spleen, pancreas, trachea, umbilical cord, adipose tissue, and synovial membrane, but the percentage is as sparse as 0.01%–3% [5, 28, 29]. Muse cells are even found in the pia mater and arachnoid of the brain (unpublished data by Mari Dezawa [30]).

When used in preclinical research, millions of purified Muse cells are needed to meet the transplantation standards. The most popular isolation method is fluorescence-activated cell sorting (FACS) [2]. Briefly, it amplificated the MSC cells in adhesion culture and got the cell suspension. Then it stained the cells with anti-SSEA3 antibody and the fluorescent probe-labeled secondary antibody. Finally, it isolated Muse cells by FACS. Researchers also have used severe culturing conditions to isolate and purify Muse cells, such as
long-time trypsin [1], peroxide hydrogen or UV irradiation [28], low oxygen and temperature [6]. But personally, the isolated Muse cells may change their characteristics under the severe culture stress. And two published studies are involved in magnetically activated cell sorting (MACS), but their isolation rate were only 77.1% and 71.3% [15, 31].

3 Preclinical research of Muse cells in Neurorestoratology

3.1 Muse cells treated glioma

Herpes simplex virus thymidine kinase (HSVtk) combined with Ganciclovir (GCV) is the most popular treatment in the research of glioma [32]. Hiroki Namba’s team [16] transplanted Muse-tk cells (HSVtk-IRES2-EGFP-Muse cells) and U87-luc glioma cells together into the brain of SCID mice, and GCV was administrated by intraperitoneal injection (IP). Results showed that mice survived for 100 days when the transplanted cells’ ratio was 1:32 (Muse-tk cells: U87-luc cells) and no glioma was observed in immunohistology staining. On the other hand, if U87-luc cells were injected into the SCID mice brain 7 days before the Muse-tk cells’ transplantation and GCV administration, all the SCID mice died in 60 days in the blank control and GCV administration-only groups. However, all the mice survived 200 days in the Muse-tk/GCV group, and no glioma was observed. The cell migration experiment in vitro showed that the U87-luc cells-conditioned medium could attract Muse-tk cells to migrate but Non-Muse-tk did not. This study demonstrated that intracranial injection of Muse-tk combined with GCV IP was safe and effective in SCID glioma treatment.

3.2 Muse cells treated stroke

Mari Dezawa, the first to describe Muse cells, demonstrated that human Muse cells reconstructed neuronal circuity in subacute lacunar stroke SCID mice [15]. In the study, stroke was induced in SCID mice using the stroke model. After two weeks, Muse cells from human bone marrow stromal cells were transplanted into the local stroke area. The results showed that 28% of injected Muse cells survived 8 weeks later, and the positive markers were NeuN (neuron, 62%), MAP-2 (neuron, 30%) and GST-pi (oligodendrocyte, 12%). Dextran tracer was used to label Muse cells, demonstrating that Muse cells reconstructed synaptic connections with host neurons. Incredibly, the nerve fibers derived from Muse cells surpassed the decussation level of the pyramid, crossed to the offside pyramidal tract, and descended to the level of the second cervical spinal cord. In the cylinder function test, the score of the Muse cell transplantation group was significantly higher than that of the non-Muse cells group. 10 months after transplantation, the human specific Alu gene sequence only occurred in the brains of the mice and no teratomas were found. Another interesting finding was that the immunostaining was GFAP negative and Iba-1 negative, which meant Muse cells did not differentiate into astrocytes or microglia. Muse cells mainly differentiated into the injured target cells, neurons, according to the microenvironment. This study indicated the Muse cells’ therapeutic potential for lacunar stroke.

In another study, SCID mice were subjected to permanent middle cerebral artery occlusion, according to the ischemic stroke model [33]. Muse cells and non-Muse cells were transplanted into the ipsilateral striatum 7 days after the onset of stroke. 42 days after transplantation, the immunostaining results showed that 45.3% ± 13.9% of GFP+ Muse cells were Tuj-1+, 20.5% ± 8.7% were NeuN+, and 1.4% ± 1.2% were GFAP+, which corresponded with the Dezawa lab’s research. Muse cells mainly differentiated into neurons not astrocytes to regenerate injured tissue, while non-Muse cells did not survive for 42 days. In the Muse group, functional recovery was apparent 35 days post-transplantation.

Both studies above demonstrated that non-Muse cells could secrete nutrition factors to immunomodulate, but they could not remain long-term in vivo to replace injured cells. Contrastingly, Muse cells spontaneously differentiated into target cells according to the microenvironment and recovered the host’s function.

3.3 Muse cells were highly effective in treating ICH

In Hiroki Ohkuma’s experiment, GFP+ Muse cells derived from human bone marrow MSC were injected into the left putamen of SCID mice five days later than the ICH modeling [34]. 69 days post-injection, immunostaining results showed that 57.3% ± 3.5%
were NeuN+, 41.6% ± 4.7% were MAP-2+, and none were GFAP+. In the water maze and motor function tests, the scores in the Muse-engrafted group were significantly higher than those in the non-Muse-engrafted group. No teratomas were found in either group. This study demonstrates that Muse cells can be used to treat ICH.

3.4 Muse cells and spinal cord injury

Xue Chen reported a partition-type tubular scaffold for spinal cord injury repair [35]. The scaffold was designed to match the anatomical features of the T8-10 spinal cord of the rat, and it included chitosan with platelet-derived growth factor (PDNF). Results showed that this scaffold was suitable biocompatibility towards Muse-NPCs (Muse-neural progenitor cells) and could promote the directional migration and growth of these cells. This in vitro study indicated that the combination of a tubular scaffold, PDGF and Muse-NPCs may be a promising model for spinal cord grafts in vivo.

To explore the treatment of Muse cells in spinal cord injury, we initially transplanted human umbilical cord MSCs-Muse cells into contused Sprague-Dawley rats’ spinal cords. 4 weeks post-transplantation, Muse cells survived and migrated into the injury site from four injection sites around the injury area. Moreover, we successfully induced Muse cells into neural spheres and they were Nestin positive. As it needs millions of mesenchymal stromal cells to sort out enough Muse cells for transplantation [5, 36], we also developed a systemic culturing method to obtain millions of Muse cells quickly and efficiently by MACS (unpublished data). We are confident that Muse cell therapy would benefit spinal cord neural regeneration.

4 Clinical research of Muse cells in Neurorestoratology

4.1 Muse cells in acute stroke patients

Dr. Satoshi Kuroda enrolled 29 acute stroke patients and 5 normal healthy persons to evaluate the number trending of Muse cells in the peripheral blood after ischemic stroke [37]. Results showed that the number of Muse cells was 3.5 ± 4.3 cells/μL in the peripheral blood of healthy persons. But in the stroke patient group, the number of Muse cells sharply increased to 81.9 ± 78.0 cells/μL after 24 hours. The number of Muse cells dropped slowly to 68.7 ± 64.9 cells/μL 30 days later, but this change was not significant. Further multivariate factor analysis revealed smoking and alcohol consumption were two independent factors that affected the level of Muse cells. Another 8 cadavers analysis indicated that the percentage of Muse cells was 0.20% ± 0.17% in bone marrow and decreased with aging. This study showed that Muse cells were mobilized from the bone marrow into peripheral blood in the acute stage of ischemic stroke. Increasing endogenous Muse cells or exogenous administration of Muse cells may recover patients’ function after ischemic stroke.

4.2 Clinical trials of Muse cells

Since Muse cells are non-tumorigenic and account for a part of MSCs, which are already widely used in clinical trials, they are feasible for clinical trials from the viewpoint of safety. Besides, many preclinical studies also demonstrate Muse cells are safe and effective in vivo. Although there are no related clinical trials in Neurorestoratology field, a phase I clinical trial to evaluate Muse cell application for the treatment of acute myocardial infarction has been initiated in Japan by Mari Dezawa [30]. After verification of the safety of Muse cell administration, the range of application will be expanded.

5 Future of Muse cells

Muse cells may be the real stem cells in mesenchymal stromal cells.

The concept of “mesenchymal stem cells” dated back to 1991 when US biologist Arnold Caplan claimed to isolate a type of stem cell found in bone marrow [38]. Over the next decades, the number of reported tissue types containing “mesenchymal stem cells” exploded. Studies also suggested that these cells could differentiate into cells of all three lineages. During this period, researchers confused the concepts “mesenchymal stem cells” and “mesenchymal stromal cells”. So International Society for Cellular Therapy (ISCT) believes that “mesenchymal stem cells” are not really stem cells. Naming them as “mesenchymal stromal cells” is more rational. Then ISCT made the standard of
identification of “mesenchymal stromal cells” [39–41]. In the past few years, there has been questioning of “mesenchymal stem cells” as a valid biological entity [42]. Many authors reported that after in vivo transplantation, no more than 3% of “mesenchymal stem cells” could survive and few could differentiate into functional cells, especially in the Neurorestoratology field [23, 43]. Last year, Caplan declared that he no longer believes that “mesenchymal stem cells” are stem cells [44]. Huang also appealed to use the name correctly [45]. Douglas Sipp declared the existence of a tissue-specific stem cell in bone-marrow stroma, albeit one with a limited ability to differentiate into other cell types [42]. Muse cells are a subpopulation of mesenchymal stromal cells, accounting for about 0.01%–3% [13]. They portray the stemness characteristics as described above, and Muse cells may be the real stem cells in mesenchymal stromal cells. The future of Muse cells is bright.

6 Summary: Muse cells may revolutionize regenerative medicine

Cell therapy is well known as the most promising treatment in regenerative medicine. Embryonic stem cells, induced pluripotent stem cells, neural stem cells and other functional cells, such as Schwann cells have been demonstrated effectiveness in treating certain diseases. However, these cell groups face barriers in cell source, ethics, tumorigenicity or limited functional recovery [46]. Studies have shown that Muse cells could migrate to and home into damaged areas by intravenous (IV) injection [22, 31, 47–52], which makes them practical for regenerative therapy. Only three steps are necessary for Muse cell transplantation. First Muse cells are isolated from mesenchymal stromal cells derived from bone marrow, fat or umbilical cords. The second step is Muse cell expansion. Finally, Muse cells are injected into patients by IV. Allogenic Muse cell function mimics autologous cell function, and differentiation does not need to be induced. As a result, Muse cells spontaneously differentiate into target cells after homing. As naturally existing stem cells, Muse cells may revolutionize regenerative medicine. More basic research and high-quality studies, such as multicenter, random, double blind, and placebo-control clinical trials need to be carried out.

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Disclosure

The authors declare no conflict of interests for this paper.

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