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Application prospects of multilineage differentiating stress-enduring (Muse) cells in the treatment of skin diseases

Abstract

Multilineage differentiating stress-enduring (Muse) cells are a subgroup of mesenchymal stem cells (MSCs) that express stage-specific embryonic antigen-3 (SSEA-3) and CD105. It is capable of generating cells representing all three germ layers from a single cell and is non-tumorigenic. Muse cells can efficiently migrate and integrate into damaged tissues that produce sphingosine-1-phosphate (S1P), while Muse cells specifically express S1P receptor 2. The factors secreted by Muse cells play anti-inflammatory, anti-fibrosis, and anti-apoptotic effects, and cooperate to complete the function and structure repair of the damaged group. This review summarizes the basic characteristics of Muse cells and describes their application in skin repair.

Keywords: multilineage differentiating stress-enduring (muse) cells, stage-specific embryonic antigen-3 (SSEA-3), mesenchymal stem cells (MSC), repair, differentiation

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Introduction

Muse cells were discovered by scientist from the University of Tohoku (Sendai, Japan) in 2010 and were recognized as a kind of naturally pluripotent cells within mesenchymal tissues.[1] Neither related to special structures, nor observed in any specific niche.[2] The distribution of Muse cells is a dynamic process.[3] Muse cells reside in bone marrow (BM) in adherence status and occupy around 0.01~0.03 % of the mononucleated cells.[4] A small amount of Muse cells from BM could mobilize to the peripheral blood (PB) becoming overactive in suspension status and comprise nearly 0.01-0.2 % of the mononucleated cells.[1] The proportion of Muse cells in PB increased rapidly after injury or in the presence of diseases.[5] Other than the BM and PB, Muse cells sparsely distribute in the dermis, spleen, pancreas, trachea, umbilical cord and adipose tissue.[4] In a physiological state, Muse cells are surrounded by affluent matrix and display as a circular shape.[3] Muse cells have a highly conserved cellular mechanism in response to cellular stress and acute injury.[6] Muse cells can home into damaged tissues and differentiate into specific cells leading to tissue regeneration and functional recovery.[5] After homing, Muse cells spontaneously differentiate into cells compatible with homing tissue, not necessary to induce the target cell type before transplantation. Furthermore, Muse cells have immunomodulatory properties,[7] which could promote tissue generation and functional repair in vivo. Muse cells could differentiate into cells representative of all three germ cell layers both spontaneously and under media-specific induction, and exhibit low telomerase activity and do not undergo tumorigenesis once implanted in animal models.[8,9] Muse cells are injected intravenously to repair various tissues including the brain and skin. Muse cells express human leucocyte antigen (HLA)-G with strong immune-inhibitory properties, do not require HLA-matching or long-term immunosuppressant treatment before transplantation. This review details the therapeutic characteristics of Muse cells, highlighting their potential application in skin diseases.

Biological characteristics of muse cells suitable for clinical application

Genetic basis of stress tolerance

Persistent stress stimuli may promote massive DNA damage and induce apoptosis or senescence of an organism.[6] Muse cells may have a powerful non-homologous end joining (NHEJ) system to survive strong genotoxic stress.[6,10] One of the first activated proteins after genomic injuries is the ataxia-telangiectasia mutated kinase (ATM), whose activation is switched off some hours later.[6,11] It has been reported that the increased ATM value in the dispose-derived Muse cells had a gradual decline to basal level by 48h following stresses.[11] Proteome analysis revealed that five proteins that might play a major role in the stress enduring of Muse cells: SELONOM, SELENOBP, OXR1, GPX8 and FANCM.[12] In Muse cells, the DNA damage repair (DDR) was activated correctly after injury.[7] The phosphorylated H2AX (γ-H2AX) contributes to the recruitment of DNA repair factor and the presence of γ-H2AX foci indicates the regions with damaged DNA is undergoing repair.[6,11]

Serine protease inhibitors (also known as Serpin), a group of protein superfamily with protease inhibitory activity, are only expressed in Muse cells, which can partially explain the long-time trypsin (LTT) endurance of Muse cells in vitro.[13] The recent study has also shown that the high expression of CCNA2 gene was associated with the enhanced tolerance.[14] The secretomes of Muse cells contains a large number of 14-3-3 protein (also called YWHAQ) isomers, which plays a key role in regulating the response to DNA damage following cellular injury, indicating that the stress-enduring ability involves the secretion of pro-survival factors.[7]

Pluripotent and non-tumorigenesis

Muse cells have not tumorigenic and ethical sources of problems and widely accepted as a multipotent cell population.[15] Muse cells

express pluripotent markers such as NANOG, Oct3/4, Par-4, Sox2, and TRA1-60,[16] and have potential for triploblastic differentiation from a single cell.[3,17] Muse cells exhibit lower telomerase activity, normal karyotype and asymmetric growth.[3] They express low levels of Lin28, a RNA binding protein gene involved in tumorigenesis and maintenance of pluripotency and express high levels of Let-7, as a potential counteracting protective mechanism against Lin 28 and against tumorigenesis.[3] Therefore, Muse cells do not undergo tumorigenesis when transplanted into a host organism, are the most promising cells in regenerative fields.[18] Another unique characteristic of Muse cells is their unique immune privilege system.[19]

Abilities to homing to the injured sites

When tissues are injured, the damaged cells produce sphingosine-1-phosphate (S1P), which is an alerting signal and attracts Muse cells that specifically express S1P receptor 2 to the damaged sites.[20] After homing, Muse cells spontaneously differentiate into tissue-compatible cells and replenish new functional cells for tissue repair.[16] Human leucocyte antigen (HLA)-G is the nonclassical HLA allele with strong immune-inhibitory properties, was much more expressed on Muse cells than on non-Muse cells.[13,21] So Muse cells has a good tolerance in allogenic transplantation and do not require HLA-matching or long-term immunosuppressant treatment.[21]

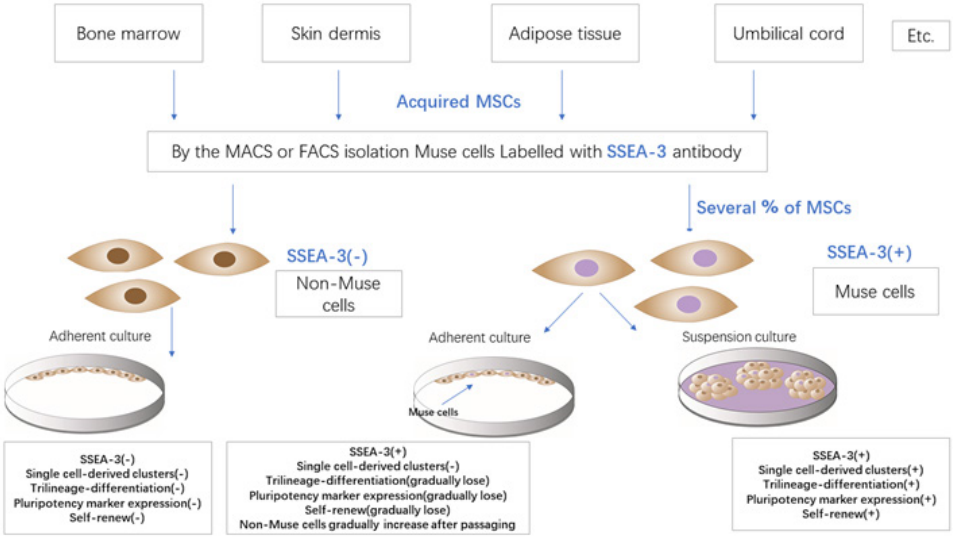

Figure 1 Acquisition of Muse cells.

Pleiotropic effects

Muse cells release more cytokines than non-Muse cells, including EGF, PDGF-bb, NGF-b, SCF, TNF-α, bFGF and TGF-β, and the levels of these factors are higher under hypoxic conditions.[22] A scientific team has reported that the mRNA levels of TGF-β and IL-10 in Muse cells are significantly higher than MSCs in the case of inflammation.[23] Therefore, Muse cells can play a better role in stress conditions (such as hypoxia) and coordinate wound healing by releasing soluble cytokines.

Acquisition of muse cells

Tissue source

The number of Muse cells extracted from different tissues or experimental environments was inconsistent.[4] Culturing BM-derived Muse cells should use the Low-glucose DMEM supplemented with 10%FBS and FGF-2. It was reported that approximately 16.50% ± 2.01% rat BM-MSCs maintained normal morphologies and intact cell membranes after an 8h trypsin incubation, and single-cell culture displaying M-cluster accounted for approximately 2.03% ± 0.14% of total BM-MSCs.[24,25] An experiment separated the Muse cells from human umbilical cords (UCs) by collagenase, and quantified the percentage of SSEA-3 positive cells, and found that the first passage had 5.0% ± 4.8% SSEA3+ cells, but the percentage decreased significantly between P0 and P2. Upon culture of the sorted population, the percentage of SSEA3+ cells ranged from 62.5%-76.0% in P2–P5 to 42.0%–54.7% in P6-P9.[26] The results indicated that the process of passaging may gradually decrease pluripotency of cells. Dermis can be regarded as an ideal source to obtain Muse cells, because it can be obtained from various surgical procedures.[24] The expansion of Muse cells from dermis need to use the specific Poly-HEMA (poly 2-hydroxyethyl methacrylate) coated wells.[24,27] By contrasted other tissues in the body, AT is the most promising source because of convenient and painless obtain from the liposuction, millions of Muse cells can be extracted from 1-2 liters of Adipose tissues.[22]

Sorting technologies

SSEA-3, an antibody to recognize sugar epitopes that no gene encodes SSEA-3 and no species variation, is a specific marker by which to distinguish Muse cells from other cells in MSCs. Muse cells have been isolated with SSEA-3 positive expression from BM, umbilical cords (UCs), adipose tissue, dermis and the cultured fibroblasts.[24] Fluorescence activated cell sorting (FACS) is a precise method for sorting cells, but requires expensive equipment and complicated operating procedures. Magnetic-activated cell sorting (MACS) has been used to collect SSEA-3 positive Muse cells and enrich the target cells to 91.4% ±3.2%.[26] In 2018, Wakao et al. had provided a protocol for enriching Muse cells by long-term trypsin (LTT), which is now considered to be one of the feasible methods to obtain Muse-rich cell population.[24]

Expansion in vitro

Muse cells can survive and proliferate in both suspension or adhesion environments, but their activities and behaviors were

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affected by culture system.[4] More importantly, their pluripotency is regulated by the "adhesion suspension switch".[4] Muse cells are natural adult stem cells so that they proliferate through symmetrical and asymmetric cell division. When Muse cells were cultured in suspended fluids, they generated slender non-Muse cells through asymmetric cell division. The non-Muse cells wrapped Muse cells, and those wrapped Muse cells clusters continued to proliferate and form embryoid-like cell clusters whose proliferation gradually slowed down and ceased after about 14 days maybe due to the presence of overlying cells or intracellular signaling.[3] When the cell clusters were transferred to the adherent culture, the outermost non-Muse cells open the coating layer, and Muse cells are beginning to proliferate again, migrating out of the cell clusters, and producing the expanded cells until they get reached the Hayflick limited. In the status of adhesion, the cells divide once in about 1.3 days, almost the same as or slightly slower than FBs. Nanog, OCT3/4 and Sox2 distributed in the cytoplasm of Muse cells in adhesion status, will transfer to the nucleus when Muse cells are transferred to suspension status, and their expression will increase 50 to hundreds of times.[4,28] The expression level of pluripotent genes between adherent and suspended states is reversible. How suspension states are associated with demethylation is unclear.

Application in skin damage repair

The number of endogenous Muse cells is a potential parameter of the body's reparative activity.[13] If the number is insufficient for repair, or if the endogenous Muse cells have low reparative activity due to underlying diseases, exogenous Muse cells can be supplied via intravenous infusion.[29] This is the basic concept of Muse cell therapy.[23]

Three-dimensional skin model derived from muse cells

It is reported that Muse cells could differentiate into fibroblasts (FBs), keratinocytes (KCs) and melanocytes (MCs).[30] When Muse cells were suspended cultured with Low gluco-DMEM (DMEM-L) containing transforming growth factor β2 (TGF-β2) for 1 week to form embryoid bodies, which subsequently were cultured adherently in DMEM-L containing ascorbic acid and fetal calf serum for 1 week, most cells expressed CD10 and CD73, and their mRNA and protein levels of collagen 1 and 3 were similar to normal human FBs. When Muse cells were cultured on matrigel-coating dishes with defined keratinocyte serum-free medium including bone morphogenetic protein 4 and all-trans retinoic acid, 4 weeks culture later, the cells became similar to normal human KCs in morphology and expressed cytokeratin-14, p63 and mRNA of desmoglein-3.[30,31]

To induce MCs, Muse cells need to be cultured in melanocyte induction medium (MIM) containing Wnt3a, stem cell factor (SCF), endothelin-3 (ET-3), basic FB growth factor (b-FGF), linoleic acid, cholera toxin, L-ascorbic acid, 12-o-tetradecane-phorbol-13 acetate (TPA), insulin transferrin selenium (ITS) and dexamethasone for 6 weeks. Among of them, Wnt3a, SCF and ET-3 are necessary to differentiate stem cells into MCs. During the process of induction, the morphology of Muse cells gradually changed, gradually expressing microphthalmia-associated transcription factor (MITF), kit, tyrosinase-related protein 1 (TYRP-1) and PMEL (gp100) (in the third week), dopachrome tautomerase (DCT) (in the 5th week) and tyrosinase (TYR) (in the 6th week).[32] The 3D skin reconstituted with Muse-derived cells showed visible pigmentation. However, the original Muse cells could not spontaneously differentiate into MCs even if they were integrated into the epidermis of 3D cultured skin.[30,34]

Application for skin ulcers in mice model

Muse cells can spontaneously home to the injured area and secrete growth factors, such as PDGF, bFGF, TGF-b and EGF, which are required for wound healing and inflammation in the proliferation phase.[34] The skin wounds of diabetic DM-SCID mice were treated by subcutaneous injection of Muse cells and non-Muse cells. The results showed that the ulcers in the Muse cell treatment group heal quickly, the epidermis is thick, and the wound healing time is short. With hyaluronic acid as a carrier, Muse cells are injected locally to avoid cell migration and achieve a better concentrated treatment effect. After homing, Muse cells differentiate into KCs, blood vessels and FBs, and participate in the body's reconstruction through the reconstructed epidermis and dermis.[3,34]

Therapeutic approach for epidermolysis bullosa

Scientists have created knockout mice of type XVII collagen (Co117) that mimic epidermolysis bullosa (EB). The labeled human SSEA-3+ Muse cells were injected intravenously through the tail vein.[35] The imaging confirmed the homing of the injected Muse cells, expressing keratin 14 and desmoglein-3 in the epidermis. Studies have shown that intravenously injected human Muse cells preferentially implant in damaged skin and spontaneously differentiate into skin components, such as keratinocytes, hair follicle cells, vascular endothelial cells and sebaceous gland cells.

Therapeutic approach for topic dermatitis

Previous study had reported that MSC-mediated immune regulation could be used to treat skin inflammatory diseases. Wen et al. cultivated human BM-derived MSCs through LTT to obtain Muse cells, and then injected obtained Muse cells into mice model of atopic dermatitis. Their qPCR results had found that the expression levels of the inflammatory factors interleukin (IL)-6, IL-17α, and IL-33 in both the spinal cord and skin were suppressed by Muse cells.[36] Since Muse cells are an ideal type of pluripotent stem cell that may also have the potential to treat topic dermatitis.

Conclusion

Muse cells are endogenous pluripotent stem cells and therefore elicit few safety concerns in future clinical applications. Muse cells can survive in harsh environments and switch pluripotency expression in suspension culture and adhesion culture. After being injected intravenously, Muse cells can home to the injured site and differentiate into cells needed for tissue repair. Muse cells have pleiotropic effects, could synergistically deliver long-lasting functional and structural recovery. Muse cells have been used in the mouse model of diabetic ulcer or with EB, and have achieved perfect clinical effects. The clinical application prospects of Muse cells is very promising in the field of skin disease treatment

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