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Stem Cell Discovery

Benefits of dedifferentiated stem cells for neural regeneration

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ABSTRACT

Dedifferentiation, as one of the mechanisms rerouting cell fate, regresses cells from a differentiated status to a more primitive one. Due to its potential of amplifying the stem/progenitor cell pool and reproducing sizable and desirable cellular elements, it has been attended in the field of regenerative medicine, which will hopefully provide novel therapeutic strategies for currently incurable diseases, such as varieties of central nervous system (CNS) diseases and injuries. In this article, we will first discuss naturally occurring and experimentally induced dedifferentiation, and then set forth principles in stem-cell based therapy in the neural field; beyond that, we will introduce two recent studies that show dedifferentiated stem cells contribute to neural regeneration. Moreover, we also present our recent research results of dedifferentiated muscle stem cells for neurogenic differentiation study *in vitro*. Further work will be conducted to elucidate the mechanism underlying the dedifferentiation process to facilitate the development of new strategies in regenerative medicine.

Keywords: Dedifferentiation; Neural Stem Cells; Muscle Stem Cells; Neurogenesis; Regenerative Medicine

1. DEDIFFERENTIATION, A POTENTIAL APPROACH INVOLVED IN REGENERATIVE MEDICINE

The ability of animals to regenerate lost tissues is a dramatic and poorly understood aspect of biology. The sources of the new cells and the routes to these regenerative phenomena have been sought after for decades. Dedifferentiation, which is one process involved in natural regeneration, refers to the reversion of a terminally differentiated cell back to a less differentiated stage within its own lineage as part of regenerative process. It was first used to describe ascidian stolon regeneration in 1902 [1] but there was little evidence for this concept besides cellular morphology. Since then, intensive studies have been carried out in this field and accumulating evidence of this naturally occurring process has emerged from lower organisms as well as from mammalian tissues; this evidence will shed light on the basic mechanism underlying regeneration and aid in conceiving new strategies in regenerative medicine.

In non-mammalian vertebrate species, complete regeneration of zebrafish heart following ventricle amputation can be achieved by dedifferentiation of cardiomyocytes through disassembling the sarcomeric contractile apparatus, which contains a large proportion of terminally differentiated cells that physically impede cytokinesis [2-9]. Another intensively studied case is the blastema formation after limb amputation in the urodele amphibians. Shortly after limb amputation, cells adjacent to the wound dedifferentiate and form a blastema that consists of undifferentiated cells, which subsequently proliferate and eventually redifferentiate to create all the components of the lost limb [10-17]. In mammals, evidence of dedifferentiation has also been observed. In the case of peripheral nerve injury, Schwann cells are capable of dedifferentiating and proliferating when they lose contact with the axon that they are myelinating [18-21]. Astrocytes, another type of mature glial cell, can upregulate proteins that are characteristic of neural stem cells (NSCs) and re-enter the cell cycle after brain injury [22-30]. As determined *in vitro*, a fraction of these reactive astrocytes also shows long-term self-renewal and multipotency by forming neurospheres [22]. Another recent study showed that reversion of spermatogonia to germline stem cells

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occurs in the murine spermatogenic compartment [31-33]. Most recently, using an ingenious cre/lox system, our research demonstrated for the first time that dedifferentiation of skeletal muscle cells to early progenitor cells, including myoblasts and muscle-derived stem cells (MDSCs), occurs in an injured mouse model *in vivo* and can enhance cell proliferation and myogenesis [34].

In addition to the evidence demonstrating that dedifferentiation takes place naturally under certain stresses in a wide spectrum of species, recent studies have also clearly shown that this process can be achieved by experimental induction. After treatment with the extract isolated from regenerating newt limbs, mouse myotubes reduce the expression of the myoblast determination genes MyoD and myogenin, and subsequently dedifferentiate and proliferate [35]. Similarly, another group has shown that small chemical molecules can dedifferentiate lineage-committed myoblasts to multipotent mesenchymal progenitor cells, which can further go through adipogenesis and osteogenesis to generate fat cells and bone cells, respectively [36,37]. There are also examples of more dramatic dedifferentiation induction resulting in even pluripotency. A plethora of differentiated cell types can be induced to undergo an almost complete reprogramming through overexpression of a cocktail of transcriptiton factors, generating induced pluripotent stem cells (iPS cells) [38-42], which can be argued as the ultimate form of dedifferentiation by a broader definition, that is, a developmental event involving reduction in the molecular and/or functional properties of a differentiated cell type. According to this paradigm, cells might be "formatted" through dedifferentiation to a primitive status and then re-differentiate towards a new lineage to generate new types of cells. This lineage switch initiated through dedifferentiation makes it possible to use cell types that are relatively accessible and numerous in order to replace lost cell types that are scarcer and difficult to obtain through the progenitor cells of their own lineage. Given this possibility, some promising strategies might be conceived for the cell-based therapy for intractable diseases in certain organs and systems, such as the injured or degenerated central nervous system (CNS).

2. REGENERATION IN CNS: NEURAL STEM CELLS AND ADULT NEUROGENESIS

With respect to other organs, the CNS shows structural peculiarities, and owing to the relative lack of recovery from CNS injury, the dogmatic view of a "fixed, ended and immutable" neural tissue in mammals has been prevalent since the early 1900s [43-45]. The word "regeneration" in neuroscience was originally restricted to axonal regeneration by surviving cell bodies after injury [46-48]. Along with early emerging evidence of ongoing cell division in adult mammalian brain [49-51], technical advances such as the use of the S-phase marker Bromodeoxyuridine (BrdU) [52-54], the development of immunocytochemical reagents that could identify the phenotype of various neural cells [55-57], and more recently, the delicate manipulation of genetic methods for cell labeling and mutation, have led to an explosion of research in the field [58-60]. After Reynolds and Weiss showed in 1992 that precursor cells could be isolated from the forebrain and differentiate into neurons *in vitro* [61], neural stem cells (NSCs) have been characterized as self-renewing, proliferative and multipotent for the different neuroectodermal lineages of the CNS, including the multitude of neuronal and glial subtypes [62,63]. Since then, the meaning of the word "regeneration" in CNS could be extended from axonogenesis and synaptogenesis to the replacement of lost cells with newly generated elements coming from stem/progenitor cells, *i.e.*, adult neurogenesis. Such a possibility for cell renewal theoretically brings our nervous system into the context of regenerative medicine. However, before developing new strategies to figure out efficacious therapeutic approaches, it is a crucial point to determine how NSCs and adult neurogenesis provide the CNS with regenerative potential.

Adult neurogenesis is regulated by physiological and pathological activities at all levels, including the proliferation of adult neural stem cells or progenitors, differentiation and fate determination of progenitor cells, and the survival, maturation, and integration of newborn neurons. In normal conditions, adult neurogenesis has consistently been found to be restricted within two small germinal layer-derived areas, e.g., the Subventricular zone (SVZ) of the lateral ventricles and the Subgranular zone (SGZ) in the dentate gyrus of hippocampus [64-67]. In the remaining CNS parenchyma, local progenitor cells might support a "potential" neurogenesis, which in spite of their proliferative capacity and retention of potentialities *in vitro*, they do not perform neurogenesis *in vivo* [68,69]. Meanwhile, other studies believe that some local parenchymal progenitors actually sustain spontaneous neurogenesis *in vivo*. For example, in rodents and even some non-human primates, some newly generated neocortical neurons have been found [70-72], as well as some neurons of the piriform cortex originating from Ng^{2+} progenitor cells [73-75]. Thus, whether neurogenesis occurs in areas outside of the two widely accepted "neurogenic regions" remains controversial [76,77].

After unraveling the neurogenic potential in normal adult mammalian CNS, the question of how the neural stem/progenitor cells behave in different injury/pathological contexts need to be addressed. Although topographically restricted, neurogenic sites in mammalian

brain that are active throughout life can react to injury [78-81], and adult neurogenesis may also be substantially augmented in neurodegenerative diseases [82,83]. For instance, experiments carried out in rodent models of stroke revealed that reactive neurogenesis does occur from the SVZ, leading to increased cell production and migration of neuronal precursors to the lesion site [79,80]. In addition to reactive neurogenesis from stem cell-containing "neurogenic regions", accumulating evidence indicates that different paradigms of brain lesion can induce neurogenic events from the local progenitors resident in normally "non-neurogenic sites", including the neocortex [84-86], striatum [87-89], amygdale [90], hypothalamus [89,91,92], substantianigra [93,94] and brainstem [95-97]. For example, local progenitors that are in a relatively quiescent state in layer I of the rat cerebral cortex were activated after ischemia, giving rise to new cortical interneurons [86]. These examples support the hypothesis that the mature CNS parenchyma may retain a latent stem/progenitor cell potential that is normally inhibited *in vivo*, but that, if properly evoked, might be exploited *in situ* for cell replacement.

3. STRATEGIES AND CHALLENGES FOR NEURAL REGENERATION WITH STEM CELLS

Given their ability to generate neuronal and glial cells in response to damage, neural stem cells are believed to play a core role in cell-based therapy for various neurobiological disorders, ranging from acute injury such as brain trauma and stroke, to chronic neurodegenerative diseases including Alzheimer's Disease (AD) and Parkinson's Disease (PD), all of which are characterized by neuronal loss. As described above, it can be concluded that the brain has an endogenous regenerative potential and that in some pathological conditions, it becomes more permissive. Based on this point, one conceivable strategy for neural regeneration is to enhance the endogenous neurogenesis *in situ* [73,78,85,86,98,99]. The advantage of this approach is that it takes advantage of the intrinsic potential of endogenous neural stem/progenitor cells, and as a result, is less invasive and has fewer side effects in comparison with strategies relying on cellular transplantation [100-103], which will be discussed later. However, in most cases, such neurogenic potential cannot be utilized in a successful way. First, the magnitude of the neurogenic response to injury appears small, and it remains unclear as to what extent this is, because new neurons fail to develop at a sufficiently rapid rate versus cell death prior to sufficient integration into the host environment. In one case of brain injury, the great majority of the newborn cells survive < 1 month, and fail to replace lost neuronal populations and to restore damaged neuronal circuits [104]. Newly born neurons could replace only 0.2% of the dead striatal neurons in another rat cerebral ischemia model [79]. The exact mechanism for this overall inability of the endogenous stem cell compartment to promote full and long-lasting neural regeneration remains unclear. Recent data suggest that an altered neurogenic niche, including various overlapping local interactions between growth factors [105-108], extracellular proteins [109,110], metalloproteases [111-113], neurotransmitters [108,114,115], and angiogenesis [116-118], can be responsible for this failure. Even if enhanced cellular survival can be achieved, there are still significant impediments to neural maturation and integration, such as glial scar formation [119,120], cell death [121,122], inflammation [123,124] and aging [125, 126], all of which are topics of an open field of research. Although encouraging results from various experiments involving the administration of neurotrophin [127-129] or anti-inflammatory drugs [130-132] have shown some evidence that functional recovery is related to enhanced endogenous neurogenesis, the road ahead is still rocky and full of obstacles.

Paralleling this new understanding of endogenous neurogenesis, much progress has been made in the area of exogenous neuronal transplantation [100-103]. Early transplantation of embryonic midbrain tissue to the brain was first performed for PD and Huntington's disease (HD) in animal models as well as human clinical applications [133-136]. However, these experiments demonstrated a limited efficacy, along with other problems such as tissue availability and ethical questions. Today, it seems possible to achieve such therapeutic effects by using various sources of stem cells, due to their ability to replace the lost tissue as well as their "bystander" effects like neuroprotection and immunomodulation [101]. NSCs can be extracted directly from fetal or adult tissue via the dissection and digestion of CNS regions. In serum-free cultures with Epidermal growth factor (EGF) and Fibroblast growth factor 2 (FGF2), they can proliferate and spontaneously differentiate into both neuronal and glial cells after withdrawal of growth factors [61,137]. This possibility of stable expansion and *in vitro* differentiation into desired neural cells makes human NSCs an attractive cell source for transplantation strategies. Fetal-tissue-derived NSCs are the only source of stem-cell-derived neural cells that have entered the clinical arena for treatment of Neuronal ceroidlipofuscinosis (NCL, Batten's disease) [138] and Pelizaeus-Merzbacher disease (PMD). Application of oncogene-immortalized NSCs [139] are also approved in a phase I clinical trial in the United Kingdom for stroke therapy. Results so far have been favorable and encouraging. Autologous NPCs obtained at the site of focal damage would be an even more attractive option since they avoid immunogenicity, al

logenicity and ethical issues related to NSCs from other sources above [140]. The risk of tumor formation cannot be excluded and the long-term safety of such cells remains to be determined [141].

Embryonic stem cells (ESCs) are derived from blastocysts during the 16 cell stage and have an almost unlimited capacity to self-renew [142]. They can be expanded for many years and differentiated into neural stem or precursor cells and subsequently into brain cells, which makes them a feasible exogenous source [143-146]. On the other hand, ESCs also bear considerable teratogenic potential after implantation into host tissue, although protocols for inducing them into relatively pure differenttiated population before transplantation have been developed [147]. Immunosupression is also needed. In addition, immense ethical concerns exist regarding the use of human ESCs as well as government restrictions that continue to limit clinical applications [148].

The generation of iPS cells is considered the main breakthrough in regenerative medicine [38-42]. As mentioned in the first section in this article, these cells are reprogrammed via a thorough reversion from a terminal stageback to a pluripotent status, which can be considered a complete dedifferentiation. By re-differentiating along a neural lineage, such cells offer another autologous source that is ethically acceptable and eliminates the risk of immunological complications. However, these cells are also under risk of tumor formation, and safety cannot yet be guaranteed [149]. In addition, differences seem to remain between ESCs and iPS cells that render the differentiation of the latter cells into mature neurons much more difficult [150-152]. Therefore, their clinical application does not seem feasible in the near future. Most recently, induced neurons (iN) have been obtained by reprogramming adult somatic cells directly into mature neurons without the intermediate step of iPS cells [153-159]. Future studies are necessary to show whether it is possible to generate such specific neurons that are sufficiently mature for transplantation and also lack the risk of tumor formation.

Additionally, it has been shown that terminal neural differentiation can also be seen with non-CNS-derived multipotent somatic stem cells, such as mesenchymal stem cells (MSCs) [160,161], muscle stem cells (MuSCs) [162-164], placental cord blood stem cells [165,166], skin stem cells [167] and adipose derived stem cells [168,169]. These cells are relatively numerous and easy to collect from patients, presenting another autologous source without immune reaction. It is also possible that these stem cells provide trophic support to damaged neural tissue and as a result, enhances the endogenous approach [170]. However, the proof of functional neurons derived from MSCs has not been provided. Although there are already some ongoing clinical trials that show some possible clinical improvement [171], many questions concerning how to enhance their survival and the potential of neural differentiation remain to be addressed.

In summary, two main therapeutic strategies have been developed in neural regeneration. Exploring the potentialities of resident, endogenous adult stem/progenitor cells is an ideal approach for the future. In parallel, an intense effort has been made to produce stem/progenitor cells that could be used as transplantation tools so as to replace lost elements in pathologies. Immune reaction and ethical controversy are the primary issues related to the allogeneic approach of mainly utilizing embryonic/fetal oriented cells, while the powerful iPS cells cannot avoid the risk of tumor formation and genetic instability. Figuring out how to enhance the survival, migration and neural differentiation potential of non-neural somatic stem cells will be the problem that needs to be resolved before these accessible autologous resources can be clinically applied.

4. DEDIFFERENTIATED SOMATIC STEM CELLS, A BETTER SOLUSTION?

Addressing the last point summarized above, we here introduce two lines of evidence that show dedifferentiation might contribute to the resolution. One is from our work that focuses on muscle stem cells (MuSCs), and the other one published most recently is related to mesenchymal stem cells (MSCs) [172]. They start from distinct approaches of induction of dedifferentiation, but they arrive at the same conclusion that dedifferentiated MuSCs or MSCs present improved neural regenerative potential.

4.1. Dedifferentiation-Reprogrammed MSCs

Hsiao Chang Chan and his group used a culture induction to perform dedifferentiation [172]. After establishing monoclonal MSC clones from primary rat bone marrow MSCs, they first initiated neuronal differentiation by transferring the clones into neuronal induction media and then returned them to stem cell characteristics by withdrawal of the induction media and reincubation in serum. These cells are considered dedifferentiated MSCs (De-MSCs). First, compared with uncommitted MSCs, De-MSCs are demonstrated to represent a previously undescribed distinct population of stem cells with several distinguishing features. Apart from the morphological and phenotypical similarity and the potential for multilineage differentiation into osteoblasts, adipocytes and chondrocytes, De-MSCs exhibit a predisposition to the neuronal lineages as demonstrated by both genetic and functional assays. Global gene expression profiling and PCR data show that dedifferentiated cells express in-

creased levels of both neurogenesis-related genes and growth factors. The increase of nestin- and musashi-positive cells in De-MSCs suggests that these cells carry additional neuronal potentiality that is ready to be activated under appropriate conditions, which is representative of an immature neural phenotype, most likely neural stem/progenitor cells. Taken together, De-MSCs appear to represent a distinct population of stem cells with a higher potential for re-differentiation into neurons compared to their original counterparts.

Next, they asked the question of whether or not De-MSCs have significant advantages over undifferentiated MSCs with respect to proliferation and survival. Indeed, proliferating cellular nuclear antigen (PCNA) staining confirmed that De-MSCs proliferated vigorously at 24 hours after dedifferentiation occurred and indicated this might be a result of acute reentry into the cell cycle. De-MSCs also exhibited a survival advantage over undifferentiated MSCs under conditions of hydrogen peroxide (H_2O_2) oxidative stress, as demonstrated by FACS sorting analysis of Annexin-V/propidium iodide staining after H_2O_2 treatment. More importantly, they have found that De-MSCs maintained their anti-apoptotic properties after *in vitro* culture and passaging. Increased expression of bcl-2 family proteins was observed and appeared to play a role in the anti-apoptotic action. All of these results explained the observation of an increase in viable cells in De-MSCs compared to the uncommitted MSCs during the *in vitro* differentiation and dedifferentiation process and demonstrated that De-MSCs are advanced in cell survival and proliferation.

They went further to demonstrate the therapeutic advantage of De-MSCs *in vivo* in a rat model of neonatal hypoxic-ischemic brain damage (HIBD) via lateral ventricular transplantation of fluorescent cells isolated from GFP-transgenic animal. On day 7, GFP expression could only be detected in De-MSCs group, indicating improved cell survival. Moreover, a number of the surviving GFP-De-MSCs were found outside of the injection site, indicating migration of the cells. Immunostaining revealed that some GFP-positive De-MSCs expressed differentiated neuronal markers NF-M or MAP2, indicating neuronal differentiation from the De-MSCs *in vivo*. Of note, they also showed that the better survival of De-MSCs might lie in their greater ability to promote angiogenesis in the ischemic region. Finally, shuttle box tests confirmed a more significant improvement of functional recovery of HIBD animals after De-MSCs treatment. Taken together, these results indicated that De-MSCs had survival and neuronal differentiation advantages over undifferentiated MSCs under both *in vitro* and *in vivo* conditions. This makes them a promising cellular source in therapeutic strategies based on autologous transplantation for neural regeneration.

4.2. Update Study of Muscle Cell Dedifferentiation for Neurogenic Differentiation

As mentioned at the beginning of our discussion on dedifferentiation, various studies in the amphibian limb regeneration field have demonstrated that dedifferentiation plays the core role by which the multipotent stem cells are generated via the formation of the "blastema" that consequently regenerates the entire limb [10-17]. However, the occurrence of this process in mammalian skeletal muscle has been questioned, partially due to the contamination of other endogenous progenitor cells which might not be excluded using regular cell isolation techniques, leaving the possibility that they are the source of dedifferentiation rather than the terminally differentiated cells [173,174]. Therefore, as recently reported, we developed a conditional transgenic model based on cre/lox-β-galactosidase (gal) system to specifically and effectively isolate differentiated myofibers both *in vitro* and *in vivo* to obtain the purified source [34]. Using this model, we have successfully determined the superior myogenesis potential of the injury-induced dedifferentiated muscle stem cells (De-MuSCs) that were dedifferentiated from β-gal positive multinuclear myofibers in comparison with the non-injury counterpart. Moreover, some β-gal and CD31 (a marker for endothelial cells) dual positive signals were also found in the blood vasculation, raising the question of whether these De-MuSCs-could advantageously contribute to differentiation down other lineages, such as neurogenesis [34]. In the present experiment, we explored further with our previous novel cre/lox model in order to address this question.

De-MuSCs were obtained as previously described [34]. Briefly, Muscle Creatine Kinase-cre muscle derived cells (MCK-cre MDCs) and ROSA-lox-β-gal MDCs were implanted into the gastrocnemius (GM) muscles of SCID mice via intramuscular injection of equal populations. Three weeks later, a laceration injury was created at the cell implantation site in the GMs. Four days after injury, β-gal positive cells were isolated by flow cytometry. The pre-plate technique was then applied to isolate and expand the β-gal positive slow adhering cells (*i.e.*, PP5 and PP6), which were convectively demonstrated to be De-MuSCs. We used primary mouse myoblasts, a muscle progenitor cell, as the control counterpart.

After isolation, both cell lines were kept in muscle cell growth media for one week before being transferred into NSC media for the induction of neurosphere proliferation. For further neural differentiation analysis, single cells were transferred into neural differentiation media. Cultured in NSC media, De-MuSCs successfully presented neural stem/progenitor characteristics. By day 3 in NSC

media, some De-MuSCs had aggregated to form neurosphere-like structures that floated in suspension (**Figure 1(c)**), a hallmark of the structure of NSCs or neural progenitor cells, while control cells showed no signs of forming these special structures (**Figure 1(a)**). By day 6, the majority of the De-MuSCs were floating as spheres (**Figure 1(d)**), while the control cells still retained muscle cell morphology and stayed attached to the flask (**Figure 1(b)**).

The phenotype of the cells within the De-MuSCs derived neurosphere was analyzed by immunocytochemistry with typical neural markers. These spheres stained positive for Nestin, a marker for neural progenitor/stem cells, as well as the markers for more mature neural lineage cells: Glial fibrilllary acidic protein (GFAP) for astrocytes, CNPase for oligodentrocytes and Neurofilament (NFm) for neurons. EdU was detected in a select group of cells in each neurosphere (**Figures 2(a)-(d)**), indicating the proliferative status of these cells when cultured with mitotic reagents EGF and bFGF.

RT-PCR was performed on De-MuSCs and myoblasts, under both non-induced and NSC medium-induced conditions to detect changes that occurred on a transcriptional level. Within the non-induced group, the control cells had higher mRNA levels of myogenin compared to the De-MuSCs (**Figure 3**). In the induced group, both the control cells and De-MuSCs had lost myogenin mRNA expression (**Figure 3**). Meanwhile, nestin expression increased significantly for control cells and only slightly for MuSCs after NSC media induction (**Figure 3**). The expression of the stem cell marker Sca-1, which was absent in control cells and low in De-MuSCs at the beginning,

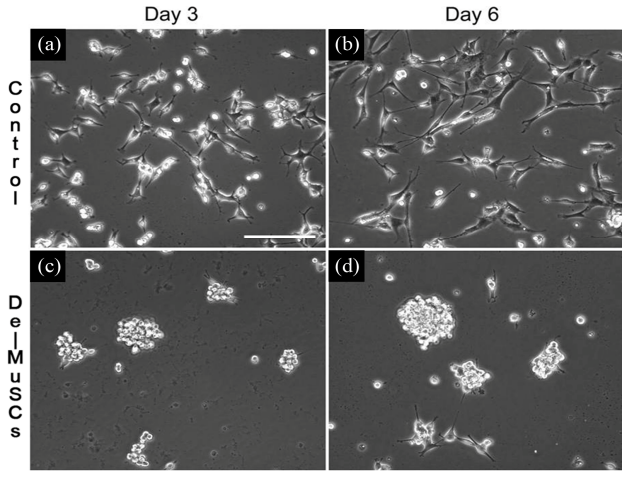


Figure 1. Results show that De-MuSCs Commit to Neural Lineage. Neurosphere-like structures were formed by De-MuSCs cultured in NSC proliferative media. De-MuSCs began to aggregate on day 3 (c) and presented morphology and architecture resembling that of neurosphere by day 6 (d), while the control primary myoblasts showed no signs of forming these structures (a)-(b) during these time period (3 and 6 days).

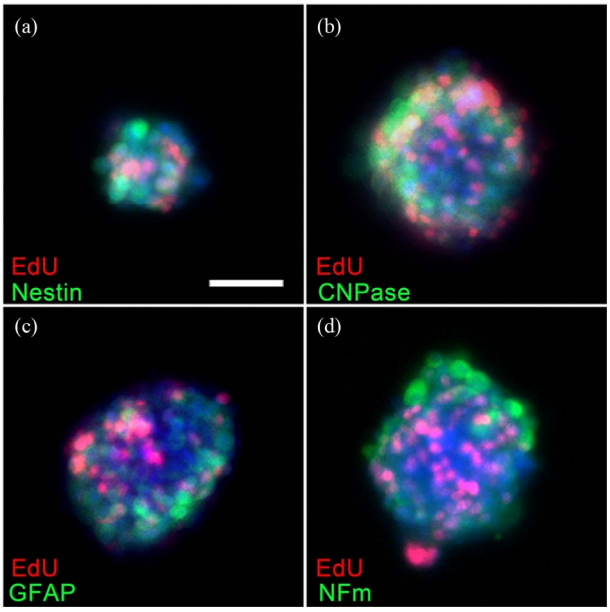


Figure 2. Immunocytochemistry showed the neural phenotype and proliferative status of the cells in the spheres that derived from De-MuSCs. On the fifth day after culturing in the NSC medium, these spheres stained positive for Nestin, a marker of neural progenitor/stem cells (a), as well as for other markers of mature neural cells, such as GFAP, NFm and CNPase (b)-(d). Mitosis assay performed 2 hours after EdU administration demonstrated the proliferative status of the cells in these spheres (a)-(d).

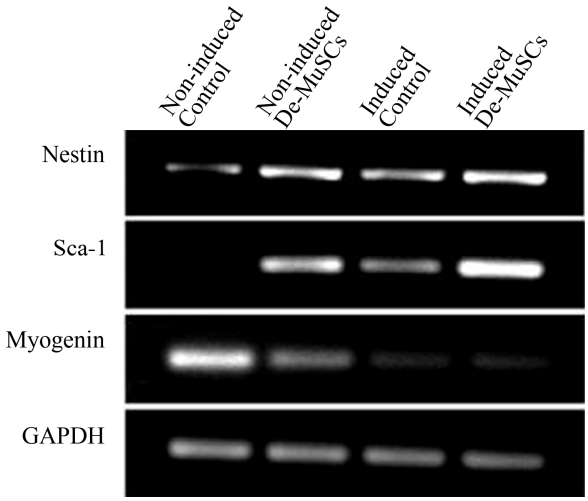


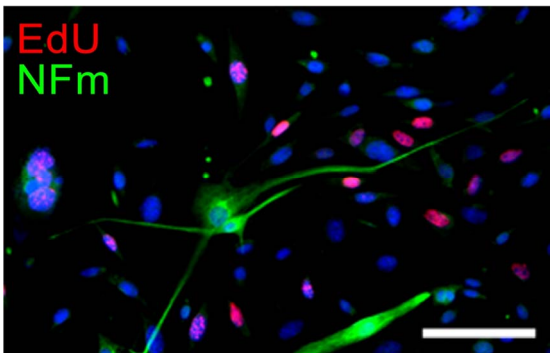
Figure 3. RT-PCR explored the alteration of the cell profile during culture with NSC medium. At the transcriptional level, the stem cell related markers Nestin and Sca-1 increased in both control cells and De-MuSCs after induction, although the De-MuSCs maintained a greater level. Meanwhile, the expression of Myogenin gradually decreased.

increased for both cell types after NSC medium induction (**Figure 3**).

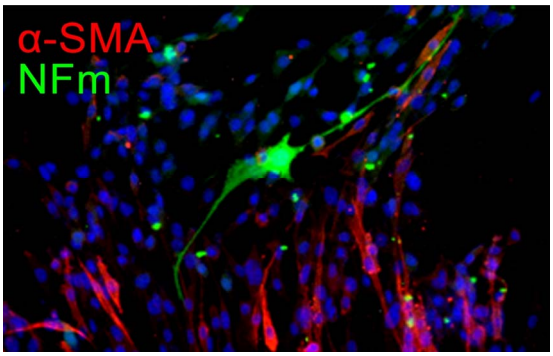
As for neural differentiation (ND) induction, the De-MuSCs but not the myoblasts successfully differentiated

into neural lineage cells. After 3 days in ND media, cells which were solely positive for NFM and possessed long, thin projections that resembled neuronal processes were observed. These cells were EdU negative, indicating their postmitotic status (**Figure 4(a)**). After 8 days, although some of the cells were positive for α-smooth muscle actin (α-SMA), which might count for myofibroblast differentiation, the NFM positive cells were still detectable (**Figure 4(b)**). Meanwhile, other glial markers such as GFAP, CNPase were also positive in a subgroup of the cells, further suggesting the multiple potential of the De-MuSCs for neural differentiation.

In summary, our results potentially showed that De-MuSCs successfully formed neurosphere-like structures that contained neural stem/progenitor cells within NSC medium culture. RT-PCR confirmed that they were endowed with the capacity of differentiating along the neural lineage while they gradually lost myogenic potential. After being transferred into ND medium, De-MuSCs presented with neuronal morphology and immunophenotype, confirming they became terminally differentiated neuron-like cells. Since our data implies that De-MuSCs have the potential to commit to the neural lineage, they may be able to aid recovery from neurological diseases by providing an easily accessible cell source for neural regeneration.



(a)



(b)

Figure 4. De-MuSCs differentiated into neuron-like cells after induction within ND medium. After 3 days in ND medium culture, some cells which were solely positive for NFm and possessed long, thin projections that resembled neuronal processes were observed. These cells were EdU negative, indicating their post-mitotic status (a); Eight days later, some of the cells began expressing for α-smooth muscle actin (α-SMA), while there still scattered some NFm positive cells reminded scattered among the population (b).

Taken together, the two studies above indicate that through various approaches, lineage committed somatic stem cells can be further dedifferentiated to a more primitive status, such as De-MSCs and De-MuSCs, which appear to have survival, proliferation, migration and neuronal differentiation advantages over their original counterparts under both *in vitro* and *in vivo* conditions. Because of the aforementioned characteristics as well as their accessibility, substantial population, and autologous orientation, they provide hope for finding a novel treatment strategy with improved therapeutic efficacy.

5. CONCLUSION

Regenerative medicine carries the responsibility of tackling various neurological diseases with limited treatment efficacies. Stem cell-based therapy can restore neural function by either enhancing the endogenous neurogenesis that is normally quiescent or replacing the lost cellular elements via exogenous transplantation of stem cell-derived cells. Dedifferentiation, as one significant approach involved in naturally occurring regeneration and experimental reprogramming, gives us more insight into these strategies. Among a variety of theoretically available cellular sources, dedifferentiated somatic stem cells with enhanced survival, proliferation, migration and neural differentiation in addition to easy accessibility and low tendency of tumor formation may offer an optimal solution. We introduced a recent study that depicted the advantages of De-MSCs along with our preliminary results that showed the remarkable superiority of De-MuSCs in neural regeneration. The detailed characterization of this novel stem cell population and the precise mechanism behind their beneficial effects still remain unclear, so further investigation must be conducted before the benefits of these stem cells can be clinically applied. However, the insight into dedifferentiation that we have already gained will bring us one step closer to finding effective bedside treatments for neurological disease.

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