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Therapeutic Advancement in Neuronal Transdifferentiation of Mesenchymal Stromal Cells for Neurological Disorders

Princy Choudhary[1] · Ayushi Gupta[1] · Sangeeta Singh[1]

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Abstract

Neurodegenerative disorders have become the leading cause of chronic pain and death. Treatments available are not sufficient to help the patients as they only alleviate the symptoms and not the cause. In this regard, stem cells therapy has emerged as an upcoming option for the replacement of dead and damaged neurons. Stem cells, in general, are characterized as cells exhibiting potency properties, i.e., on being subjected to specific conditions they transform into cells of another lineage. Of all the types, mesenchymal stem cells (MSCs) are known for their pluripotent nature without the obstacle of ethical concern surrounding the procurement of other cell types. Although fibroblasts are quite similar to MSCs morphologically, certain markers like CD73, CD 90 are specific to MSCs, making both the cell types distinguishable from each other. This is implemented while procuring MSCs from a plethora of sources like umbilical cord blood, adipose tissue, bone marrow, etc. Among these, bone marrow MSCs are the most widely used type for neural regeneration. Neural regeneration is achieved via transdifferentiation. Several studies have either transplanted the stem cells into rodent models or have carried out transdifferentiation in vitro. The process involves a combination of growth factors, pre-treatment factors, and neuronal differentiation inducing mediums. The results obtained are characterized by neuron-like morphology, expression of markers, along with electrophysical activity in some. Recent attempts involve exploring biomaterials that may mimic the native ECM and therefore can be directly introduced at the site of interest. The review gives a brief description of MSCs, their sources and markers, and the different attempts that have been made towards achieving the goal of differentiating MSCs into neurons.

Keywords Stem cells · Mesenchymal stem cells · Neuronal cells · Transdifferentiation

Introduction

Brain and spinal disease, especially neurodegenerative disorders, affect millions of people worldwide. Furthermore, spinal cord injury caused due to accidents poses a major problem globally. The symptoms that are presented in such patients are treatable but the disease itself is incurable. In such cases, the dopamine-producing neurons play a crucial role in the nervous system as dopamine communicates with that part of the brain which controls the movement of the body. In patients suffering from Parkinson's disease, there is an inadequate production of dopamine, as the cells producing the same are destroyed in the wake of the disease. As a result of this, the patient experiences muscle rigidity and tremors, which in turn slows down their movement. In addition to this, in MND, 'motor neurons' which relay signals from the brain to muscles in the body to control movement, are affected, which leads to progressive paralysis, resulting in the patients suffering from a variety of problems such as uncontrolled twitching, muscle stiffness, difficulty in speaking, swallowing, and even breathing. A certain amount of relief can be given to the patients with the help of a combination of certain drugs, physiotherapy, a healthy diet, and exercise. Unfortunately, these treatments relieve the symptoms but are unable to reverse the damage that has been done to these nerve cells.

Stem cells provide a very alluring method that can result in the regeneration of cells leading to cell therapy. In cases of neurodegenerative diseases, the mesenchymal stem cells are the major type that can be developed to form distinct types of neurons. These include the peptidergic neurons, dopaminergic neurons, and the cholinergic neurons (Takeda and Xu 2015; Ye et al. 2016). The mesenchymal stem cells, which are procured from the bone marrow, are known to sustain while residing within the damaged brain and spinal cord tissue. Subsequently, the MSCs tend to divide, migrate, and transform into precursors of neurons. These precursors take over

✉ Sangeeta Singh
sangeeta@iiita.ac.in

[1] Applied Science Department, Indian Institute of Information Technology, Allahabad, UP, India

the function of damaged neurons, efficiently improving the neurological state of the patients (Fairbairn 2015; Zheng et al. 2017b; Ren et al. 2018; Alshawaf et al. 2018). Several studies have shown differentiation of BM-MSCs into neural-like modality constructing a network of connection and expression of neural markers (Xu et al. 2020). This helps in enhancing neural regeneration considerably.

The study of neural regeneration has now moved on to the application part of the process, where partial successful attempts have been made in terms of peripheral nerve regeneration and functional recovery (Zheng et al. 2017a). Results have also confirmed successful transplantation of differentiated cholinergic neurons in rat models for treatment of sciatic nerve defects (Jang et al. 2018), and amyotrophic lateral sclerosis (Ciervo et al. 2017).

The major effect of the decades of study conducted on stem cells, especially on neural cell construction have led to the development of pathways where stem cells are used for spinal cord reconstruction along with neural regeneration, remyelination, neural protection, and replacement of neural cells that have been lost or damaged due to an injury or disease (Lu 2017).

Mesenchymal Stem Cells: The Distinct Preference for Differentiation into Neuronal Lineage

The International Society for Cellular Therapy (ISCT) termed MSCs as "multipotent mesenchymal stromal cells." MSCs have been proven to have a wide range of differentiation potential and known to develop into all three germ layer cells. For mesodermal origin cells, MSCs transform into chondrocytes, adipocytes, and osteoblasts. Under very specific conditions, the MSCs are known to mature into cells of ectodermal and endodermal origin like retinal pigment epithelium, skin, lungs, hepatocytes, renal tubular cells, pancreatic islets, sebaceous duct cells, and neural cells (Kobolak et al. 2016). The in vitro culture of MSCs shows genomic stability over several passages and negligible occurrence of ethical issues (Ullah et al. 2015; Kobolak et al. 2016). MSCs are known to possess low immunogenicity and therefore have the ability to function as universal donor stem cells. The hypoimmunogenic nature of MSCs is due to the expression of low levels of human leukocyte antigen (HLA) class I, which are responsible for protecting the cells against natural killer (NK) cell-mediated cytotoxicity. The lack of expression of HLA-DR enables them to escape the nature of immune surveillance (Rawat et al. 2019). Morphologically, fibroblasts are quite similar to mesenchymal stem cells, and certain markers such as CD73, CD 90, and more can be used to distinguish between them. Different characteristic markers of MSCs are described in Table 1.

This knowledge becomes crucial in procurement of stem cells from various sources such as umbilical cord blood, adipose tissue, dental pulp, and bone marrow. Advantages and limitations of MSCs from these sources is listed in Table 2.

The MSCs exist almost in each and every type of tissue. They are easily extracted, and can differentiate into almost any type of end-stage lineage cells. It has been observed that MSCs express neuronal markers and those markers associated with astrocytes (Han et al. 2019). Stem cells, in general, are known to simply change their morphology under DMSO/BHA (neural inducers) treatment that promotes retraction of cell margins and morphological changes to attain a stellate pseudo neural appearance. In the case of MSCs, even a slight chemical manipulation is able to induce the expression of markers that are neuron-specific such as GFAP, NSE, NF-200, Tau, and NeuN (Bertani 2005).

In contrast to MSCs, the neural stem cells show that there is only limited ability for the NSCs to differentiate into neuroglia when placed in an adult mammalian brain and are provided with suitable conditions to grow. Therefore, MSCs are preferred as they are the adult stem cells from mesoderm that can easily differentiate neuronal and glial cells when treated with various growth inducers. The MSCs are also known to exert autocrine and paracrine effects in order to replace the genes and proteins that are responsible for different neurodegenerative disorders for the improperly functioning neuroglia (George et al. 2019).

Although recent developments have not reached to such a point where the MSCs can totally replace the damaged neurons, they can still initiate angiogenesis and therefore help in the migration of the neurogenic cells of the host to the site of damage in the central nervous system. Several other characteristics of the MSCs have led to the inference that MSCs are clearly the better choice for initiating the process of transdifferentiation into neural cells. These include their allogenicity that has allowed easy transplantation and migration of cells to the site of injury (Castorina et al. 2015). Although MSCs have come up as the clear choice for tissue regeneration into all the three germ layers, special references have been used in case of neural tissue regeneration where the native neurons were damaged as a result of oxidative stress and consequent telomere shortening. This is due to the fact that the MSCs are involved in paracrine secretion that protects the cells from oxidation and apoptosis (Castorina et al. 2015; Vono et al. 2018).

Transdifferentiation of MSCs into Neurons: History and Progress

Transdifferentiation of MSCs into neurons is a topic that has been sought after since the discovery of the fact that MSCs can be differentiated into cells of all three germ layers. Several

Table 1 Characteristic markers of MSCs

Marker	Chemical name	Expressed in	Features	References
CD73	Ecto-5′-nucleotidase	Smooth muscle cells, lymphocytes, epithelial cells, endothelial cells, fibroblasts	Monoclonal antibodies SH3 and SH4 (anti-CD73) has specificity for MSCs, which implies MSCs express a CD73 molecule that is different from other cell types	(Lin et al. 2013)
CD90 (Thy1)	Glycosylphosphatidylinositol-linked protein	Lymphocytes, fibroblasts, endothelial cells (vascular and lymphatic), hematopoietic stem cells, and neurons	The function of CD90 is evaluated by hindering the expression of the marker through the application of CD90-targeted hairpin RNA lentiviral vectors and the inhibition causes in vitro differentiation of MSCs into adipogenic and osteogenic cell line lineages	(Lin et al. 2013; Moraes et al. 2016)
CD105 (Endoglin)	Type I membrane glycoprotein	Vascular endothelial cells, syncytiotrophoblasts (high) and monocytes, fibroblasts, chondrocytes, and hematopoietic progenitor cells (low)	CD105 expression shown to have a positive influence on hMSC (human mesenchymal stem cells) cardiac regenerative potential	(Kleinsorge et al. 2013; Lin et al. 2013)
CD34	Transmembrane phospho-glycoprotein	First recognized in the hematopoietic stem as well as hematopoietic progenitor cells (HSC and HPC)	CD34 marker is expressed in MSCs that are present in vivo. While the MSCs procured and further cultured showed negative expression of CD34	(Simmons et al. 1992; Satterthwaite et al. 1992; Sidney et al. 2014)
Stro-1	Anti-CD 34^+ MSC monoclonal antibody	Stromal elements in hBM-MSCs and early adipocytes	• Stro–1 mesenchymal precursor cells (MPC) possess excellent immuno-modulatory features and have the capability to invade and diffuse in various types of tissues • also known to assist in the process of hematopoiesis • It is also recognized as a highly distinct marker for BM CFU-F	(Gonçalves et al. 2006; Nasef et al. 2009; Lin et al. 2011; Fitter et al. 2017)
Stage-specific embryonic antigen-4 (SSEA-4)	A sialic acid-containing glycolipid	Pluripotent stem cells, human umbilical cord mesenchymal stem cells (hUC-MSCs), cancer cells	• Standard MSCs markers viz. CD105, CD90, CD73, and CD29 and pluripotent SC markers viz. Nanog, Oct4, and Sox2 were both expressed by SSEA-4^+ cells • These cells differentiate into cells that have their origin in all the three germ layers without the obstacle of teratoma formation	(Li et al. 2017)
MSC antigen 1 (MSCA-1)	Tissue-nonspecific alkaline phosphatase (TNAP)	Human adult MSCs including dental pulp, jaw periosteum, Wharton jelly and heart	• involved in a lot of MSCs features like cell differentiation, immunomodulatory properties, adipocyte, and neuron differentiation	(Diaz-Hernandez et al. 2015; Estève et al. 2016)
CD271/NGFR	p75NTR (neurotrophin receptor)	Particularly expressed by BM – MSCs. Also, by, adipose tissue as well as in trabecular bone cavity and dermis	• associated with the tumor necrosis factor superfamily • It is an optimum marker that refers to the presence of human BM-MSCs	(Quirici et al. 2010; Cuevas-Diaz Duran et al. 2013; Álvarez-Viejo 2015)
CD146 (cell adhesion molecule (MCAM, MelCAM)	Cell-surface glycoprotein Muc18	Endothelial cells	Its expression enhances the process of angiogenesis and interaction of the cells with the matrix	(Kasten et al. 2008)

Table 2 Comparison of MSCs sources and their characteristics

Source	Advantages	Limitations	References
Bone marrow	• Can differentiate into neuronal progenitor cells and other neuronal cells Differentiation to dopaminergic neurons has also been reported • Acts an accessible cellular reservoir for the treatment of a variety of neurologic diseases • It is most extensively investigated stem cell source and is considered to be the gold standard	• Procurement of cells is often painful and carries risk of infection • Differentiation potential and cell yield depends on donor attributes (e.g. age) • 0.001–0.01%) of the harvested bone marrow cells are MSCs	(Woodbury et al. 2000; Kim et al. 2002; Jin et al. 2003; Abouelfetouh et al. 2004; Tropel et al. 2006; Nandy et al. 2014; Berebichez-Fridman and Montero-Olvera 2018)
Adipose tissue	• Successfully implemented in peripheral nerve regeneration • Potential to differentiate into cholinergic and dopaminergic neurons	• It has lower chondrogenic and osteogenic differentiation potential compared to BM-MSCs	(Moon et al. 2018; Marei et al. 2018; lo Furno et al. 2018; Berebichez-Fridman and Montero-Olvera 2018)
Dental MSCs	• Can be procured during dental surgeries or even from decayed tooth • Can be obtained from different dental tissues, viz. follicle, papilla, pulp • Can differentiate into cholinergic neurons and other neuronal cells	• Procurement of the cells can be difficult or invasive	(Ullah et al. 2016; Geng et al. 2017; Kang et al. 2019)
Umbilical cord blood MSCs	• No invasive process required • No ethical issues involved • Immunosuppressive potential • It does not age over passages (i.e., senescence)	• Although various clinical trials are ongoing, there is no clear report regarding the long-term safety of hUCB-MSCs in humans	(Divya et al. 2012; Nan et al. 2016; Lee 2018; Berebichez-Fridman and Montero-Olvera 2018)
Wharton's jelly	• Capable of transplantation without rejection • Induced better neural differentiation and neural cell migration than BM-MSCs • Potentially used in MI treatment and peripheral nerve regeneration	• In terms of osteogenic potential, this source is not as useful as BM, blood or liver	(Hsieh et al. 2013; Berebichez-Fridman and Montero-Olvera 2018)
Skin	• Have a high proliferation rate • Can induce prominent nerve regeneration and can differentiate into neuronal lineages as Schwann cells	• Invasive procedure is involved for procurement • Safety and effectiveness of this source of MSCs has yet to be evaluated by clinical trials	(Park et al. 2012; Saulite et al. 2018; Berebichez-Fridman and Montero-Olvera 2018)
Amniotic fluid and placenta	• No invasive process required • No ethical issues involved • They produce immunosuppressive factors • Potential treatment for nerve injuries and neurodegenerative diseases	• Various donor-specific factors required in AF stem cell therapy have yet to be determined • Therapy needs use of xenogeneic reagents that makes clinical use quite challenging	(Kunisaki 2018; Berebichez-Fridman and Montero-Olvera 2018)
Muscle	• Muscle-MSCs can be obtained from virtually any muscle in the body • Used in promoting peripheral nerve regeneration	• Invasive procedures involved for cell procurement	(Berebichez-Fridman and Montero-Olvera 2018)

attempts have been made to develop a conclusive process for obtaining the same. Over the years, various endeavors have also indulged in the differentiation of MSCs into neurons glial tissues. The progress in achieving the same has been continuous, with every experiment acting as a step further in achieving the goal.

Following are the endeavors that have taken place in the decades following the discovery of the fact that neural transdifferentiation can occur from MSCs.

From 1990 to 2000

The step–wise procedure involving transdifferentiation of MSCs into neuronal lineage started with the discovery of Nestin as the gene or marker that governs the development for the next generation of cell types of the brain (Lendahl et al. 1990). Keeping the involvement of Nestin in deciding the type of cell, CNS progenitor cells were applied with bFGF-induced medium, exogenously. It was observed that two progenitor cells types were developed. The first type gave rise to cells showing similar morphological and antigenic properties as shown by neurons and astrocytes. However, the second type of cells generated showed only neural characteristics. The progress in generating neural-like properties in progenitor cells shows that regulations of growth factors and regulatory genes play a major role in defining the neuronal regeneration (Vescovi et al. 1993). In addition to these factors, the usage of 2–mercaptophenol in the culture medium increases the chances of neurite outgrowth (Ishii et al. 1993).

The summation of the above discoveries was applied to the cells that were isolated from the adult rat hippocampus in the presence of FGF–2. The resultant cells expressed neuronal and glial markers. When maintained for 1 year and transplanted into adult rat hippocampus, the cells were found to differentiate into neurons (Gage et al. 1995). The earliest reference of development of neuron-like cells is when genetically marked donor marrow cells were transplanted into adult female mice; some astroglia and microglia cells emerged from a precursor, which is a usual component of adult bone marrow (Eglitis and Mezey 1997).

MSCs from wild-type mice were systemically infused into irradiated 3-week-old mice and the donor DNA was observed in various tissues including the brain (Pereira et al. 1998). Later on, it was observed that upon direct injection of human BM-MSCs into the rat brain, a relatively large recovery of almost 20% of the infused cells could be achieved. It can be inferred by this that the MSCs possess a high proliferation potential even when subjected to a host environment (Azizi et al. 1998).

NSCs isolated from the human fetal telencephalon were transplanted into the germinal zones of the newborn mouse. These cells were later observed to migrate to the established pathways of the central nervous systems. It was seen that the transplanted cells were tending to replace the specific deficient neuronal populations pointing to the fact that these cells may further help to elaborate the process of normal neuronal development (Flax et al. 1998).

After this, several attempts of neurotransplantation have been made where the procedure involved direct injection of MSCs into the rat brain's corpus striatum. When sections of the brain were taken 5–72 days postinjection, it could be observed that the cells migrated from the site of injection to the successive layers of the brain along the established paths of migration (Azizi et al. 1998). The migration of MSCs was achieved, all through the forebrain and cerebellum by injecting murine MSCs into the lateral ventricle of neonatal mice. The injected MSCs were found to mimic the neural progenitors. The cells further differentiated into astrocytes and possibly in neurons, too. A population of donor-derived cells was detected in brain and neural phenotypes. Even when MSCs were injected into the lateral ventricle of the neonatal mice, the resultant cells were seen to migrate to the cerebellum and the forebrain. In these cases, the major positive point was that there was no detection of tumor formation because of the injected cells (Kopen et al. 1999).

Subsequent studies showed that the generation of neural phenotypes and cells expressing neuronal gene products (NeuN, 200-kiloDalton neurofilament, and class III β-tubulin) were present in adult mouse brain 1–6 months post bone marrow transplant (Brazelton 2000). The marrow cells were also found to express neuron-specific antigens after differentiation and migration to the brain (Mezey 2000).

Under specific conditions, mouse and human BM-MSCs, when treated with EGF or BDNF in the culture showed positive expression of a neuron-specific nuclear protein (NeuN), Nestin, and Glial fibrillary acidic protein (GFAP). Limited cases of BM-MSC-derived cells were also known to differentiate into neuron-like cells with expression of glial cells and NeuN, with positive levels of GFAP (Sanchez-Ramos et al. 2000).

The first major breakthrough was the development of adult rat stromal cells into neural precursor cells using the neural inducing growth factors. The results showed expression of neural phenotype. The cells formed showed positive expression of NSE (neuron-specific enolase), NeuN, neurofilament – M (NF-M) and Tau. Nestin, a characteristic of neural precursor cells, was also expressed during the initial days of neural induction. Later stages showed minimal or no detection of the precursor marker, suggesting further development of the precursor cells (Woodbury et al. 2000).

From 2001 to 2010

The peripheral mesenchymal cells were developed into neurons through an in vitro culture. The cultured human and rat BM-MSCs, under specific conditions, lead to 80% of cells

expressing nerve growth factor (bNGF) and neuron-specific enolase (NSE). In such cases, Retinoic acid (RA) and neurofilament-M (NF-M) were known to serve as a potent enhancer for neural differentiation and were recommended for transformation of human ES cells into a potent unlimited cell source for neurons (Schuldiner et al. 2001).

In cases where hMSCs were treated with a specific composition of FGF and RA, retinoic acid led to the initiation of differentiation of cells into potential neurons (Kim et al. 2002).

Various growth factors have been recognized as agents that can bring BM-MSCs towards neuronal phenotypes. However, the features and expressions related to neuronal proteins or neurotransmitters may not get equalized with the potential to attain usual neuronal functions (Jin et al. 2003).

Cultured adult green fluorescent protein (GFP)-transgenic mice MSCs in the presence of hippocampal slice, mandates contact with the host brain tissue for differentiation of marrow stromal cells into neurons (Abouelfetouh et al. 2004). The presence of retinoic acid was seen to help in enhancing the number of differentiated cells and synaptic transmission (Abouelfetouh et al. 2004; Cho et al. 2005). On the other hand, the differentiation of MSCs into neurons was also accompanied by the formation of several types of neuronal supporting cells like Schwann cells (Keilhoff et al. 2006; Caddick et al. 2006; Yang et al. 2008).

Various techniques like culture surface modification (Qian and Saltzman 2004), stimulation with various factors like interleukin (IL)-1α (Cho et al. 2005), retinoic acid, synthetic nanostructures (Yim et al. 2007), cocktail of induction agents (Greco et al. 2007), epidermal and basic fibroblast growth factors (EGF-bFGF) (Delcroix et al. 2010), recombinant human erythropoietin [rhEPO] (Koh et al. 2009), extracellular matrix (ECM) proteins—fibronectin, laminin-, laminin-10/11,collagen-1, collagen-IV (Mruthyunjaya et al. 2010), astrocyte-derived soluble factor (Oh et al. 2009) were implemented to effectively accomplish neuronal differentiation or enhancement of neurotrophic factors' production. Increased expression of neurotransmitters and neuronal markers like class III β-tubulin, NF-L (neurofilament- light, or neurofilament 70 kDa) (Tropel et al. 2006), microtubule-associated protein 2 (MAP2) (Yim et al. 2007), transcription factors (Greco et al. 2007), Schwann cell markers S100, P75, and GFAP (Caddick et al. 2006; Yang et al. 2008), Nestin, Ngn2, Pax6, neurotrophin receptor tyrosine kinase1 and kinase3 have been reported.

The adult human BM-MSCs were induced to transform into dopamine neurons (DA) in an in vitro culture by using a cocktail of factors like sonic hedgehog and fibroblast growth factors. Electrophysiological studies revealed that the formed DA cells were actually DA neural progenitors. They expressed DA-specific genes and also secreted DA-specific markers. However, the Na^+ and Ca^{2+}-gated channels were found to be poorly formed, suggesting that the cells are still immature (Trzaska et al. 2007).

It has also been reported that miR-124 suppresses non-neuronal genes in neural tissue that complements the role of RE-1 silencing transcription factor (REST/ NRSF) and miR-9 facilitates the neuronal precursor production by inhibiting pro-neural transcription factors (Lim et al. 2010).

The surface topography has a crucial role in stem cell differentiation. The hBM-MSC were differentiated into neurons in the absence of BDNF by being subjected to specific surface topography. Hydrogenated amorphous carbon (α-C:H) groove topography has been known to drive the differentiation of hBMMSCs towards neural lineage (D'Angelo et al. 2010).

From 2011 to 2020

Micro-RNAs were also found to perform a prerequisite function role in stimulating neural differentiation. miR-9 (microRNA-9) takes an active part in promoting neuronal differentiation of mouse MSCs by notch signaling (Zhang et al. 2015).

When the differentiation of hBMSCs into neurons like cells was accomplished using several differentiating factors including edaravone, the differentiated neuron-like cells expressed membrane channel proteins with ion current formation. However, in spite of the expression of sodium channels, sodium currents were absent. Therefore, it can be inferred that the cells formed by the method were immature in nature (Zeng et al. 2011).

Co-effects of low elasticity and aligned topography of AFG were seen in neuro-differentiation of human umbilical cord mesenchymal cells, suggesting that aligned topography and matrix stiffness also plays a significant role in differentiation (Yao et al. 2016).

When MSCs from umbilical cord blood (UCB) were investigated with innate neurogenic potential (Divya et al. 2012), the WJ-MSCs were found to express secreted factors involved in angiogenesis and neurogenesis. The cells were known to exhibit better neuroprotection efficiency when compared to BM-MSCS. As WJ-MSCS possess a unique secretome, they are recommended as better MSC sources for promoting neurorestoration (Hsieh et al. 2013).

Several factors have been used over the years in order to enhance effective neuronal cell differentiation. These include the following –

- Chemicals such as valproic acid as a pre-treatment for hMSCs (Jeong et al. 2013); salidroside, which is a known neuroprotective phenylpropanoid glycoside as one of the neuronal inducers for rat MSCs (Zhao et al. 2014); cobalt chloride treatment of hMSCs, which resulted in upregulation of miR-124a and downregulation of anti-neural proteins SOX9 and SCP1 (Jeon et al. 2014); treatment with antidepressants like imipramine, desipramine, fluoxetine, and tianeptine (Borkowska et al. 2015b); induction with

TMP (tetramethylpyrazine) (Nan et al. 2016), or resveratrol, which is a natural polyphenolic and is known for anti-inflammatory properties (Geng et al. 2017), and zinc when applied to undifferentiated stage od ADMSCs (Moon et al. 2018), have led to enhanced neuronal differentiation and expression of neuronal markers.

- Usage of a different induction medium such as KoSR (synthetic serum replacement) along with low concentration of β–methionine in adipose-derived stem cells (Taha et al. 2014), hippocampal astrocyte conditioned medium, and glioblastoma conditioned medium (Borkowska et al. 2015a).
- Several attempts have induced certain conditions to attain neuronal transdifferentiation, such as induction of autophagy of BMSCs by rifampicin, which decreases the S-phase population (Li et al. 2016), mediation of Schwann–cell like development from BMSCs using lentivirus (Zheng et al. 2016), and usage of pulsated electromagnetic field (Urnukhsaikhan et al. 2016).

The differentiation of MSCs into neuron-like cells has not been limited to specific sources. Different sources from all over the body have been reported for the differentiation of MSCs into neural lineages. Along with bone marrow, adipose tissue (Xu et al. 2017; Marei et al. 2018) is used as a common MSC source, dental pulp MSCs (Ullah et al. 2016; Singh et al. 2017), dermal MSCs (Saulite et al. 2018), and menstrual blood (Wu et al. 2018)-derived MSCs have also been reported recently for having the capability to differentiate into neuronal lineages. On comparing the ability to generate dopaminergic (DAergic) neurons by bone marrow (BM), adipose tissue (AD), and dental pulp (DP)-derived MSCs, it was found that DP MSCs possess remarkably better characteristics and can serve as a better candidate for future studies on dopaminergic neurons (Singh et al. 2017). The Men-MSCs transplantation and their subsequent differentiation showed improved hind limb motor functions when implanted for the treatment of incomplete thoracic (T10) spinal cord injury (SCI) rats. From the above observation, it can be implicated that the MenSCs uphold therapeutic potential and can be used for SCI (spinal cord injury) patients in the future (Wu et al. 2018).

Several efforts have been made to perform successful differentiation of MSCs into neuron-like cells for a couple of decades. However, no studies have reported fully functional neurons that are formed as a result of MSCs differentiation.

Criteria for Characterization of Differentiated Neuronal Cell Functionality

Several procedures that have been implemented over the years have led to several criteria to decide whether the process followed has given the desired result of the formation of neuronal lineage cells. Depending on the basis on which the degree of transformation of MSCs is accessed, we have attempted to discuss below the resultant cells for the process of neuronal transdifferentiation.

On the Basis of Morphology

Morphology of the derived cells from the MSCs have greatly differed depending of the source of the initial cells, i.e., the source from which the MSCs are derived as well as on the growth factors and the conditions that the MSCs have been subjected to in order to produce the desired neuronal lineage cells.

In cases where neurons are differentiated from adipose-derived hMSCs, the cells present an elliptical or spherical-shaped morphology when differentiated using two different sets of differentiation medium, one consisting of a combination of DMEM, FBS, antibiotic and retinoic acid, and the other consisting of DMEM, FBS, antibiotic, FGF2, and heparin. Similar results were obtained while using a mixture of DMEM, FBS, antibiotics, FGF2, EGF, BMP-9, and retinoic acid as the differentiation medium (Marei et al. 2018). When the same cells were used to obtain Schwann cells as a product of transdifferentiation by using appropriate differentiation medium, the morphology of the resultant cells showed a complex cytoplasm with increased number of cells (lo Furno et al. 2018).

When human nucleus pulposus MSCs were subjected to neural differentiation by using an induction media that was composed of DMEM-F12 along-with B27, antibiotics, FGF-basic, EGF, IGF (insulin-like growth factor), neural- like cells were obtained, demonstrating a morphology having a small oval-shaped cell body and emerging protrusions were recognized (Lazzarini et al. 2019).

However, instances where hMSCs derived from various sources were induced with FGF2 and BDNF, the neuronal morphology in regard to the perikaryal feature of neuronal cells depicted that the nucleus of the cells showed a shift towards the periphery of the cell body. Long and distinct axons emerging from axon hillock and multiple neuritis from nucleus were also observed irrespective of the source of hMSCs. The average neurite length was found to be higher when BDNF was added to the induction media (Singh et al. 2017).

On the Basis of Neuronal Markers

The functionality of the differentiated neurons is evaluated by analyzing some neuronal-specific markers at the protein and mRNA level. The markers are categorized based on the type of cell that expresses them. However, the groups may overlap, as one marker may be expressed by more than one cell type.

For example, both Nestin and Notch1 are categorized as neuroepithelial markers where, Nestin is an intermediate filament protein whose expression endured in radial glia until astrocyte development (Kriegstein and Götz 2003), whereas Notch1 regulates generation, migration, and differentiation of neural crest cells (Noisa et al. 2014). Neuroepithelial cells get converted into glial cells through neurogenesis.

Similarly, radial glial cells show markers like PAX6, Nestin, and Vimentin, along with some astrocyte markers like GFAP (present as chief ingredient of intermediate filaments in mature astrocyte and provide mechanical strength) (Hol et al. 2003), GLAST (Glutamate transporter), BLBP, SOX2 (transcriptional factor; expressed in proliferating cells and cells acquiring glial fate) (Papanayotou et al. 2008). Markers like Nestin and NeuN are considered to be early neuronal markers (Kriegstein and Götz 2003; Gusel'nikova and Korzhevskiy 2015), whereas MAP2 are a property of mature neurons (Soltani et al. 2005).

Oligodendrocytes are accountable for myelin production in the central nervous system and are marked by transcription factors like Olig 1,2,3 (they aid in oligodendrocyte development) (Dennis et al. 2019), SOX10 (directs neural stem cells to differentiate into cells for glial lineage) (Pozniak et al. 2010), and several oligodendrocyte surface proteins such as OSP (oligodendrocyte-specific protein), and MOG (myelin oligodendrocyte glycoprotein).

Schwann cells, which are also known for their myelin-producing properties in the peripheral nervous system, show markers like NCAM and S100 (Liu et al. 2015).

Differentiated neurons derived from dental MSCs have been reported to express markers like MAP2, β-tubulin III, and NFs along with synaptic markers like synapsin and synaptophysin (Ullah et al. 2016) as well as cholinergic neuron-specific markers like ChAT. ISL1, BETA-3, HB9 (Kang et al. 2019).

Dopaminergic neuron-specific markers include FOXA2, NR4A2, EN1, PITX3, TH (Nandy et al. 2014; Chabrat et al. 2019). Induced neurons from different sources were observed with expression of DCX, NDM, TAU, NCAM, GABA, NeuN, ENO2, Nestin, NSE, NeuN, S100, NF-200, GFAP (glial fibrillary acidic protein) at different levels (Lu et al. 2004; Neuhuber et al. 2004; Barnabé et al. 2009; Cortés-Medina et al. 2019).

On the Basis of Electrophysiology

The presence of electrophysiological activity, viz. action potential and synaptic transmission, is another one of the vital factors to substantiate functionality of induced neurons. These studies are carried out by using the patch clamp technique.

Differentiated neuronal cells are first cultured on poly–L–lysine-coated glass coverslips and then a patch clamp amplifier is used for recording Na^+ and K^+ potentials.

Ca^{2+}, Na^+, and K^+ voltage-gated channels have been found to co-exist along with Na^+ and K^+ currents in induced neurons (Ullah et al. 2016; Li et al. 2019). Electrophysiological recordings show the presence of Ca^{2+}, Na^+, and K^+ voltage-gated channels on the membranes of the differentiated neuronal cells (Subbarao et al. 2015; Ullah et al. 2016; Li et al. 2019).

Calcium ion imaging is used to analyze synaptic plasticity. Higher extracellular K^+ causes changes in the intracellular calcium concentration of the cell, which indicates cellular excitability. Calcium activity at a certain level has been observed after depolarization with KCl in differentiated neurons (Nandy et al. 2014; Singh et al. 2017). When hMSCs were induced for neuronal transdifferentiation by the addition of any chemical stimulatory, the Ca^{2+}displays spontaneous activity (Karakaş et al. 2020).

In many studies, despite showing good neuronal morphology along with neuronal markers, differentiated neurons either failed or expressed partial electrophysiological activity (Barnabé et al. 2009; Zhu et al. 2017; Lazzarini et al. 2019; Cortés-Medina et al. 2019). Therefore, these cannot be considered as functional neurons. The exact criteria for considering differentiated neurons as functional ones are still elusive.

According to a study of stem cell differentiation, the niche in which the cell is differentiated directly affects them (Rahimi-Sherbaf et al. 2020). The fate of the stem cells is defined by the design of the scaffold and its interface with the growth factors. Therefore, the scaffold has to be implemented in accordance with neuronal transdifferentiation. Some 3-D nanostructured microarchitectures have also been shown to encourage cell alignment, leading to efficient neural differentiation of hMSCs (Poudineh et al. 2018).

Studies have been conducted to see the effect of scaffolds, both of natural and synthetic origin, on the differentiation of MSCs into neurons (Guo et al. 2016). Induced neurons on PLLA/PCL scaffolds (Rahimi-Sherbaf et al. 2020), PCL/collagen scaffold (Guo et al. 2016; Bagher et al. 2016) and PCL nanofibrous scaffold (Shirian et al. 2016) showed better results in terms of higher gene expression and survival percentage of cells compared to the cultures grown on tissue culture plates. Some special scaffolds have been engineered that can target effective neural differentiation of MSCs. These include PVA/SA nanofibrous scaffold (with 30 wt% SA) (Hazeri et al. 2020), 3D rGO-collagen hybrid scaffold (Guo et al. 2016) (Shirian et al. 2016) and 3-D Col–HA (Her et al. 2013).

Scaffolds that have been regularly implemented to enhance neuronal differentiation are listed below –

- Electrospun poly (ε–caprolactone) scaffold: The PCL nanofibrous scaffold and TCP (tissue culture polystyrene) have been successfully used to differentiate hBM-MSCs and hEnSCs into motor neuron-like cells. The resultant cells showed high expression of markers like β-tubulin –

III, NF – H, HB9, Islet 1, Pax6, ChAT. This is due to the fact that the polymer imitates the local tissue environment. The process of electrospinning that is implemented in the production of the scaffold enables the user to define the diameter of the individual fibers and their alignment. It was found that a diameter of 200–300 nm is ideal for neurite outgrowth and neural differentiation (Shirian et al. 2016).

- 3D rGO – collagen hybrid scaffold: This scaffold is formed by layers of reduced graphene oxide (rGO) nanosheets, assembled on 3D porcine acellular dermal matrix (PADM) channels that are mainly composed of collagen type I. The result is a conductive, biocompatible, and biodegradable PADM–rGO hybrid scaffold. When rat BM–MSCs were cultured on both PADM and the hybrid scaffold and induced for neuronal differentiation, then the cells cultured on the hybrid scaffold showed better results. This was because of the increased cell-to-cell communication that occurs due to the rGO. The enhanced communication increases the neurite outgrowth, as a result of which the electrical conductivity between the formed cells increases (Guo et al. 2016).
- PLLA/PCL scaffold: This scaffold is prepared by electrospinning poly–L–lactide acid (PLLA) and Polycaprolactone (PCL). The PDMSCs cultured on the said scaffold along with neural induction medium showed neural genes for β–tubulin, GFAP, and Nestin, thus resulting in a better outcome than the cells that were cultured as control having only the neural induction medium (Rahimi-Sherbaf et al. 2020).
- PCL/collagen scaffold: This scaffold is generally used for seeding WJMSCs along with several neurotrophic factors. The resultant cells have shown expression of biomarkers Islet 1, HB9, ChAt, and NF–H (Ebrahimi-Barough et al. 2017).
- PVA/SA nanofiber scaffold: The fabricated polyvinyl alcohol/sulfated alginate (PVA/SA) nanofiber scaffold is the preferable substrate for hBMSCs proliferation and neurogenesis. It has been observed that neural cells started to form 2 weeks after seeding without any external addition of growth factors (Hazeri et al. 2020).

Conclusions

There is no doubt in considering MSCs as one of the preferred sources for trans-differentiation of cells into neuronal lineage. Its presence in almost every type of tissue in the body makes it an accessible source too. The differentiation potential of MSCs towards neuronal lineage has been explored since the 1990s. A number of strategies, growth factors, and neural inducers have been implemented to differentiate them. While reviewing hundreds of research articles, we have seen that no study has reported functionality of differentiated neurons in all aspects. Also, different studies have considered different aspects in defining differentiation, most of time contradicting each other. Many of those show neuronal morphology and specific markers, but fail to possess an electrophysiological function, which is a crucial factor for defining neuronal functionality.

Although in the recent past most of the studies have been focused on exploiting both allogeneic and autologous potential of MSCs into neural cell lines, the exact procedure for achieving the same remains elusive. Therefore, making MSCs a controversial mode of treatment for neurodegenerative diseases among the scientific minds. Henceforth, successful transdifferentiation of MSCs into fully functional neurons is still to be achieved, marking scope for more experiments that can pave the way in achieving the same.

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Author's Contribution Pricncy Choudhary: Data collection, analysis and writing manuscript; Ayushi Gupta: data collection and manuscript writing and editing: Sangeeta Singh: Conceptualization, supervision, manuscript drafting, editing, and review.

Compliance with Ethical Standards

Declaration of Competing Interests All authors have asserted that there are no conflicts of interest to declare.

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