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An overview of the role of Wnt signalling pathway in governing transdifferentiation of stem cells towards neuronal lineage

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Abstract: Mesenchymal stem cells possess the potential to differentiate into many lineages, which regulate diverse signalling cascades. This unique property of stem cells is called transdifferentiation/linear reprogramming and this characteristic is of immense importance in regenerative medicine and tissue repair. The mechanism involved in the regeneration process is still unclear and requires further analysis. Extracellular mediators are found to be linked to the growth and differentiation of mesenchymal stem cells. In recent research, Wnt and its downstream signalling pathways are also found to have a significant function in the self-renewal and differentiation of mesenchymal stem cells. Consequently, cellular reprogramming may enable the application of clinical research to cell therapy, disease modelling, drug screening, and the fabrication of artificial organs. Studies related to this distinctive phenomenon of stem cells, where the cells could reprogram themselves into entirely different cell lineages, show promising therapeutic applications in the future. However, unrelenting development in cellular reprogramming has paved the way for novel strategies in which signalling pathway manipulation might be used to decide cellular destiny. This cellular reprogramming has got bright prospects in the studies of regenerative medicine. Understanding the link underlying stochasticity and cell destiny might therefore aid in unravelling the molecular modulatory mechanism associated with cellular reprogramming.

Introduction

The undifferentiated cells in the human body, known as stem cells, have the potential to self-renew and can grow into any other cell in an organism. The process of specialisation may be subdivided into numerous stages. The developmental potential of a stem cell decreases with each subsequent stage. Consequently, unlike pluripotent cells, unipotent stem cells can differentiate only into a restricted count of different types of cells (Zakrzewski et al., 2019). Although, stem cells have been majorly associated with regeneration studies, additional knowledge and information on their novel characteristics and applications are still under further exploration (Lai and Aboobaker, 2018). In response to organ injuries, the specialised cells dedifferentiate in the specific organs to repair the injured site (Ahmed et al., 2018). For disorders such as heart attack, neuronal diseases and hepatobiliary diseases, which are otherwise difficult to treat, stem cells promise to be of great therapeutic value (Kim et al., 2020). Using the particular transcription factors, SOX2, OCT4, c-Myc, and KLF4 somatic cells are transformed into induced pluripotent stem cells (iPSCs) (Patel and Yang, 2010; Kim et al., 2008). Similar methods are used to create human iPSCs, which can differentiate into various cellular fates without the pluripotent state. However, in the clinical scenario, these reprogramming methods defeat the purpose due to the integration of oncogenes or external factors. Few of the other methods,

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based on non-viral genes, non-integrative vectors, miRNA, and small molecules, are under extensive study as an alternative to the clinical scenario (González et al., 2019). Among the alternatives, the use of small molecules such as HDAC inhibitors sodium butyrate, trichostatin A, additionally retinoic acid, vitamin C, and numerous others for reprogramming is thought to be suitable because of their simplicity of synthesis and standardization, cost-effectiveness, time-dependence, ease of concentration monitoring (Xu et al., 2015, Zentelytė et al., 2021). Thus, in the current review, we have summarised the role of stem cells and their signalling pathways in mediating towards specific lineage. Further, we have also discussed the use of small molecules as a step towards regenerative medicine in the future, as limited studies are available describing the function of small molecules in getting somatic cells to differentiate and in deciding the fate of the differentiated cells. Transdifferentiation, whereby the cells commit to one lineage are reprogrammed into another, this property of stem cells is widely studied and exploited in the field of research. This property of transdifferentiation has a promising future in various research disciplines such as regenerative medicine, cell therapy, cancer research and transplantation. Stem cells arising from different sources will likely give rise to lineage-specific cells. Some properties enable stem cells to expand and maintain their pluripotency. They are briefly discussed below:

Self-renewal

Among the key features of stem cells is the capability of self-renewal and simultaneously maintaining their undifferentiated state. For this, stem cells should develop a network in accordance with genomic integrity (Shenghui et al., 2009). Self-renewal property is vital in deciding when to proliferate or differentiate in the case of stem cell biology and also in cancer and aging (Fuchs and Chen, 2013).

Pluripotency

The other important aspect of stem cells is pluripotency, which describes the potential of stem cells to endure self-renewal and form into nearly all distinct tissue-specific cell types (Romito and Cobellis, 2016). Recent research has shown that transcription factors maintain the pluripotent state of stem cells and that an imbalance in their production might compromise self-renewal or differentiation (Han et al., 2018).

Differentiation

A stem cell (unspecialized) can give rise to differentiated (specialized) lineage-specific tissue. These differentiated cells aid as a source for cellular transplantation. The differentiation process continues in adulthood, and the cells divide and repopulate to repair injured sites and maintain cell turnover number. This may also occur in response to antigen exposure.

Dedifferentiation

Dedifferentiation is a fundamental biological mechanism in which cells dedifferentiate from a specialized role to a simpler condition similar to stem cells, which may give rise to differentiated cells when given the right stimuli. These differentiated cells do not risk immune rejection or genetic incompatibility, so they can be used as a new source for regenerative medicine.

Transdifferentiation

Transdifferentiation is also known as direct linear reprogramming, in which one cell type is converted to another cell type without undergoing any intermediate pluripotent state. This unique property of adult stem cells (ASCs) can form any cell (Cho et al., 2019). Transdifferentiation of somatic cells into a fraction of patient-specific neuronal cells is one therapeutic option for neurodegenerative illnesses. Transdifferentiation was first observed when the cuticle-producing cells differentiated into salt-secreting cells during the metamorphosis of silk moths from larval to adult (Cho et al., 2019). Further, analysis has discovered that when human and rat MSCs were fed with neural induction media, they differentiated into cells with neuronal characteristics within a few hours (Black and Woodbury, 2001). According to one research, co-culturing MSCs with mouse mesencephalic cells caused them to develop into neuronal-like cells. Increased expression of NeuN and GFAP, which are widely expressed in neuronal cells, validated this conclusion. Studies have also found that combining human mesenchymal stem cells (hMSCs) with drugs such as dibutyl cyclic AMP (dbcAMP) and isobutyl methyl xanthine (IBMX) led to the differentiation of around 25% of MSCs into neuronal-like cells, as shown by increased vimentin intracellular expression (Sanchez-Ramos et al., 2000; Grijalvo and Díaz, 2021).The transdifferentiating cells thus emerged as a potent source of generating different cell lineage types. They hold promising therapy methods in regenerative medicine since it is less time-consuming and cost-effective. The ability to modify a cell's genetic code using particular transcription factor combinations has revolutionised the field of regenerative medicine and made it possible for patients or donors who match their immune systems to generate iPSCs (Takahashi and Yamanaka, 2016). Several scientists are looking into transdifferentiation as a viable technique for combating neuronal loss in the brain. Induced neural progenitor cells would also be valuable for obtaining target cells for transplantation treatment,

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developing disease models, screening medication, and monitoring therapeutic effects.

Roles of stem cells in the clinical field

The integration of the surviving neurons into the brain network was aided by the transdifferentiation of RAS into neurons, which helped to replace the damaged neurons. Furthermore, glial scarring might be suppressed, removing the axonal extension-blocking strain (Chen and Li, 2022). When exposed to certain induction factors, human adipose tissue was observed to be an abundant source of adult multipotent adipocytes derived stem cells (ADSCs). These ADSCs are the potential for transdifferentiation and produce a variety of cells of endodermal, ectodermal and mesodermal lineages. Transdifferentiation of these ADSCs into neurons (Darvishi et al., 2017; Qin et al., 2015), oligodendrocytes, and Schwann cells (Fu et al., 2016) has been documented. Thus, adipose tissues were acknowledged to be a promising source of stem cells capable of transdifferentiating brain cells and may be useful in therapeutic applications. Studies have also shown the successful transplantation of HSCs in patients, which paved a path to various activities to ascertain patient treatment (Giordano et al., 2007; Cable et al., 2020).

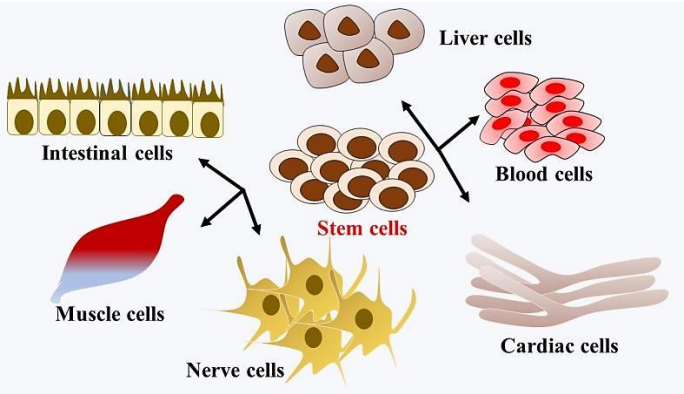

Figure 1. Diagram representation of differentiation potential of stem cells into different lineages

Analysis has also shown that cells harvested from the bone marrow displayed marked plasticity, which could develop along a neural lineage and could be used as an autologous source for brain repair (Krabbe C et al., 2005; Wright et al., 2011). Recent research suggests that neural crest (NC)-derived origins might be a source of an endogenous transdifferentiation response. The NC is a distinct and transitional embryonic cell populace that emerges from the ectoderm at the neural tube's borders. All through the epithelial-mesenchymal transition and major migratory phases, NC-derived stem cells (NCSCs) settle in various places of the body to help establish a variety of organs and tissues by transforming into neurone, glia and mesenchymal derivatives (Egawa et al., 2020). Figure 1 represents the ability of stem cells to differentiate into various lineages.

Stem cell interventions are also used for screening drugs and chemicals, as shown in Figure 2. Apart from this, the therapeutic potential of embryonic stem cell-derived somatic cells has been observed in *in-vivo* models of Parkinson's disease (PD), retinal blindness, type I diabetes mellitus, Alzheimer's disease, myocardial infarction, epilepsy, trauma, sickle cell anaemia, lymphomas, neuroblastoma and many more. The majority of neural illnesses are marked by the death of neuronal cells, which is assisted by reduced function and impairments (Katsuda et al., 2013; Zhang et al., 2016; Miki and Grubbs, 2014; López-González and Velasco, 2012). So, several approaches have been practised to repopulate the lost neuronal cells. Utilization of neural stem cells (NSCs) in recovering neuronal-related disorders is one of these approaches, which is being widely studied in the form of cell therapy and cell factory therapy (Chiu and Rao, 2011).

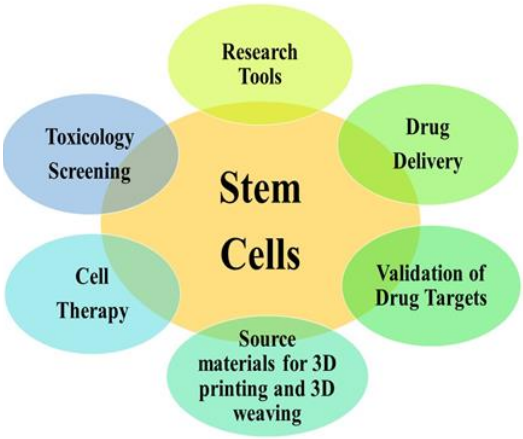

Figure 2. Diagram representing the various applications of stem cells

Cellular therapy

Human embryonic stem cells (hESCs) are being actively produced for clinical phase trials in PD by both academia and industry. This approach was primarily characterized by resting tremors, rigidity, and bradykinesia, caused by the deterioration of brain neurons (Brederlau et al., 2006; Parmar et al., 2020). Few clinical and experimental evidence showed that the fetal dopaminergic neuroblasts obtained from early substantia nigra could potentially replace and restore the functional abilities of the lost dopaminergic neurons and thus can be used to treat PD. Thus, PD is much more complex regarding cell survival, maintenance, and differentiation (Ringe et al., 2002; Buchbinder and Desai, 2016). Due to the low survival rate and less abundant sources of fetal dopaminergic neuroblasts, the focus shifted towards transplantable dopaminergic neuroblasts differentiated

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from embryonic stem cells (ESCs) or tissue-derived ASCs, but their availability and isolation procedures are laborious. Hence, the focus is now on non-neuronal stem cells like mesenchymal stem cells (MSCs) and fibroblasts, which appear to be a suitable alternative in treating diseases through the process of transdifferentiation. The recipient's mesenchymal stem cells are harvested, transdifferentiated, and grafted as autologous grafts without the risk of immunological rejection (Dezawa et al., 2004). In therapeutic brain transplantation models, neurons of various phenotypes, astrocytes, and oligodendrocytes may all be useful. The ability to govern differentiation is necessary to use brain stem cells' multipotency to produce these various cell types (Armstrong and Svendsen, 2000). These induced neuronal (iN) cells produce action potentials, neurons-specific proteins, and useful connections. Another study documented that the transcription factors Ngn2, Mash1, Sox2, Ptx3 and Nurr1 could transform human fibroblasts into dopaminergic (DA) neurons effectively and directly. The reprogrammed cells showed positive staining for DA neuron cell type-specific markers (Pang et al., 2011; Liu et al., 2012). Bone marrow MSCs, HSCs, amniotic fluid stem cells, NSCs and adipose tissue-derived stem cells are just some of the tissues with more advanced maturation phases from which tissue-specific stem cells can be isolated. In developing of adult mouse brains, multipotent NSCs are potent of differentiating into the three major cell types of the central nervous system (CNS) neurons, oligodendrocytes, astrocytes have been identified (Biswas et al., 2019).

Transcriptional factors associated with ADSC transdifferentiation

Overexpressing OCT4, KLF4, c-Myc and SOX2 transcription factor (TFs) on mouse fibroblast cells allowed Yamanaka and his group to successfully reprogram these cells into iPSCs in 2006 (Takahashi and Yamanaka, 2006). Since then, numerous research teams have analyzed the epigenetic and transcriptional modifications at various periods after inducing the factors in somatic cells to understand better the techniques and processes of somatic cell reprogramming better (Tat et al., 2010; Sun et al., 2009). Few analyses have been published so far on the topic of TF mediated transdifferentiation of ADSCs. With the help of a single transcription factor, SOX2, ADSCs may be transformed into induced NSC-like cells (Qin et al., 2015). During neurogenic transdifferentiation of hADSCs, expression patterns of important transcription factors were altered. These factors included PAX6, NGN2, MASH1, TBR1 NeuroD1 and TBR2 (Cardozo et al., 2012; Roybon et al., 2009). In general, studies examining ADSC transdifferentiation are seldom published. Although many studies have used ADSCs as a cell model for transdifferentiation, few exquisite investigations have documented TF transfections and reprogramming procedures that cause mesodermal-derived fibroblasts to develop into neural cells or NSCs. Some notable examples of such TFs are PAX6, SOX2, BRN2, ASCL1, NG, and many more (Bielefeld et al., 2017). The ability of these TFs to alter pertinent epigenetic alterations or initiate particular programs raises the possibility that they are crucial for transforming ADSCs into brain cells. These analyses further signify that ADSCs might be induced to undergo direct transdifferentiation into brain cells by overexpressing a small number of critical proteins.

Alterations associated with ADSC's transdifferentiation into neural cells

Epigenetic factors are critical in deciding what will happen to stem cells and how they will change. Currently, there are challenges associated with the transdifferentiation of ADSCs. A deep understanding of how transdifferentiation works through epigenetics is required to solve these problems. When MSCs change into neural cells, two main things should happen: NSCs, or neural cell-specific modifications, are stabilized, and the equilibrium of epigenetic modifications in the original cell is disrupted. An epigenetic barrier strictly protects the ADSCs because they can cross this barrier leading to reprogramming factors of neural cells, making them pluripotent (Encinas and Fitzsimons, 2017).

It has been observed that lysine methylation, at H3K9, H3K4, H3K20, H3K27, H3K79, or H3K36 sites, is the key reason for transcriptionally suppressed or active chromatin state (Huang et al., 2015). Dynamic alterations in the NES locus methylation of histones are detected during the neurogenic transdifferentiation of ADSCs (Boulland et al., 2013). Collectively, these results elucidate the epigenetic mechanisms by which ADSCs undergo neuronal differentiation and provide mechanistic models for the relationships between the essential factors and histone modifications in ADSCs. Therefore, histone alterations need to be explored in ADSC transdifferentiation of ADSC. However, methylation, demethylation, deacetylation, and acetylation, of histones coexist in the process and intimately connect and govern the whole transdifferentiation process.

When properly stimulated, mesenchymal stem cells (MSCs) may undergo transdifferentiation into NSCs or other neural cells. It has been postulated that modifying the mutation rate of lineage-specific genes is a vital

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stage in the neural cell development processes (Noer et al., 2006). Analyses have shown that miR-124 increases during ADSC neurogenic transdifferentiation, and its knockdown inhibited this process (Luo et al., 2018; Yang et al., 2018). Lentiviral vectors were used to transduce ADSCs carrying miRNA-34a to promote nerve regeneration in a rat model of medically induced sciatic nerve injury, resulting in nerve continuity and recovery (He et al., 2016).

Pathways associated with proliferation and differentiation of neuronal progenitor cells

NSCs' diversification into neuronal-specific lineages is mediated by many signalling mechanisms. Some of the most predominant pathways include the BMP signalling pathway, Notch signalling pathway, sonic hedgehog (Shh) signalling pathways, and Wnt/β-catenin signalling system that mediate neural plasticity (Lessard et al., 2007; Gonzalez and Medici, 2014; Yang et al., 2016). In recent times, studies have confirmed the association of BMP signalling pathway in the initiation of bone and cartilage formation (Gonzalez and Medici, 2014; Yang et al., 2016; Hoffmann and Gross, 2001). Additionally, it functions in various physiological activities in our bodies, including therapy for chronic kidney disease (CKD). In the past, a few reports have established the utilization of BMP 2 and BMP 7 in certain therapies (Knippenberg et al., 2006; Rauch et al., 2000; von Rüden et al., 2016). Likewise, promoting or suppressing the BMP signalling pathway can result in several issues. A few researches have shown that when the BMP signalling system is suppressed, a subset of epiblast cells commences mammalian neural induction (Di-Gregorio et al., 2007; Wawersik et al., 2005). The activation of BMP influences neural stem cell fate and maturation through all stages of neuronal development and also differentiates into astrocytes (Bond et al., 2012). The Notch signalling system is found mostly in multicellular animals and processes like so mitogenesis and the development of embryonic, cardiac, endocrine, respiratory, and neuronal (Siebel and Lendahl et al., 2017; McIntyre et al., 2020; Liu et al., 2010) tissue lineage specialisation, as well as guiding their flexibility. Another substantial role that the pathway plays is to regulate the fate of cell determination in *Caenorhabditis elegans.* It has a potential role in therapeutics, especially in osteoporosis and bone regeneration treatment. This pathway's inhibition can help treat cancer and its recurrence when combined with chemotherapeutic drugs (Venkatesh et al., 2018). These pathways have a significant role in the different tissue lineages and their specificity in organ expansion. Correspondingly, the Wnt signalling pathway has been immensely studied and has a remarkable efficiency in understanding its function in organ development and its relevance in stem cell plasticity (Banerjee et al., 2019). Their functions are also widely distributed in certain tissue-specific proliferation, differentiation, and development. According to recent research, the pathway of Wnt/β-catenin monitors the status and differentiation of neural stem cells throughout their neuronal development (Zhang and Wang, 2020).

Wnt/β-catenin signalling in stems cells

Wnt signalling cascade is necessary for ESCs development and self-renewal (Dravid et al., 2005; Ombrato et al., 2012). In the neurogenic regions of the adult mammalian brain and the ventricular zones of the embryonic nervous system, the self-renewal of neural stem cells is maintained by Wnt/β-catenin signalling. Shh is essential upstream of Wnt to modulate neural progenitor proliferation all through the development, and cyclin D1 generation is resistant to Wnt signalling in the absence of Shh signalling (Alvarez-Medina et al., 2009). Wnt3a, or a GSK3 inhibitor, has been demonstrated to aid in creating ESC-like colonies. It stimulates self-renewal by suppressing TCF3 repressor activity, which is generated endogenously. It can, however, produce differentiation (Merrill, 2012; Ho et al., 2013; Sineva and Pospelov, 2014). Experiments on the embryoid body and teratoma differentiation show that a lack of proper differentiation occurs when either APC is mutated or endogenous GSK3 limits the stimulation of Wnt/β-catenin signalling. (Jothimani et al., 2022). Thus, pluripotency can be maintained as long as the essential transcription factors Nanog, OCT4 and SOX2 are expressed favourably (Merrill, 2012). MSCs are controlled biphasically by canonical Wnt signals, depending on signal amplitude (Kim et al., 2015; Yang et al., 2016). It is also required for successful gut sustainment and growth of stem cells. Numerous ligands and receptors of the Wnt signalling pathway are found in intestinal crypt epithelial cells (Gregorieff et al., 2005; Clevers and Batlle, 2006). It targets genes that are seen to be found in considerable quantities in the intestine. Notch and Wnt signalling is essential for the maintenance of stem cells along with a good equilibrium of differentiation between secretory and absorptive cell lineages (Valkenburg et al., 2011).

Association of Wnt signalling in Neuronal stem cells

Wnt proteins are important in many biological processes, including cellular proliferation, motility, polarity, and cell differentiation determination (Girigoswami et al., 2021; Etheridge et al., 2004). They are also reported to be pivotal in the early phases of

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Table 1. Table representing the list of Wnt activators

Sl. No	Wnt activators	Wnt Target
1	Licl	GSK3 Inhibitor
2	LY2090314	GSK3 Inhibitor
3	CHIR99021	GSK3 Inhibitor
4	SB-216763	GSK3 Inhibitor
5	6-bromoindirubin-3-oxime (BIO)	GSK3 Inhibitor
6	DCA	B-catenin activator
7	QS11	ARFGAP1activator
8	WAY-316606	SFRP Inhibitor
9	IQ1	PP2A Activator

Table 2. Table representing the list of Wnt Inhibitors

Sl. No	Wnt inhibitors	Wnt Target
1	Quercetin	TCF Inhibitor
2	Niclosamide	Frizzled Inhibitor
3	PKF115-584	TCF/β-catenin Inhibitor
4	NSC668036	DVl Inhibitor
5	LGK974	Porcupine Inhibitor
6	IWP	Porcupine Inhibitor
7	C59	Porcupine Inhibitor
8	Apircularen and bafilomycin	Vacolar ATPase Inhibitor
9	Pyrvinium	CK1 Inhibitor
10	IWR	Axin Activator
11	Shizokaol D	Axin Activator
12	XAV939	Tankyrase 1/Axin Activator
13	Ant 1.4Br/Ant 1.4cl	Wnt Inhibitor

development and the homeostasis of mature tissues (Nusse, 2008; Wexler et al., 2009). Wnt proteins bind to secreted growth factors, which act as ligands for the cell membrane's low-density lipoprotein receptor-related protein (LRP) and Frizzled receptors (Mao et al., 2001; Kang, 2020). There are at least 19 different types of Wnt ligands, transcription factors, receptors, competitors and transducers in mammals (Komiya and Habas, 2008; Wieschaus, 2016). Wnt signalling is an essential and conserved cascade in vertebrate development, especially in the formation of the peripheral nervous system (Herman et al., 2018; Petrova et al., 2014). Migration of neural crest cells to the ventral regions of the embryo requires Wnt signalling. Furthermore, sympathetic trunk ganglia maturation doesn't always appear to be stimulated by Wnt signals (Becker and Wilting, 2018; Dorsky et al., 2000). BMPs from the dorsal aorta stimulate the growth of sympathetic neurons and the creation of ganglia (Huber, 2006; Wieschaus, 2016). Wnt pathway activators, which inhibit the characteristics of early cells, are commonly utilized. Wnt proteins activate Dishevelled (Dvl), a cytoplasmic protein, via binding to Frizzled receptors and LRP-4, LRP-5, and LRP-6 receptors (Anastas and Moon, 2013; Klaus and Birchmeier, 2008; Barker, 2008; Florian et al., 2013; Sheikh and Grohom, 2021). The active Dvl protein is inhibited by GSK-3 phosphorylation of β-catenin, causing β-catenin to concentrate and stabilize in the cytoplasm. β-catenin then accumulates in the nucleus, where it may bind to T-cell factor (TCF) and lymphoid enhancer factor (LEF), activating downstream Wnt signalling pathways (Sheikh and Grohom, 2021). The Frizzled receptor, β-catenin, LRP, and TCF transcription factors are mostly required for the Wnt pathway but not for the non-canonical one. The Wnt/Ca^{2+} signalling pathway increases intracellular Ca^{2+} transient response, which regulates intercellular communication. The intracellular release of Ca^{2+} by the Wnt molecule activates protein kinase C and Ca^{2+} calmodulin-dependent kinase II (Gentzel et al., 2015). According to research, Wnt signalling has been linked to the regulation of skin stem cell differentiation, HSC differentiation, as well as neurogenesis, adipogenesis, chondrogenesis, and myogenesis. Wnt activation hastens the senescence of stem cells in intestinal crypts, bone, and hair follicles. According to a few studies, Wnt/β-catenin signalling activity is manifested in the transdifferentiation of ADSCs to neuronal cells (Girigoswami et al., 2021; Etheridge et al., 2004). Studies have reported that neurotrophins (NTs) have been shown to boost Wnt1 and Wnt7a expression in hMSCs. Only Wnt7a, on the contrary, promotes cholinergic and dopaminergic neuron growth by promoting the formation of synapsin-1, a synaptic marker in mature neurons. Human recombinant (hr) Wnt7a was shown to be concentration and time-dependently related. Additionally, Wnt7a and lithium, a glycogen synthase kinase-3 inhibitor, enhanced synaptic markers and neurites in NT-generated hMSCs via the canonical/β-catenin pathway, although this was inhibited by Wnt inhibitors and frizzled-5 (Frz5) inhibiting antibodies. Finally, via numerous Frz receptors, hrWnt7a stimulates synapse creation and supports neuronal development in hMSCs. These processes might be used to transdifferentiate other ASCs in the future (Tsai et al., 2014). This approach of targeting Wnt signalling might govern to the formation of lineage-specific cells for a wider therapeutic approach.

Repression of CEBP and PPAR expression is considered the crucial adipocyte development receptor that leads the Wnt signalling pathway to control the differentiation of adipocytes. Analysis has documented that the Wnt/β-catenin signalling pathway is activated when human adipose-derived stem cells are

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transdifferentiated into neural cells (Feng et al., 2014; Jang et al., 2015). Wnt5a helps hADSC transform into neural cells by attaching to the Fz3/Fz5 receptor and relaying signals via the Wnt5a-JNK cascade (Jang et al., 2015). In the early stages of differentiation, the expression of genes like cyclin D1 and Stat3 downstream of the Wnt/β-catenin pathway increased (Bizen et al., 2014), but the expression of genes like BMP2 and BMP4 decreased (Kléber et al., 2005). Genetic research has shown that active Wnt/β-catenin signalling is essential for forming neural cells (Bowman et al., 2013). Non-phosphorylated β-catenin is initially expressed in the cytoplasm of NSCs in the Wnt/β-catenin pathway. It binds to the transcription factors LEF/TCF after it reaches the nucleus. Lastly, it stimulates the transcription of downstream genes, including Neurod1 and Prox1, which are transcription factors involved in neural development (Wisniewska, 2013). In a nutshell, the stimulation of Wnt/β-catenin signalling is a significant factor that plays a part in encouraging the transdifferentiation of ADSCs towards a neuronal destiny. The list of Wnt activators and Wnt inhibitors are depicted below in Tables 1 and 2.

Association of Wnt signalling pathway in other stem cells

Significant breakthroughs in the function of Wnt signalling in hematopoietic development *in-vitro* have been accomplished. The mesodermal lineage is initially followed by a specialised subset of cells that develop from the vascular endothelium called the hemogenic endothelium, which originates from hematopoietic stem cells and progenitor cells (Richter et al., 2017). Inhibitors of GSK3β, which activate β-catenin, have also been used to activate Wnt signalling in murine and human HSCs. GSK3β inhibitors increased HSC engraftment in the bone marrow when given *in-vivo* (Lento et al., 2013; Ko et al., 2011). Adult B-cells in the mouse bone marrow produce Wnt5a, whereas myeloid cells, erythrocytes, and immature B-cells produce Wnt10b (Lento et al., 2013; Memarian et al., 2012). Wnt3a, Wnt5a, and Wnt10b are expressed in the homeostatic murine bone marrow microenvironment, but Wnt10b is significantly upregulated within the injured murine bone marrow microenvironment, indicating that unique Wnts may be more adept at or used for the course of regeneration (Gold and Brückner, 2004). The exact intracellular pathways inspired by how each kind of Wnt ligand is expressed with the aid of MSCs and their linkages must be discovered if the mechanisms driving MSC cell fate destiny choices are to be effectively investigated and understood. (Ling et al., 2009). At 24 hours post fertilization (hpf), knocking out Wnt16 decreased HSC marker gene expression, suggesting a failure in HSC specification. The lack of notch ligand delta C and delta D expression in the somites is caused by this Wnt16 gene deletion, and overexpression of delta C and delta D is sufficient to restore the HSC phenotype (Richter et al., 2017).

Modulation of stem cells by Wnt signalling

A study demonstrated that amyloid-β could inhibit β-catenin signalling from influencing hippocampal neurogenesis. An increase in levels of amyloid-β in amyloid-β treated NPCs block neuronal stimulation by hindering the β-catenin signalling pathway, reducing pro-neural gene expression (Oh et al., 2015). Wnt3 was observed to enhance axonal branching and growth cone size in sensory axons emerging from dorsal root ganglion neurons during sensory-motor link development in the mouse spinal cord. According to a recent study, the canonical ligand Wnt7a can cause presynaptic protein clustering and synaptic vesicle recycling (Toledo et al., 2008). According to the research, Wnt/β-catenin signalling was critical in controlling the differentiation and multiplication of living progenitor cells and metabolic zonation. In mice, the non-canonical Wnts were able to suppress Wnt3a-induced β-catenin/TCF reporter activity, diminish immortalised hepatic progenitor cell stemness, and increase hepatic differentiation (Fan et al., 2017). Studies conducted by overexpressing Wnt protein in quail mesodermal stem cell lines revealed that either Wnt11 or Wnt5a inhibited differentiation in macrophages. These Wnt proteins influenced the formation of blood cells by inducing shifting cell fate from a macrophage to red blood cells (RBCs) phenotype (Undi et al., 2016). Wnt proteins, including Wnt2, 6, 11, and 16b, as well as four inhibitors of the Wnt, like Wnt inhibitory factor, DKK, FRZB, and secreted frizzled-associated proteins, were discovered in the limbus. During quiescent stages, Wnt inhibitors balance Wnt signalling, and these inhibitors are carefully controlled to facilitate limbal stem cell development and differentiation (González et al., 2011).

Regulation of Wnt signalling for clinical benefits

In the field of conventional medicine, tremendous research approaches and clinical successive cases contributed to translational medicine in recent years. The approach of targeting Wnt signalling might govern to formation of lineage-specific cells for a wider therapeutic approach due to its remarkable role in lineage specificity (Atlasi et al., 2013). Direct reprogramming of a somatic cell is another approach with wider clinical application. Instead of this, the reprogramming of somatic cells to differentiate into neuronal cells by using small molecules

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will have an approach to treating neurological disorders. Small molecules, such as quercetin, and niclosamide, are found to have a role in regulating Wnt protein expression. Few small molecules like BC2059 have an inhibitory role, causing the decreased expression of the Wnt cascade, as a result, functional neural cells are formed from stem cells (Xie et al., 2017; Xie et al., 2017). Being a well-distinct pathway in regulating the chief cellular activities and modulation of this pathway by using the small molecules or chemical inducers would be a better approach for the therapeutics. Small molecules which were predominantly obtained from natural sources had wider applications in the treatment of cancer and neuronal disorders, and especially flavonoids obtained from plant sources have a potential role in triggering many cellular pathways (Taura et al., 2009).

Conclusion

Stem cells and its pathways have made considerable advancement in the last two decades. Current review, aims at the characteristics of stem cells, transdifferentiation, applications of stem cells, ADSC transdifferentiation and its associated signalling pathways, further Wnt signalling, and its role in neuronal stem cell differentiation. MSCs show a promising future in therapeutics and stem cell regenerative technology due to their vast diversification and potentiality to delineate and proliferate. The identification of numerous molecules like the transcription factors that have enhanced the reprogramming of stem cells through mechanisms of epigenetic regulation. Several research is being conducted on the transdifferentiation of MSCs into neural cells. The neuronal stem cells are known for its ability to not proliferate and differentiate, hence making it an active field of study to treat neuronal-related diseases and regeneration. The concept of regenerative medicine is a promising field of study for patient-specific therapy and treatment. The Wnt signalling proteins play a crucial role in maintaining the property of pluripotency and self-renewal in ASCs. Additionally, previous research has confirmed the role of Wnt signalling pathways in stem cell differentiation and embryogenesis. Wnt signalling is hypothesized to be important in the development and progression of neurodegenerative disorders; therefore, concentrating on this aspect of treatment will ensure a prosperous future. Thereafter, the activation and inhibition of Wnt signalling pathways could be considered as a hopeful strategy for restraining the advancement of neurodegenerative diseases. Analyses have also demonstrated the function of Wnt signalling in the modulation of transdifferentiation of ADSCs as discussed in this review, thus resulting in their activation. ADSCs vary in their therapeutic effects. They are identified to possess characteristics that help in the differentiation into epithelial cells. Thus, it is noteworthy to mention that ADSC can be utilized as an alternative source in therapeutics and cell-based treatment for neuronal-related degenerative diseases in inducing ADSC transdifferentiation into neuronal cell lineage.

Therefore, MSCs have enormous potential to differentiate into multiple lineages by targeting several signalling cascades for the therapeutic approaches that have been examined. The rise in the occurrence of neurodegenerative diseases has made an impact on stem cell therapy, where researchers have accomplished ways to efficiently achieve neuronal transdifferentiation. Transdifferentiation may occur across germ layers, according to novel direct reprogramming procedures with specific variables. Epigenetic modulation and gene activation may govern transdifferentiation. Alternative approaches have been explored to boost the number of neuronal cells; however, the cellular activities do not last long. Hence, cellular reprogramming may open the way for clinical investigations in cell therapy, disease modelling, drug screening, and artificial organ formation. However, the indefinite progress in cellular reprogramming paved new methods by the modulation of signalling pathways might determine the cellular fate, and hopefully, in the future, the usage of small molecules could opt as an additive method in regenerative medicine. Due to challenges such as a lack of donor cells and the need for immunosuppressive medication, experts all around the globe are looking for a new source of stem cells. *In-vivo* analysis has revealed that selective changes in Wnt signalling are useful for maintaining a multipotent pool of cells and controlling their transformation into certain cell types.

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Conflict of interest

The authors declared that there is no conflict of interest.

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