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REVIEW

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Mesenchymal stromal/stem cells from perinatal sources: biological facts, molecular biomarkers, and therapeutic promises

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Abstract

The use of mesenchymal stem cells (MSCs) from perinatal tissue sources has gained attention due to their availability and lack of significant ethical or moral concerns. These cells have a higher proliferative capability than adult MSCs and less immunogenic or tumorigenesis risk than fetal and embryonic stem cells. Additionally, they do not require invasive isolation methods like fetal and adult MSCs. We reviewed the main biological and therapeutic aspects of perinatal MSCs in a three-part article. In the first part, we revised the main biological features and characteristics of MSCs and the advantages of perinatal MSCs over other types of SCs. In the second part, we provided a detailed molecular background for the main biomarkers that can be used to identify MSCs. In the final part, we appraised the therapeutic application of perinatal MSCs in four major degenerative disorders: degenerative disc disease, retinal degenerative diseases, ischemic heart disease, and neurodegenerative diseases. In conclusion, there is no single specific molecular marker to identify MSCs. We recommend using at least two positive markers of stemness (CD29, CD73, CD90, or CD105) and two negative markers (CD34, CD45, or CD14) to exclude the hematopoietic origin. Moreover, utilizing perinatal MSCs for managing degenerative diseases presents a promising therapeutic approach. This review emphasizes the significance of employing more specialized progenitor cells that originated from the perinatal MSCs. The review provides scientific evidence from the literature that applying these progenitor cells in therapeutic procedures provides a greater regenerative capacity than the original primitive MSCs. Finally, this review provides a valuable reference for researchers exploring perinatal MSCs and their therapeutic applications.

Keywords Stem cells, Progenitor cells, Multipotent, Regenerative medicine, Degenerative disease

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Biological facts

Mesenchymal stem cells (MSCs) are multipotent regenerative cells that can differentiate into several tissue cells [1, 2]. They have been designated the name "multipotent mesenchymal stromal cells" by the International Society for Cellular Therapy (ISCT) [2]. However, the term "MSCs" remains the most widely used name in literature. According to the Mesenchymal and Tissue Stem Cell Committee of the ISCT, human MSCs must exhibit the following standards to be considered for scientific investigations and/or pre-clinical trials:

- They must be plastic-adherent.
- They must express the following cell surface antigen biomarkers: CD73 (ecto 5' nucleotidase), CD90 (Thy–1), and CD105 (endoglin).
- They must lack the expression of hematopoietic antigens. These include the following: the hematopoietic progenitor cells marker CD34; the pan-leukocyte marker CD45; the monocyte and macrophage markers CD14 and CD11b; the B-cell markers CD19 and CD79α.
- They must display trilineage differentiation in vitro. Thus, the cells can differentiate into osteoblasts, chondroblasts, and adipocytes in culture.
- In addition, the following fifth criterion of MSCs has been emphasized in the literature:

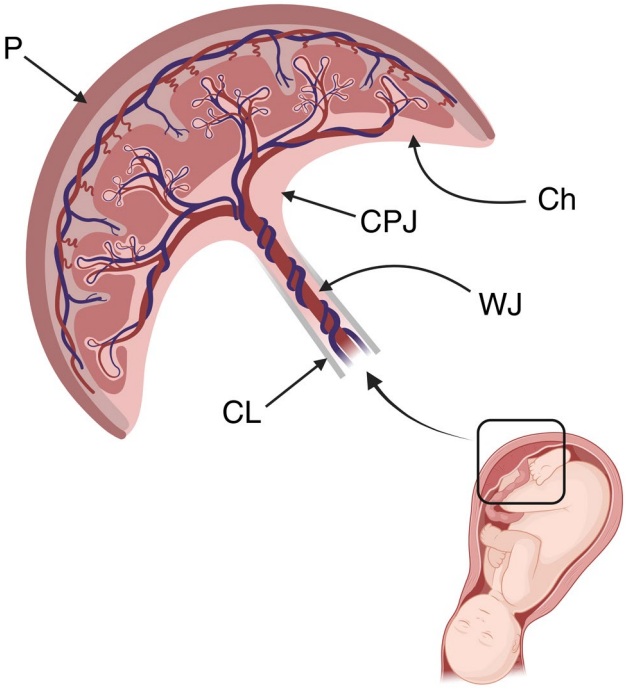

Fig. 1 Illustration of the five different regions of the placentome to isolate perinatal MSCs. These are cord lining (CL), Wharton's jelly (WJ), cordplacenta junction (CPJ), the chorion (Ch), and the maternal placenta (P). Created in https://BioRender.com

- They must be able to show a fibroblastoid morphology [1, 3, 4].

Despite sharing the aforementioned standards, human MSCs can still be classified into three types based on their source tissue. These include (i) adult MSCs, isolated from adult tissues, especially bone marrow (BM) and adipose tissue; (ii) fetal MSCs, isolated from collected samples of amniotic fluid or fetal tissues after terminated pregnancies; and (iii) perinatal MSCs, which are isolated from the placentome tissues (i.e., umbilical cord and placenta membranes). Perinatal MSCs can be harvested from five distinct regions of the placentome (Fig. 1), including the umbilical cord (UC) lining membrane, Wharton's jelly (WJ), The junction between the UC and placenta, the fetal chorion, and the maternal part of the placenta (i.e., *Decidua basalis*) [1].

The MSCs from perinatal sources have attracted the attention of many researchers due to their abundance in easily accessible tissue sources without significant ethical and safety concerns. For example, perinatal MSCs do not carry major ethical concerns like embryonic stem cells (ESCs). The primary ethical conflict associated with ESCs consists of using human embryos to obtain these cells [5, 6]. Therefore, the ethical debate surrounding the ESCs revolves around whether it is justifiable to develop new cell-based therapies at the cost of harming human embryos. In contrast, MSCs derived from perinatal sources do not raise significant ethical concerns. They only require institutional ethical approval along with the consent of the mother or guardian to use the discarded placentome tissue. Additionally, perinatal MSCs do not need invasive harvesting procedures, unlike MSCs from adult sources such as bone marrow and adipose tissue. The invasive procedures required to harvest adult MSCs can cause tissue damage, discomfort or pain to the donor, and place him at risk. Therefore, harvesting adult MSCs requires more ethical and safety considerations than the perinatal MSCs.

Perinatal MSCs have numerous advantages over MSCs from other sources (Fetal and adult), making them the center of attention for both experimental research and therapeutic applications. These benefits include:

- Higher proliferation capability than adult MSCs [7].
- Lower immunogenicity than adult MSCs with the ability to ameliorate immune response [7, 8].
- Less likely to have mutations than adult MSCs since they are immature cells, which reduces the risk of tumorigenesis [4, 7, 9].
- They do not require invasive isolation methods like fetal and adult MSCs [1, 9].

- They have no ethical issues like fetal and adult MSCs since placentome tissues are considered medical waste that is discarded after birth [1, 9, 10].
- large-scale availability due to the abundance of placentome tissues that yield a high number of cells [4, 7].

Molecular biomarkers

Numerous surface biomarkers have been used to identify MSCs. However, there is currently no single marker that can be used specifically to identify MSCs. Herewith, we discuss the most recommended and most frequently adopted biomarkers in the literature for MSC identification. These include the three recommended markers by the ISCT committee (CD73, CD90, & CD105), the three markers of stemness, known as the pluripotency triad (Sox2, Oct4, & Nanog), and two widely used integrin biomarkers (CD29 & CD49f). A summary of the structural and functional characteristics of these markers is provided in Table 1.

Cluster of differentiation 73 (CD73, NT5E)

This is a membrane-bound enzyme (ectoenzyme) that is known as ecto-5'-nucleotidase (NT5E) and is encoded by the NT5E gene [11, 12]. It degrades extracellular AMP into adenosine [11]. The successive enzymatic activities of ectonucleotidases CD39 and CD73 form the main pathway for hydrolysis of extracellular ATP into adenosine (Fig. 2). CD39 hydrolyzes ATP into AMP, and CD73 further converts AMP into adenosine [13]. Therefore, CD73 is considered an immune modulator since it helps convert the pro-inflammatory ATP molecule into the immune suppressive nucleoside adenosine [11, 13].

As noted in the previous section, CD73 has been recognized by the ISCT as one of the three surface markers required to define the MSCs [1, 2]. However, it has been revealed that CD73 is unequally expressed among different MSC populations, with MSCs from perinatal sources (especially from the UC) having higher expression levels of CD73 than MSCs from adult sources (including BM and adipose tissue) [14]. This heterogenous pattern of CD73 expression may explain the inconsistency in

Table 1 Summary of the most commonly used biomarkers to identify MSCs from perinatal tissues

#	Marker	Name	Location	Function	Explanation	References
1	CD73 (NT5E)	Ecto-5'-nucleotidase	Anchored to the external surface of the cell membrane	Ectoenzyme (Degrades extracellular AMP into adenosine)*	Recognized by the ISCT committee	[1, 2, 11–14]
2	CD90 (Thy-1)	Thymocyte differentiation antigen 1	Anchored to the external surface of the cell membrane	Cell surface receptor that binds to various ligands (e.g., integrins, syndecans, & CD97). The activation of this receptor stimulates signaling pathways that are mainly involved in cell adhesion and migration.	Recognized by the ISCT committee	[15–22]
3	CD105 (TGF-βR3)	Endoglin or Transforming growth factor β receptor type 3	Transmembrane glycoprotein	Auxiliary coreceptor for TGF-β1 and β3 ligands**	Recognized by the ISCT committee	[23–27]
4	Sox2	Sex determining region Y-box 2	Nuclear transcription factor	interacts with Oct4 to form a binary complex that activates the transcription of several pluripotent genes and inhibits the transcription of several differentiation genes.	Member of the pluripotency triad	[28–33]
5	Oct4	Octamer-binding transcription factor 4	Nuclear transcription factor	1- interacts with Sox2 to form a binary complex that activates the transcription of several pluripotent genes. 2- interacts with Nanog to form a complex that activates the transcription of the Dnmt1 methyltransferase gene.	Member of the pluripotency triad	[9, 34, 46–49]
6	Nanog	Nanog (from Irish mythology Tir nan Og = Land of eternal youth)	Nuclear transcription factor	Interacts with Oct4 to form a complex that activates the transcription of the Dnmt1 methyltransferase gene.***	Member of the pluripotency triad	[35–49]
7	CD29 (ITGβ1)	Integrin beta 1	Transmembrane protein	associates with different integrin alpha subunits to form integrin complex receptors that contribute to various biological processes in different cells. In MSCs, it is mainly involved in cell adhesion and migration.	Widely used marker for perinatal MSCs	[50–60]
8	CD49f (ITGA6)	Integrin alpha 6	Transmembrane protein	Sustains stemness of the cell by downregulating p53 expression.****	Widely used marker for perinatal MSCs	[61–65]

*See Figure 2 for the detailed function of CD73. **See Figure 3 for the detailed function of CD105. ***See Figure 4 for the detailed function of Nanog. ****See Figure 5 for the detailed function of CD49f (ITGA6)

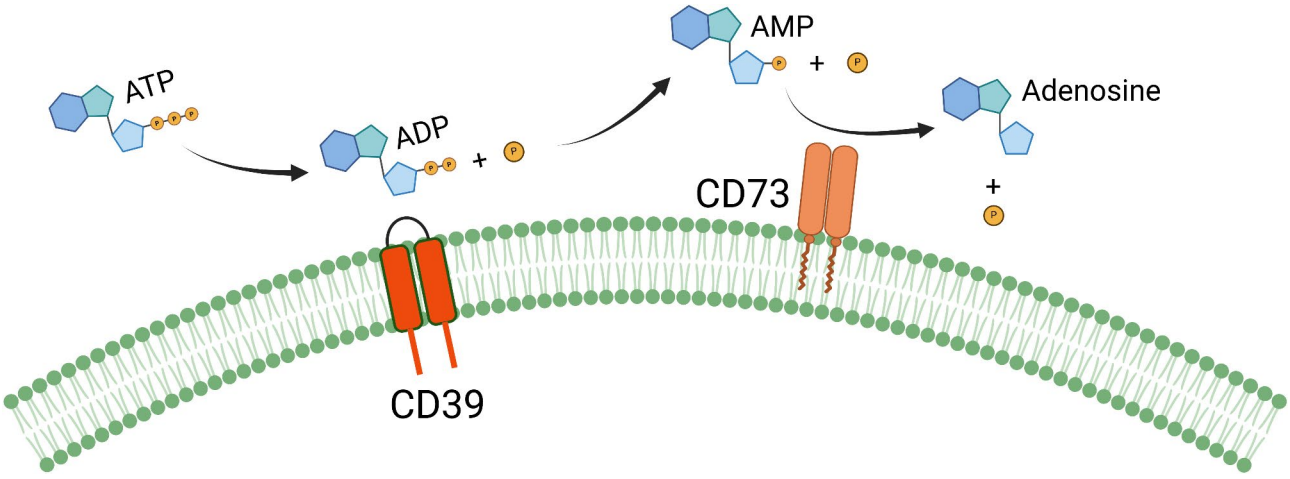

Fig. 2 Role of CD73 in the hydrolysis of extracellular ATP into Adenosine. Created in https://BioRender.com

regenerative potentials among different MSCs. For example, MSCs expressing high levels of CD73 promote significantly better cardiac repair in infarcted murine hearts than MSCs with low levels of CD73 [14].

Cluster of differentiation 90 (CD90, Thy-1)

This protein is anchored to the outer surface of the plasma membrane by a glycosylphosphatidylinositol (GPI) segment [15, 16]. It is also known as thymocyte differentiation antigen 1 (Thy-1) because it was initially discovered in murine thymocytes [17]. It is associated with many biological functions, including cell death (apoptosis), cell adhesion, cell proliferation and migration, and as a pluripotency marker for stem cells [16, 18].

CD90 is expressed in different types of stem cells, including cancer stem cells (CSCs) [19, 20], hematopoietic stem cells (HSCs) [21, 22], and MSCs [1, 2, 9]. Therefore, when using CD90 to identify MSCs, it is necessary to use additional markers to distinguish them from other stem cell populations. For example, CD34, a common marker for HSCs, is often used with CD90 to differentiate between MSCs and HSCs. HSCs are CD34+/CD90+, while MSCs are CD34-/CD90+ [18, 22].

Cluster of differentiation 105 (CD105, Endoglin)

This is a transmembrane glycoprotein commonly known as endoglin because it is predominantly expressed in the endothelial cells of blood vessels. Despite being initially identified in a lymphoblastic leukemia cell line, it has been exclusively investigated in vascular endothelial cells [23, 24]. Endoglin (CD105) is composed of three distinct domains. These include (i) an extracellular tripeptide domain, which is heavily glycosylated; (ii) a hydrophobic transmembrane domain; and (iii) an intracellular domain, which is rich in serine/threonine phosphorylation sites [24]. The intracellular domain is involved in modulating response only, and it does not commence a signaling cascade [25].

Endoglin functions as an auxiliary receptor or coreceptor since it is a component of the receptor complex for the transforming growth factor beta (TGF-β) superfamily ligands. This receptor complex is made of three types of receptors. These are TGF-β receptor type 1 (TGF-βR1), TGF-β receptor type 2 (TGF-βR2), and CD105 (endoglin). Endoglin is also known by some investigators as TGF-β receptor type 3 (TGF-βR3). CD105 is first associated with receptor TGR-βR2. This association allows the binding of TGF-β 1 or 3 ligands (Fig. 3). The binding of these ligands to the association will then activate TGF-βR2 to phosphorylate TGF-βR1. Phosphorylation of TGF-βR1, also known as activin receptor-like kinase (ALK), will initiate intracellular downstream signaling cascades. There are two downstream signaling pathways that are activated by two different subtypes of TGF-βR1. These include ALK1 or ALK5 kinase (Fig. 3) [24–26].

Endoglin is expressed in various cell types, including vascular endothelial cells, MSCs, HSCs, tumor-associated fibroblasts, and epithelial cancer cells [23, 24, 26]. Additionally, its expression levels are significantly elevated in the highly proliferating than in low proliferating and quiescent cells. Therefore, it is a predominant marker of both tumorigenesis and stemness. For example, MSCs expressing CD105 have a higher proliferation rate and better colony formation than MSCs not expressing CD105 [27]. In summary, similar to CD90, CD105 is a marker of proliferation and cannot be used as a single marker to identify MSCs. Additional markers are required to confirm the stemness of the cells (i.e., CD73 + and CD90+) and to exclude the hematopoietic origin (i.e., CD45– and CD34–).

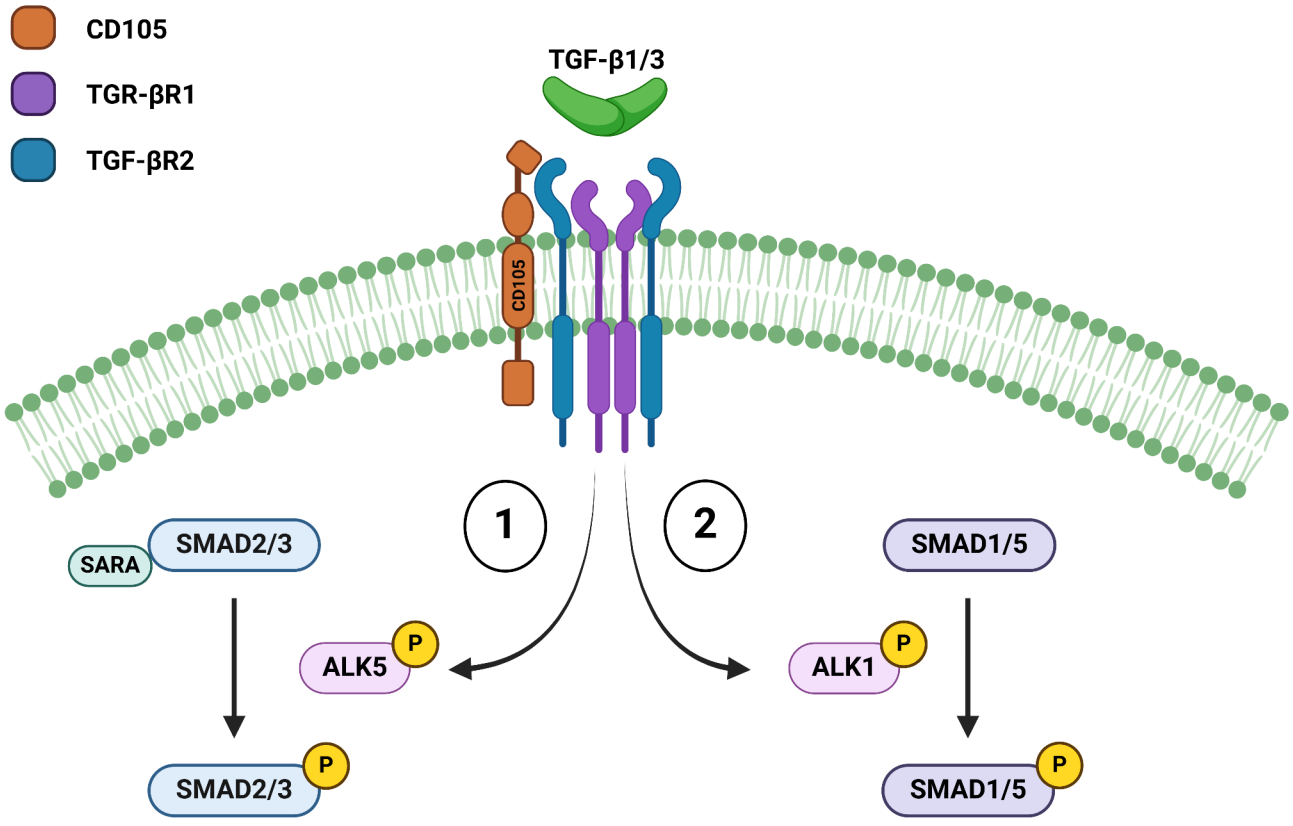

Fig. 3 Mechanism of action of CD105 through TGF-β receptor complex. CD105 is first associated with receptor TGR-βR2. This association allows the binding of TGF-β 1 or 3 ligands to the complex. The binding of these ligands to the receptor complex will then activate TGF-βR2 to phosphorylate TGF-βR1. Phosphorylation of TGF-βR1, also known as activin receptor-like kinase (ALK), will initiate two intracellular downstream signaling pathways that are activated by two different subtypes of TGF-βR1 (ALK1 or ALK5). Created in https://BioRender.com

Sex determining region Y – Box 2 (Sox2)

This nuclear transcriptional factor is essential for the maintenance and stemness of different types of stem cells [28]. It is one of three critical transcription factors that are referred to as the pluripotency triad of stem cells. These are Sox2, Oct4, and Nanog [29, 30]. Sox2 is a core transcription factor in embryonic stem cells (ESCs) and MSCs from perinatal and adult sources [28–31]. It is expressed at a higher level in MSCs from perinatal sources than MSCs in adult sources (BM and Adipose tissue). However, this level of expression is not as high as the expression level in ESCs [28].

Sox2 interacts with Oct4 to form a binary complex that activates pluripotent genes and represses differentiation genes [32]. It has been reported that Sox2 is essential for maintaining the self-renewal capability of MSCs, and without Sox2, the cells may lose their stemness [30]. In fact, the expression level of Sox2 gradually decreases as the MSC passaging number increases. Additionally, the knockdown of Sox2 inhibits proliferation and significantly reduces the colony-forming ability of MSCs [30]. On the other hand, induced expression of Sox2 in aged bone marrow-derived MSCs (BM-MSCs), which display flattened morphology and decreased proliferation, restored their normal fibroblastoid morphology and proliferation rate [33]. It is anticipated that higher expression of Sox2 is associated with greater stemness, increased proliferative potential, and better cell migration and adhesion in perinatal MSCs [31].

Octamer-binding transcription factor 4 (Oct4, POU5F1)

This is an important transcription factor found in both ESCs and MSCs. It is involved in sustaining the self-renewal ability of undifferentiated stem cells [9, 34]. It is the first and most used marker for identifying ESCs [34]. However, it is also expressed as a marker in perinatal MSCs isolated from WJ and the amniotic membrane of the placenta [9].

Nanog

Nanog is a homeobox transcription factor that is a member of the pluripotency triad (Sox2, Oct4, & Nanog) [35]. It is a 305 amino acids (AAs) long protein with a homeobox domain in the middle that spans 60 AAs from AA 95 to AA 154 [35]. In addition, it contains an N-terminal region that constitutes 94 AAs and is rich in serine residues (17 serine residues in its N-terminal).

The C-terminal region contains a tryptophan repeating domain where every fifth residue is a tryptophan (residues 200, 205, & 210). The tryptophan repeating domain is essential for Nanog's interaction with other pluripotency factors and for mediating Nanog's dimerization [36, 37]. It is important to note that in addition to the original *NANOG* gene on chromosome 12, there are 11 *NANOG* pseudogenes marked as P1 to P11 [35, 38]. The first pseudogene (*NANOG*P1) results from a tandem duplication of the original *NANOG* gene and has a high homology to the original gene. The remaining pseudogenes (*NANOG*P2-*NANOG*P11) are located on different chromosomes and show 85–90% homology to the original *NANOG* gene [35, 38].

Nanog's name is derived from the Celtic Irish mythology "Tir nan Og," which means the land of ever young, where people never age [39, 40]. This explains the main role of Nanog in stem cells, which includes maintaining the stemness and self-renewal of these cells and retaining them in an undifferentiated state [35]. Nanog is expressed in different types of stem cells, including ESCs [39, 40], CSCs [35, 38], and both perinatal [41–43] and adult [44, 45] MSCs.

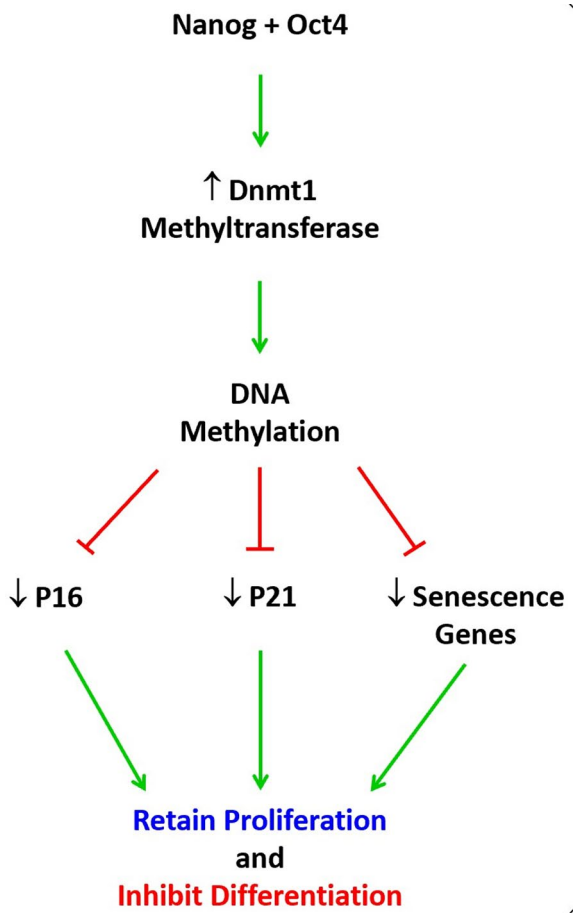

Fig. 4 Illustrating diagram for a suggested mechanism of action of Nanog in maintaining self-renewal of MSCs

Nanog is a key factor in maintaining the self-renewal of perinatal MSCs by preserving stemness, delaying senescence, and increasing the expression of both Sox2 and Oct4 [28]. It is important to clarify that Nanog, along with Sox2 and Oct 4, are forming a pluripotency triad that is essential for maintaining self-renewal and pluripotency of ESCs. In MSCs, this triad is also speculated to play similar roles in maintaining MSCs self-renewal and retaining them in an undifferentiated state [46]. This is evident by the fact that overexpression of these factors promotes the proliferation rate and enhances the colony formation of MSCs [47]. In contrast, the knockdown of these factors significantly reduces the growth rate and multipotency of MSCs [48, 49]. Furthermore, it has been established that Nanog functions by activating the repressors and suppressing the activators of differentiation [35]. Therefore, Nanog inhibits the differentiation of various types of stem cells.

One of Nanog's suggested mechanisms of action is that it collaborates with Oct4 to suppress the expression of genes that promote differentiation and senescence by enhancing DNA methylation. It has been revealed that both Nanog and Oct4 can upregulate the expression of a significant DNA methyltransferase gene called Dnmt1 by binding directly to its promoter region [46]. In turn, Dnmt1 will promote DNA methylation and inhibit the transcription of p16, p21, and other senescence genes, ultimately retaining cell proliferation and inhibiting differentiation (Fig. 4).

Cluster of differentiation 29 (CD29, ITGβ1)

This is a transmembrane adhesion molecule that is also known as integrin beta 1 (ITGβ1) [50, 51]. This molecule has four isoforms, and it can be associated with different integrin alpha subunits to form integrin complexes that bind extracellular adhesion proteins, including collagen, laminin, and fibronectin [52, 53]. CD29 is widely expressed in different types of cell lineages and in both progenitor and differentiating cells [52].

Previous studies showed that this glycoprotein biomarker is crucial for the survival, proliferation, and migration of MSCs. Interrupting interactions between ITGβ1 and extracellular matrix proteins can result in MSC death [54]. On the other hand, overexpression of ITGβ1 increases MSC survival and improves their regenerative efficacy [55]. Ode et al. 2011 reported that decreased CD29 expression results in reduced migratory capacity of MSCs [51]. Furthermore, Ip et al. (2007) found that CD29 blockade reduces the migration and engraftment accumulation of MSCs into infarcted sites of ischemic murine hearts [56].

Several previous studies used CD29 to identify MSCs from perinatal tissues [4, 57–60]. Brown et al. (2019) showed that more than 99% of MSCs isolated from the five different regions of the perinatal tissues (Fig. 1) express CD29 [1]. Additionally, researchers used CD29 as a marker to investigate the impact of maternal age on the frequency and distribution of perinatal MSCs within different areas of the placenta [59] and the UC [58]. In conclusion, to define MSCs using CD29, it is necessary to use at least two additional markers. First, a positive marker is needed to confirm the stemness of the cells, such as CD73 or CD90. Second, a negative marker is required to exclude the hematopoietic origin of the cell, such as CD34 or CD45.

Integrin alpha 6 (ITGA6, CD49f)

This is another transmembrane protein that functions as a cell adhesion receptor [61, 62]. This cell surface biomarker is expressed in about 35 different populations of stem cells, indicating its integral role in stem cell biology, which maintains the self-renewal mechanism of stem cells [62]. It has been identified in both adult and perinatal MSCs. Nieto-Nicolau et al. (2020) reported that the expression of ITGA6 can indicate the progenitor potential of BM-MSCs since BM-MSCs that express higher levels of ITGA6 have higher clonogenicity, migration, and differentiation potentials [63]. Comparable to these findings, Al-Obaide et al. (2022) revealed that higher expression of ITGA6 is correlated with higher clonogenicity and shorter doubling time in perinatal MSCs [61]. Therefore, the expression of ITGA6 is necessary for the proliferation and self-renewal of MSCs. Perinatal MSCs isolated from the WJ in the UC had higher expression levels of ITGA6 than MSCs isolated from the placenta [61].

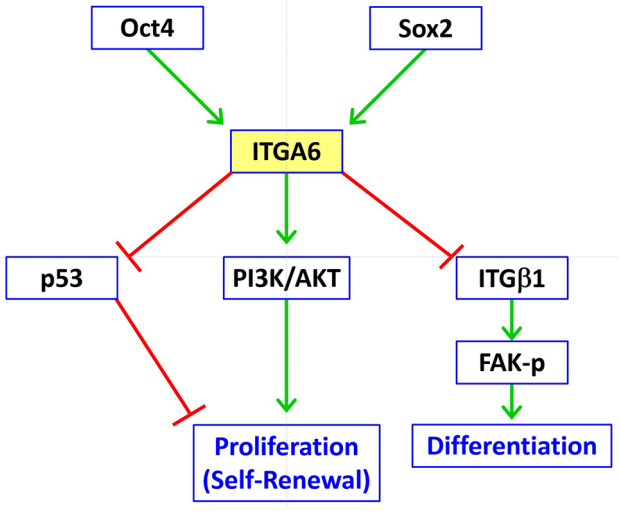

Fig. 5 Illustrating diagram for the mechanism of action of Integrin Alpha 6 (ITGA6, CD49f) in maintaining self-renewal of MSCs

The suggested mechanism of action for ITGA6 is summarized in Fig. 5 below. It has been reported that Oct4 and Sox2 transcription factors play positive roles in the expression of ITGA6. Both Oct4 and Sox2 bind to the promoter region of the *ITGA6* gene [62, 64]. On the other hand, the silencing of Oct4 and Sox2 leads to the downregulation of ITGA6 activity [64]. It is speculated that ITGA6 sustains the stemness of human MSCs by the activation of the PI3K/AKT pathway and downregulation of p53, a cell cycle regulator protein [64]. In pluripotent stem cells, ITGA6 levels diminish during differentiation, and integrin β1 is activated, leading to phosphorylation and activation of Focal adhesion kinase (FAK), which induces the cell differentiation and reduces the expression of the pluripotency factors Oct4, Sox2, and Nanog (Fig. 5) [65].

Therapeutic promises

Degenerative disc disease (DDD)

Medical research has been increasingly focused on applying stem cell-based therapy in degenerative disc diseases (DDDs). Perinatal MSCs, particularly those isolated from WJ in the UC, have shown considerable promise in cartilage regeneration and intervertebral disc (IVD) repair [66]. The first goal in using MSC therapy for DDD is to overcome the pathological catabolic microenvironment inside the disc by inhibiting the abnormal production of matrix-degrading enzymes and inflammatory cytokines, like interleukin-1β (IL-1β) and TNF-α, by the resident nucleus pulposus (NP) cells [67–69]. The second goal is to promote a healthy and more anabolic environment by promoting the implanted MSCs to produce growth factors (i.e., TGF-β, GDF5, & GDF6), anti-inflammatory agents (i.e., IL-1 receptor antagonist), and anti-catabolic factors known as tissue inhibitors of metalloproteinases (TIMPs) [66]. Previous studies revealed that MSCs are capable of improving the disc microenvironment mainly through paracrine activity by producing exosomes (extracellular vesicles) that contain proteins, microRNAs, and lipids [66, 70, 71]. These exosomes can release their contents directly into the extracellular matrix (ECM) or deliver them into a nearby NP cell after being ingested by endocytosis [71].

The MSC-secreted exosomes can produce a more favorable environment inside the IVD by several mechanisms. MSC-exosomes can release microRNAs (i.e., miR-21 & miR-31-5p) that are capable of inhibiting caspase-3 activity, thereby reducing NP cell apoptosis [71, 72]. Additionally, MSC-exosomes contain TIMPs natural proteins that bind directly to the metalloproteinases (ECM-degrading enzymes) and prevent their catabolic activity, thereby maintaining the ECM [73]. Moreover, MSC-exosomes comprise several anti-inflammatory proteins and miRNAs that suppress the inflammatory cytokines IL-1β

and TNF-α, thereby reducing the inflammatory response within the IVD [66, 71]. For example, MSC-exosomes containing miR-532-5p were found to suppress TNF-α by silencing the pro-apoptotic gene RASSF5 [74]. Also, MSC-exosomes contain factors that enhance the expression of cartilage-specific genes aggrecan, collagen II, and SOX9, thereby improving cartilage regeneration [75]. Finally, MSC-exosomes contain several anabolic growth factors like TGF-β1, HGF, BMP2, and GDF5 that contribute to the regeneration of the IVD [66, 70, 76].

Nevertheless, several challenges still hinder the clinical application of MSCs in DDDs. These are mainly technical difficulties related to pre-conditioning and preparation of implanted stem cells and overcoming the harsh pathological environment of degenerated IVD. Research has shown that implanted MSCs cannot survive in the acidic, hypoxic, and nutrient-deprived avascular environment inside the IVD [77].

In 2018, Beeravolu et al. investigated the potential of using human umbilical cord-derived MSCs (UC-MSCs) in remedying IVD degeneration. They compared the therapeutic outcomes between UC-MSCs and chondrogenic progenitor cells (ChPCs) derived from these cord cells in vitro. They showed a far greater capacity for the in vitro differentiated ChPCs to regenerate the IVD than their original UC-MSCs [3]. The UC-MSCs were differentiated into ChPCs by culturing them in a chondrogenic medium rich in TGF-β1, insulin, dexamethasone, and ascorbic acid. The ChPCs were then identified by expressing the chondrogenic markers Sox9, aggrecan, Collage type II, and the NP-specific cell marker FOXF1 [3]. Additionally, researchers at the Oakland University-William Beaumont Institute for Stem Cell and Regenerative Medicine (OU-WB ISCRM) were able to optimize in vitro differentiation of human UC-MSCs into nucleus pulposus-like cells (NPCs) by culturing them in a special differentiation medium that contained TGF-β1, BMP7, GDF5, insulin, dexamethasone, and ascorbic acid [78]. The NPCs were identified by their expression for the chondrogenic markers Sox9, Aggrecan, and Collagen type II. More importantly, the differentiated cells were able to express NP-specific markers, including FOXF1, PAX6, CA12, and KRT19. When these NPCs were injected into degenerated rabbit IVDs, they provided much better regenerative capacity than their original UC-MSCs [78].

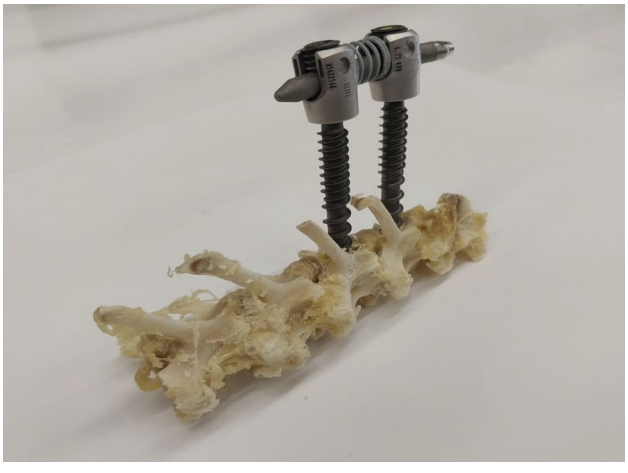

Fig. 6 Distractive medical device to restore intervertebral disc space

However, many studies revealed that MSC therapy alone in DDD is not enough. They emphasized the imperative need to use biocompatible materials with mechanical strength as scaffolds to restore mechanical stability and support spine loading [79–83]. Additionally, these scaffolds can prevent cell leakage from the injection site, therefore improving cell growth and differentiation. Most of the scaffolds investigated were injectable hydrogels to avoid invasive surgical interventions. Investigators at OU-WB ISCRM used self-assembling hydrogel scaffolds composed of polyethylene glycol biocompatible materials to examine the efficacy of combined MSC therapy and tissue engineering in managing DDD. They discovered that using self-assembling scaffolds can promote retention of the implanted cells within the IVD, which improves cellularity and ECM accumulation of the regenerated tissue [83].

Nevertheless, the hydrogel scaffolds do not provide sufficient mechanical support to completely restore the degenerated disc height. Therefore, the researchers at OU-WB ISCRM suggested a novel multi-pronged therapeutic approach for treating DDD [84]. This approach can be summarized in three main ideas: (1) Using a special distractive medical device to gradually restore the disc space to its natural height (Fig. 6). (2) Injection of self-assembling hydrogel scaffolds into the NP of the disc to prevent cell leakage. (3) The use of more chondrogenic lineage descendant progenitor cells like NPCs, which proved to be more effective in the regeneration process than the primitive UC-MSCs.

Retinal degenerative diseases (RDDs)

Retinal degenerative diseases (RDDs) refer to a group of pathologies that involve progressive degeneration of the cells in the retina, leading to low vision that subsequently worsens to induce loss of visual fields (partial blindness) and eventually end up in complete blindness [85–87]. These pathologies include (1) age-related macular degeneration, (2) retinitis pigmentosa, (3) pediatric Stargardt macular dystrophy (Stargardt disease), (4) diabetic retinopathy, and (5) glaucoma [86, 88]. The visual damage induced by RDDs is irreversible since the lost retinal cells are not replaced [86]. Hence, using MSC-based therapy provides a therapeutic promise to replace degenerated cells.

Several studies investigated the role of perinatal MSCs in managing RDDs [85, 89–91]. It is anticipated that MSCs can act through two main mechanisms to halt and overcome the damage induced by RDDs. The first mechanism is to support and protect the existing retinal cells by producing growth and anti-apoptotic factors. This mechanism is achieved in two ways: (i) secretion of several growth and neurotrophic factors that improve the persistence and growth of retinal cells, such as hepatocyte growth factor (HGF), nerve growth factor (NGF), pigment epithelium growth factor (PEGF), epidermal growth factor (EGF), Brain-derived neurotrophic factor (BDNF), and ciliary neurotrophic factor (CNTF). (ii) secretion of anti-inflammatory and anti-apoptotic agents that protect and support the survival of the retinal cells, such as TGF-β, interleukin-6 (IL-6), indoleamine 2,3-deoxygenase (IDO), TNF-α stimulated gene 6 protein (TSG-6), and programmed death-ligand 1 (PDL-1). The second mechanism is to rejuvenate the pool of degenerated cells. This mechanism is achieved by direct differentiation into new retinal cells that replace the degenerated ones. It is suggested that signals from the damaged retina stimulate this differentiation [88, 92]. Additional mechanisms are also speculated to contribute to the treatment of RDDs by MSCs, such as the ability of MSCs to release extracellular vesicles that comprise functional molecules like anti-inflammatory proteins, growth factors, and microRNAs [93–95]. These vesicles were found to be endocytosed by the existing retinal cells in a receptor-mediated, dose-dependent, and saturable manner [96]. Other mechanisms may include mitochondrial transfer and/or fusion with pre-existing retinal cells [88].

Similar to the previous principle in DDD, it is anticipated that using more specialized derivative progenitor cells would be more effective for retinal regeneration than using the original primitive MSCs. Brown et al. (2022) differentiated perinatal MSCs obtained from the human UC into retinal progenitor cells (RPCs) in vitro by culturing them for two weeks in a special neurobasal medium that contains EGF, retinoic acid, taurine, glutamine, and B27 neuronal supplement [85]. Then, they compared the therapeutic effects between the original primitive MSCs and their differentiated RPCs by intravitreally injecting these cells into a retinitis pigmentosa mouse model. They found that the primitive MSCs were restricted to the epithelial layer of the retina. In contrast, the RPCs could migrate and integrate into different neuronal layers of the retina. The RPCs significantly increased the retinal thickness and improved the visual function in the retinitis pigmentosa mouse model compared to the primitive MSCs. Additionally, it has been indicated that RPCs are the best candidate for stem cell-based therapy of RDDs [97].

In summary, RDDs are the primary sources of visual impairment and blindness worldwide. A promising therapeutic approach is available by using MSC-based therapy. The experimental findings on animal models are promising; however, these findings still need to be validated by clinical trials. Many phase I and II trials were performed and reviewed in the literature [86, 87, 97–99]. Most of these trials focused mainly on the safety and efficacy of the application of MSCs in RDDs. However, several questions still need to be investigated and verified in future clinical trials. These include questions about the best source of MSCs to be used (embryonic vs. perinatal vs. adult), the in vitro differentiation state of the cells (primitive vs. RPCs), the route of administration (intravenous vs. intravitreal), and the optimized dose required without inducing potential hazards.

Ischemic heart disease (IHD)

Ischemic heart disease, also known as coronary artery disease, is the leading cause of death worldwide [100–102]. It involves a reduction of blood supply to the cardiac muscle due to a narrowing in a coronary artery [102, 103]. The reduced blood supply can lead to damage of part of the cardiac muscle and death of cardiomyocytes, known as myocardial infarction (MI). The MI will eventually result in heart failure [102].

It has been demonstrated that intravenous infusion of perinatal MSCs can enhance heart function and improve quality of life in patients with stable heart failure [104]. The perinatal MSCs can be induced to differentiate into cardiomyocytes in vitro by either treating them with special chemical agents and supplements [105] or by coculturing them with pre-existing cardiomyocytes [106–108]. The most common and widely used chemical to induce differentiation of MSCs into cardiomyocytes is 5-azacytidine (5-AZA). This chemical agent is an analog of the pyrimidine nucleoside cytidine [109]. It can disrupt RNA metabolism and inhibit DNA methylation; therefore, it is used as an anticancer drug for treating different kinds of leukemia [109–111]. It has been anticipated that 5-AZA induces cardiomyogenic differentiation of MSCs by activating the MEK-ERK and Notch signaling pathways [105, 112]. The suggested mechanism of action of 5-AZA to induce differentiation of MSCs into cardiomyocytes is illustrated in Fig. 7.

Previous studies recommended adding culture supplements, such as fetal bovine serum and platelet lysate, to support MSC growth, expansion, and differentiation [113–116]. Platelet lysate (PL) is a media supplement that is rich in several growth and differentiation factors; therefore, it can be used as a differentiation inducer in vitro [117]. Among these factors are the platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), TGF-β, and EGF [115, 117, 118]. It has been revealed that human PL (hPL) is an effective cardiomyogenic supplement. The

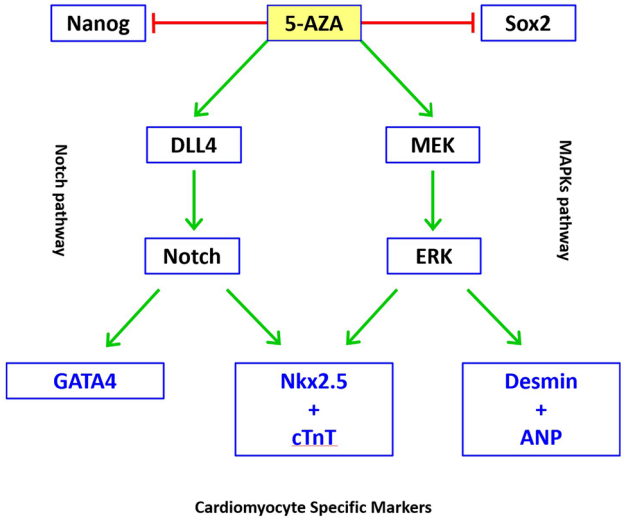

Fig. 7 The suggested mechanism of action of 5-azacytidine (5-AZA) in inducing MSC differentiation into Cardiomyocytes. 5-AZA inhibits MSC proliferation by suppressing the expression of Nanog and Sox2. It is anticipated that 5-AZA is acting through Notch and MAPK pathways to stimulate the expression of cardiac-specific genes and eventually induce differentiation into cardiomyocytes. Abbreviations: DLL4, delta-like 4 ligand; GATA4, GATA-binding protein 4; Nkx2.5, NK-2 homeobox 5 protein; cTnT, cardiac troponin T; ANP, A-type natriuretic peptide

combined treatment of perinatal MSCs with hPL and 5-AZA resulted in greater cell viability and significantly higher expression of cardiomyogenic markers than treatment with 5-AZA alone [117]. The PL is prepared by several freezing and thawing cycles of platelet concentrate or platelet-rich plasma samples, resulting in mechanical disruption and lysis of the platelets [115, 117]. To induce cardiomyogenic differentiation, the MSCs are incubated in a cardiomyogenic-induced medium containing 10 µM 5-AZA for 24 h only. After that, the cells are incubated in a basal growth medium containing 20% hPL for three weeks. The hPL medium is changed every three days [105, 117, 119].

The cardiomyogenic markers that indicate the differentiation of MSCs into cardiomyocytes are divided into two categories based on the differentiation stage [100]. Markers of early differentiation, which indicate the initiation of transformation of MSCs into cardiomyocytes, and markers of late differentiation, which indicate the maturation of cardiomyocytes. The essential markers of cardiomyogenic initiation are the GATA-binding protein 4 (GATA4) and the homeobox protein Nkx2.5 [100, 119, 120]. GATA4 belongs to the zinc finger family of transcription factors that bind to the specific DNA sequence "GATA" [121, 122]. It is well established that GATA4 is an essential factor in inducing differentiation of functionally beating cardiomyocytes in both Xenopus and mammalian systems [123]. Nkx2.5 is a homeodomain transcription factor essential in cardiomyogenesis and cardiac specification [120, 124, 125]. Moreover, mutations in Nkx2.5 are a common cause of congenital heart disease [120].

The main markers of late differentiation or cardiomyocyte maturation are cardiac troponin T (cTnT) and connexin-43 (Cx43) [100, 117]. The cTnT is one of three troponin subunits (C, I, and T) that form the troponin protein complex. This complex is a component of the cardiac thin myofilaments and plays a crucial role in cardiac muscle contraction [126]. Mutations in the cTnT gene can lead to hypertrophic cardiomyopathy [127]. Cx43 is a prominent transmembrane protein in cardiomyocytes that is also known as gap junction alpha-1 (GJA1) protein because it is encoded by the *GJA1* gene [128, 129]. It forms conductive channels between cardiomyocytes that allow the intercellular exchange of ions and metabolites [130, 131]. The cTnT is found in the cytoplasm, while Cx43 is localized to the surface membrane of cardiomyocytes [117]. Additional cardiac markers include desmin and A-type natriuretic peptide (Fig. 7).

The identification of a resident population of cardiac multipotent stem cells that are pre-committed to the cardiac lineage has reformed the stem cell-based therapeutic approaches in IHD [132, 133]. These are known as cardiac progenitor cells (CPCs), and they can differentiate into all types of cardiac cells, including cardiomyocytes, endothelial cells, and vascular smooth muscle cells [134]. Under normal conditions, CPCs are quiescent in their cardiac niche. Upon cardiac damage or stimulation, they become active, proliferate, and differentiate to repair the damaged tissue [133, 135]. They are characterized and subclassified by their expression of different surface markers. The most common markers are the tyrosine kinase receptor c-kit (CD117), the stem cell antigen 1 (Sca-1), and the insulin gene enhancer protein ISL-1 [132, 133, 135]. It is anticipated that CPCs have a higher cardiac regenerative potential than MSCs. Experimental research showed that CPCs can reduce infarct size, improve cardiac function, and promote the release of growth and angiogenic factors in the hearts of different animal models [136–138]. However, the extent of cardiac damage usually exceeds the capacity of endogenous CPCs to repair due to their very limited numbers [135]. Therefore, several studies have investigated the ex-vivo production of CPCs from different cell sources [132]. Currently, we are focusing on *the in vitro* differentiation of perinatal MSCs into CPCs and the therapeutic potential of these descendant cardiac lineage cells in IHD.

Neurodegenerative diseases (NDDs)

Neurodegenerative diseases (NDDs) are a group of debilitating disorders characterized by the progressive loss of structure and function in the nervous system. While these conditions are commonly associated with

aging, they can occur at any stage of life due to genetic, environmental, or idiopathic factors. Some of the most well-known NDDs include Parkinson's disease, Multiple Sclerosis, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. There is increasing interest in MSCs due to their regenerative, immunomodulatory, and neuroprotective properties, especially since curative options are limited.

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by a loss of motor function and the presence of tremors. The pathology of the disease involves the accumulation of misfolded α-synuclein aggregates within dopaminergic (DA) neurons, which form Lewy bodies. This buildup disrupts synaptic transmission and affects dopamine regulation [139], eventually resulting in damage and death of DA neurons in the substantia nigra pars compacta and noradrenergic neurons in the locus coeruleus of the brain [140]. The DA neurons produce and release dopamine, a neurotransmitter that relays signals controlling motor functions. Additionally, noradrenergic neurons are part of the autonomic nervous system, which, when activated by noradrenaline, is responsible for alertness [141, 142]. Since these brain areas control motor function, patients with later-stage PD exhibit symptoms such as muscle tremors, rigidity, slow movements, bradykinesia, and other motor dysfunctions [140]. PD is the second most common neurodegenerative disease and is projected to affect nearly 1.2 million people in the next 20 years; it significantly impacts the quality of life of individuals affected and causes a socioeconomic burden that accounts for almost 14.4 billion dollars annually in the United States [143]. The onset of PD symptoms commonly occurs after the age of 60 and affects about 1% of that age group (> 60 years) [144]. Although there are treatments for this disease, they are not very effective and cannot reverse or stop the progression of the disease.

Currently, there is no cure for PD. However, treatment options, such as pharmaceuticals and surgical intervention, are available to help PD patients manage their symptoms. These treatments include medications that either increase the dopamine levels in the brain or mimic dopamine's effects on the brain, such as dopamine precursors or dopamine agonists, in addition to surgical interventions such as deep brain stimulation [145]. Although these therapies can relieve some of the symptoms of PD, they cannot reverse or stop the damaging effects of the disease. The limited effectiveness of these drugs in halting disease progression and the anticipated increase in disease prevalence in future generations exhibit an immense need to develop a more effective treatment to stop and reverse the pathology of PD. We hypothesize that MSCs and DA neuron progenitors (DAPs) can halt neurodegeneration and provide neuro-regenerative effects.

We examined the effect of cell therapy on the 6-OHDA toxin-induced PD rat model. The toxin induces neural cell death via oxidative stress. Latchoumycandane et al. found that some of these pathways are via free radical generation, mitochondrial dysfunction, cytochrome c release, activation of caspase-9 and caspase-3, proteolytic activation PKCδ, and DNA fragmentation [146]. In earlier studies, perinatal MSCs have been shown to decrease oxidative stress by reducing levels of malondialdehyde and enhancing superoxide dismutase, glutathione, and the antioxidant enzyme glutathione peroxidase [147]. This may be why the MSC-treated animals displayed decreased necrosing DA neurons and increased tyrosine hydroxylase-positive (TH+) cells. Tyrosine hydroxylase (TH) is an important enzyme needed to produce dopamine. It acts by converting the amino acid tyrosine into L-DOPA, a precursor of dopamine. Our studies showed that DAPs are more effective than MSCs in mitigating disease symptoms in PD rats (*unpublished results*). This might be attributed to the high levels of neurotrophic factors GDNF and BDNF expressed by DAPs, which are known to promote neuroprotection in DA neurons. Additionally, the ability of DAPs to integrate into the damaged substantia nigra pars compacta and differentiate into DA neurons may contribute to their more promising results compared to MSCs.

On the other hand, multiple sclerosis (MS) is a chronic autoimmune disorder that affects the central nervous system (CNS) and results in demyelination, neuroinflammation, and axonal degeneration. MS presents a unique challenge due to its inflammatory and demyelinating characteristics, especially in young adults [148, 149]. The illness features either relapsing-remitting phases or progressive forms, significantly affecting motor, sensory, and cognitive functions [150, 151]. It is believed to be caused by immune-mediated attacks on myelin, the protective covering of nerve fibers. This condition arises from an unusual interaction between genetic factors and environmental triggers. Clinically, MS is characterized by symptoms such as fatigue, muscle weakness, spasticity, visual disturbances, and cognitive impairments, which can lead to varying levels of disability [152–155]. Although there have been advancements in disease-modifying therapies aimed at reducing inflammation and slowing disease progression, these treatments primarily focus on the immune response. They do not address the underlying neurodegeneration or promote repair mechanisms [156, 157]. This has generated interest in stem cell-based therapies, particularly MSCs because they can modulate the immune response, promote remyelination, and repair neuronal damage [158–160].

The MSCs derived from perinatal sources have significant potential for treating MS, due to their unique biological properties, as previously mentioned. One of

the primary mechanisms MSCs exert their therapeutic effects on MS is through immunomodulation [161, 162]. MS is driven by autoreactive T cells, particularly Th1 and Th17 subtypes, which attack the myelin sheath surrounding neurons. MSCs can suppress these pathogenic cells by secreting anti-inflammatory cytokines, including interleukin-10 (IL-10), TGF-β, and prostaglandin E2 (PGE2) [161–164]. Additionally, MSCs enhance the differentiation of regulatory T cells and reduce the activation of dendritic cells and macrophages, effectively shifting the immune response towards a more tolerogenic state [163–165].

In addition to immunomodulation, MSCs also contribute significantly to the remyelination process in MS. These cells can differentiate into oligodendrocyte progenitor-like cells, which further mature into oligodendrocytes, the myelin-producing cells in the CNS [166]. Furthermore, MSCs secrete a range of growth factors, such as BDNF, NGF, and GDNF [167, 168]. These factors enhance the survival and functionality of existing oligodendrocytes and support neuronal health by contributing to the repair of damaged neural tissue [169].

MSCs play a crucial role in remyelination and in offering neuroprotective benefits. They achieve this by secreting anti-apoptotic factors and releasing exosomes that contain microRNAs, proteins, and lipids, all supporting neuronal survival and aiding in axonal repair. By reducing oxidative stress and inflammation in the CNS, MSCs help create an environment favorable for effective tissue repair [170–177]. Additionally, the components of the extracellular matrix derived from MSCs, including laminin and fibronectin, support the structural integrity of neural networks and enhance their neuroprotective role [178].

Another critical mechanism MSCs aid in MS treatment is angiogenesis and restoring the blood-brain barrier (BBB). MSCs secrete VEGF and HGF, which enhance vascular repair and reduce BBB permeability [179–181]. This is especially crucial in multiple sclerosis, where BBB dysfunction permits immune cells to enter the central nervous system, worsening the disease [161]. Several preclinical studies provide substantial evidence supporting the efficacy of primitive MSCs in animal models of MS, such as experimental autoimmune encephalomyelitis [182–185]. These studies have demonstrated reductions in clinical scores, improved motor function, and enhanced remyelination. Similarly, clinical trials in MS patients have reported promising outcomes, including significant improvements in disability scores and reductions in relapse rates. However, the heterogeneity in trial designs, cell sources, doses, and administration routes present challenges in drawing definitive conclusions about MSC therapy's overall effectiveness [186–188].

Intravenous (IV) delivery is the most commonly used method for MSC transplantation in MS due to its safety and practicality. However, intrathecal (IT) administration, which involves directly injecting the cells into the cerebrospinal fluid, may offer enhanced therapeutic effects by improving MSC availability within the CNS [187]. Optimal dosing regimens are still actively being investigated, with new evidence suggesting that repeated administrations may be needed to maintain therapeutic benefits over time [189–191].

While there is significant potential for MSCs in treating MS, several challenges persist. The long-term safety and effectiveness of MSC therapies, especially for chronic neurodegenerative diseases, are still not completely understood [186, 192]. Additionally, variability in MSC isolation, expansion, and delivery protocols hinders the standardization required for broad clinical adoption [161, 193]. We believe more research is needed to elucidate the precise mechanisms underlying MSC-mediated neuroregeneration and immunomodulation. Additionally, novel strategies to enhance MSC functionality, such as preconditioning with hypoxia or bioactive molecules, could further improve their efficacy [162, 186].

Conclusion

This review provides the fundamental understanding of perinatal MSCs that is essential for future experimental research and therapeutic applications of these cells. It revises the main biological characteristics that make perinatal MSCs superior to stem cells from other sources. The main advantages of perinatal MSCs over MSCs from other sources can be summarized by their high proliferative capability, low immunogenicity and tumorigenesis, large-scale availability, and easy accessibility without major ethical concerns. These advantages render the perinatal MSCs a more favorable position, making them the center of attention for both medical researchers and clinicians. However, no single molecular marker can definitely identify MSCs, and a combination of several biological markers is a must. We recommend using at least two positive markers of stemness (CD29, CD73, CD90, and/or CD105) and two negative markers to exclude the hematopoietic origin of the cells (specifically CD34 and CD45).

In the realm of therapeutic applications, perinatal MSCs could be a promising solution for degenerative disorders such as degenerative disc disease, retinal degenerative diseases, ischemic heart disease, and neurodegenerative diseases. Scientific research indicates that more specialized progenitor cells derived from perinatal MSCs in vitro can provide a more potent regenerative response than the original undifferentiated MSCs. As we advance, it is crucial to continue this research trajectory, progressing from laboratory experiments to practical

applications, in order to fully exploit the regenerative abilities of perinatal MSCs. This can revolutionize the field of regenerative medicine.

Abbreviations

5-AZA	5-azacytidine
AA	Amino acid
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BM	Bone marrow
BMP	Bone morphogenetic protein
CD	Cluster of differentiation
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CPCs	Cardiac progenitor cells
ChPCs	Chondrogenic progenitor cells
CSCs	Cancer stem cells
DA	Dopaminergic
DDD	Degenerative disc disease
ECM	Extracellular matrix
EGF	Epidermal growth factor
ESCs	Embryonic stem cells
GDF	Growth and differentiation factor
GDNF	Glial cell-derived neurotrophic factor
HGF	Hepatocyte growth factor
HSCs	Hematopoietic stem cells
IGF-1	Insulin-like growth factor 1
IHD	Ischemic heart disease
IL	Interleukin
ISCT	International Society for Cellular Therapy
IVD	Intervertebral disc
MI	Myocardial infarction
MS	Multiple sclerosis
MSCs	Mesenchymal stem cells
NP	Nucleus pulposus
NPCs	Nucleus pulposus-like cells
PD	Parkinson's disease
PDGF	Platelet-derived growth factor
PEGF	Pigment epithelium growth factor
PL	Platelet lysate
RDDs	Retinal degenerative diseases
RPCs	Retinal progenitor cells
Sca-1	Stem cell antigen 1
TGF-β	Transforming growth factor beta
TIMPs	Tissue inhibitors of metalloproteinases
UC	Umbilical cord
UC-MSCs	Umbilical cord-derived mesenchymal stem cells
VEGF	Vascular endothelial growth factor
WJ	Wharton's jelly

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Declarations

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Consent for publication
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Artificial intelligence
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