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Review

The Role of the Notch Signaling Pathway in the Differentiation of Human Umbilical Cord-Derived Mesenchymal Stem Cells

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Abstract

Human umbilical cord mesenchymal stem cells (hUCMSCs) exhibit potent self-renewal and multilineage differentiation characteristics. They have garnered substantial attention within the domain of regenerative medicine owing to their therapeutic potential, such as in tissue repair, regeneration, immunomodulation, anti-inflammation, angiogenesis, wound healing, neuroprotection, and neuroregeneration. The process of fate determination is initiated by multiple signaling molecules. During development and tissue homeostasis, the Notch signaling pathway assumes a pivotal function in cell differentiation and the renewal of stem cells. A growing body of research has revealed that the Notch signaling pathway plays a pivotal role in hUCMSC proliferation and differentiation. The latest progress concerning the crucial functions of the Notch signaling pathway in maintaining homeostasis and determining the cell fate of hUCMSCs is summarized. Furthermore, the authors also summarized the mediators related to the Notch signaling pathway in hUCMSC differentiation, as well as the pathway alterations and mechanisms involved in hUCMSC therapy.

Keywords: notch signaling; human umbilical cord mesenchymal stem cells; cell differentiation; cell fate decision; stem cell therapy

1. Introduction

The Notch signaling pathway consists of a cell-to-cell communication system that functions significantly during various physiological and developmental processes [1]. Delta-like (DLL) ligands and Notch receptors are the primary components of this pathway. DLL ligands are transmembrane ligands consisting of Delta-like-1, 3, and 4, Jagged 1 and 2, and the Notch receptors (Notch1–4) present on neighboring cells. These ligands and receptors are involved in signaling interactions between neighboring cells (Fig. 1). The binding of ligands to Notch receptors initiates a series of proteolytic cleavages, leading to the release of the Notch intracellular domain (NICD) from the cell membrane [2]. Following its release, the NICD undergoes nuclear translocation, where it forms a transcriptional activation complex with an array of coactivators, among which are Mastermind-like proteins. This assembly catalyzes the upregulation of downstream target genes (Fig. 1) [3–5].

Notch signaling governs cell fate determination in various biological processes throughout development, homeostasis, and disease, and its effects on cell behavior are contingent upon the specific biological context [6]. Notably, researchers have shown pivotal roles for the Notch pathway in cell fate decisions by promoting self-renewal or differentiation of multiple stem cell populations, such as embryonic stem cells (ESCs) [7], pluripotent stem cells (PSCs) [8], hematopoietic stem cells (HSCs) [9], neural stem cells (NSCs) [10], and intestinal stem cells (ISCs) [11] (Fig. 2). Notch signaling maintains stemness by inhibiting differentiation-promoting factors and transcription factors associated with specific lineages [12–14]. In various cellular contexts, Notch has the capacity to function as both an enhancer and inhibitor of differentiation [11,15]. The Notch pathway plays an indispensable role in maintaining stem cell niches, the specialized microenvironments that support stem cell functions. Niche components can activate or suppress Notch signaling to control stem cell behavior [16,17]. In addition, Notch signaling is involved in tissue repair and regeneration processes by enabling stem cell activation and directing stem cell differentiation to replace damaged or lost cells. Notch signaling coordinates stem cell responses during tissue injury, facilitating tissue regeneration in various organs and tissues [18–20].

Derived from umbilical cord tissue, human umbilical cord mesenchymal stem cells (hUCMSCs) can undergo differentiation into a wide range of cell types, encompassing osteoblasts, chondrocytes, type II alveolar cells, hepatocytes, and cardiomyocytes [21]. hUCMSCs possess immunomodulatory properties, enabling them to regulate and suppress immune responses [22]. hUCMSCs secrete a multitude of bioactive molecules, including extracellular vesicles, cytokines, and growth factors. These secreted factors can stimulate tissue regeneration, promote angiogenesis, and modulate inflammation, apoptosis, and oxidative stress [23,24]. hUCMSC therapy holds promise in various medical fields, including tissue repair and regenera-

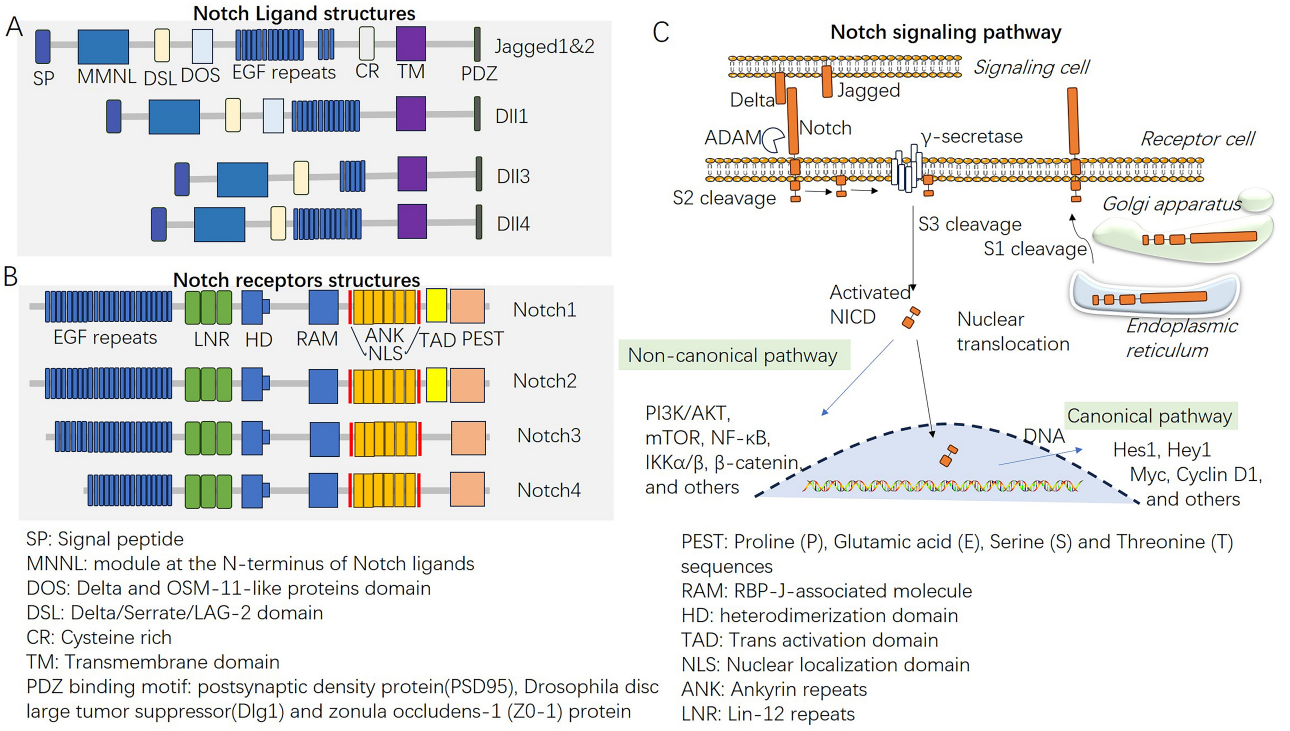

Fig. 1. The Notch signaling pathway. (A,B) The structures of Notch ligands and Notch receptors. (C) Overview of the Notch signaling pathway and therapeutic targets. EGF, epidermal growth factor; ADAM, a disintegrin and metalloprotease domain; NICD, Notch intracellular domain; PI3K/AKT, phosphatidylinositide 3-kinases/AKT; mTOR, mechanistic target of rapamycin kinase; NF-κB, nuclear factor kappa-B.

tion, treating autoimmune and inflammatory diseases, neurological disorders, liver diseases, cardiovascular diseases, lung diseases, diabetic wounds, graft-versus-host disease (GVHD), and cancer [25–28]. Hence, it is believed that hUCMSCs exhibit greater therapeutic efficacy than MSCs sourced from alternative tissues.

Therefore, understanding the function of Notch signaling within stem cells provides insights into stem cell biology, tissue regeneration processes, and disease pathogenesis. Throughout this review, an up-to-date overview of both established and emerging findings concerning Notch signaling during hUCMSC differentiation is provided. We also discuss potential therapeutic opportunities by regulating Notch signaling to improve hUCMSC-based therapies and develop targeted interventions.

2. The Mechanisms and Mediators of the Notch Signaling Pathway in Stem Cell Differentiation

2.1 The Classical Notch Signaling Pathway Effectors in Stem Cell Differentiation

The Notch signaling pathway involves several key mediators (Notch receptors, ligands, the γ-secretase complex, the NICD, coactivators, downstream target genes, and feedback regulators) that help transmit signals between stem cells [29]. Notch signaling commences through the binding of transmembrane Notch receptors (Notch1–4) to particular ligands on neighboring cells. Alterations in Notch1 [30], Notch2 [31], Notch3 [32], and Notch4 [33] can all change stem cell differentiation. By working in coordination, Notch1 and Notch2 function crucially in maintaining the stem cell population in a quiescent state, inhibiting activation, and regulating the fate decisions of stem cells by governing adult muscle regeneration [34]. Dysregulation of Notch receptor ligands, including Delta-like ligands and Jagged ligands, significantly affects the populations of airway epithelial progenitors in conducting airways [35]. The γ-secretase complex is crucial for Notch signaling stimulation and activation. After ligand–receptor binding, the γ-secretase complex cleaves the Notch receptor, after which the intracellular domain of Notch (NICD) is released from the cell membrane. The administration of a DAPT γ-secretase inhibitor can suppress the Notch signaling pathway, thus enhancing pluripotent stem cell differentiation and commitment to the osteogenic fate of mouse embryonic stem cells (ESCs) [36]. Additionally, the Notch signaling pathway regulates the expression of various downstream target genes, such as Hes1, Hes4, Hey1, Hey2, Ascl1, and MyoD. As essential Notch effectors, these genes encode proteins that serve as transcription factors, cell cycle regulators, and mediators of cell fate determination and differentiation [37,38]. Both of these proteins and

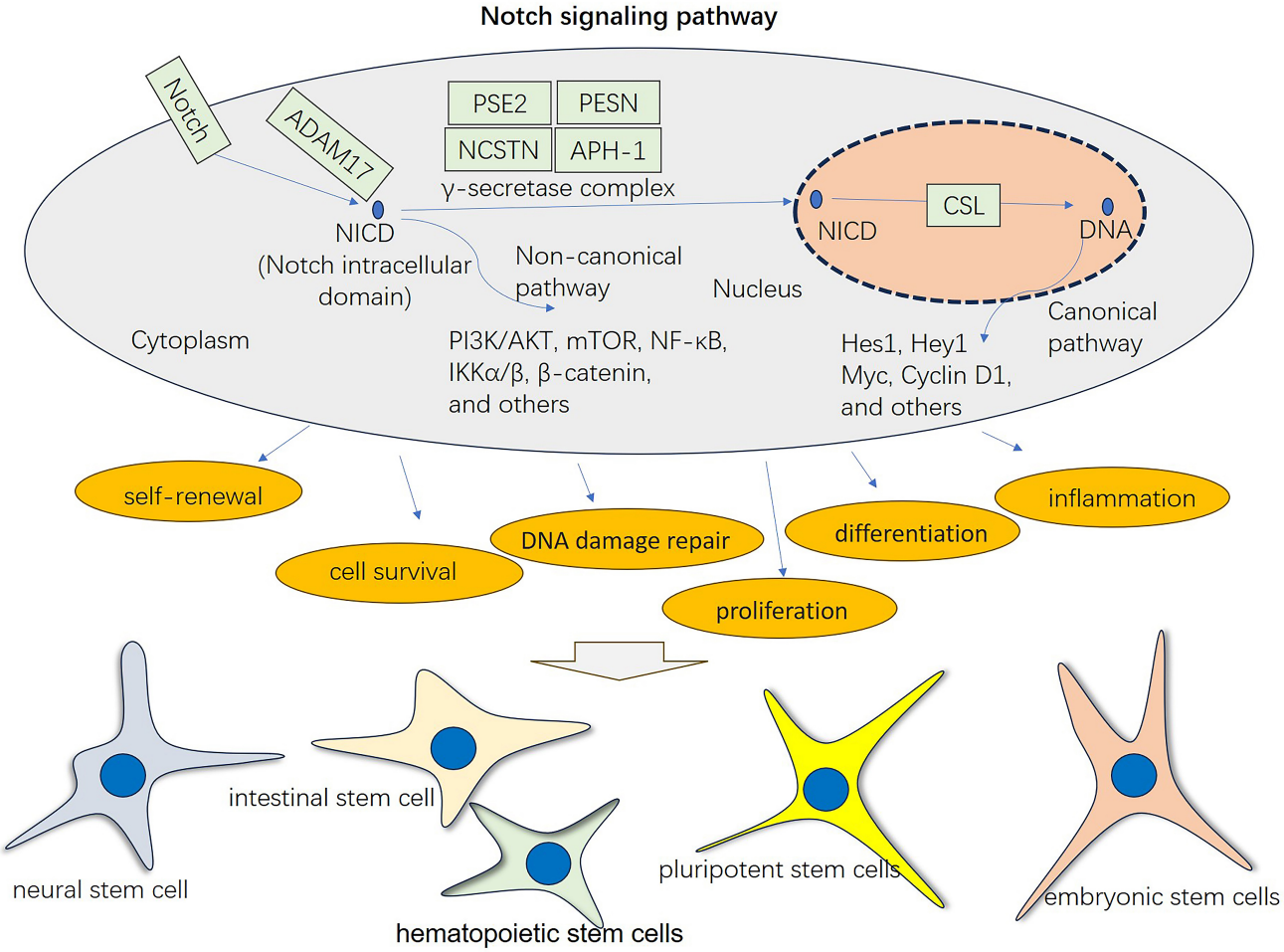

Fig. 2. The general function of the Notch signaling pathway in mediating cellular processes and stem cell differentiation. Stem cells can be differentiated into different lineages, such as neural stem cells, intestinal stem cells, hematopoietic stem cells, pluripotent stem cells, and embryonic stem cells. Notch signaling activation through the canonical pathway and noncanonical pathway has a vital role in affecting multiple biological processes, including self-renewal, cell survival, DNA damage repair, proliferation, differentiation, and inflammation. CSL refers to CBF1, Suppressor of Hairless, Lag-1.

Notch signaling work in the same direction in most other cell types. For example, Jagged1-mediated activation of the Notch signaling pathway promotes the osteogenic potential of MSCs through a mechanism dependent on Hes1 [39]. During the differentiation of quiescent NSCs, Notch and Hey1 are both upregulated and form a module for the long-term maintenance of NSCs, while other Notch effectors, such as Hes1 and Hes5, are involved in fast-cycling neural stem progenitor cells [40]. In the process of human hematopoietic stem cell (HSC) differentiation, these Notch effectors often cannot function as independent regulators. For instance, Hairy and enhancer of split-1 (HES-1) is not sufficient to induce T-cell differentiation in human HSCs [41]. A recent study revealed that double upregulation of Hes1 and Hes4 is not sufficient to induce T-lineage differentiation of HSCs, while knockdown of Hes1 or Hes4 significantly reduces human T-cell development. This study suggested that, during early human T-cell development, HES1 and HES4 play nonredundant roles [42]. However, those mediators of the Notch signaling pathway sometimes have reverse effects. For example, Hes1 expression oscillates upon induction of embryonic stem (ES) cell differentiation, and Hes1 functions as an inhibitor instead of an effector of Notch signaling [43]. The transcription of both Gata2 and HES1 is enhanced following Notch activation during HSC differentiation. The Hes1 protein represses Gata2 and forms a negative feed-forward loop [44]. Overall, the elaborate regulation of Notch and its effectors may mediate stem cell differentiation.

2.2 Feedback Regulators of the Notch Signaling Pathway in Stem Cell Differentiation

Several feedback regulators modulate and fine-tune Notch signaling [45]. For example, MyoD, which functions as a master controller of myogenesis, is a basic helix-loop-helix (bHLH) transcription factor. It plays a vital role in activating muscle-specific gene programs and driving

the differentiation of myoblasts into mature muscle fibers [46]. Deletion of MyoD by CRISPR/CAS9 leads to brown adipocyte differentiation in myoblasts [47]. Overexpression of Delta1 (a Notch ligand) during early myogenesis inhibits MyoD expression and activity, thereby maintaining myogenic progenitor cells in an undifferentiated state [48]. MyoD functions as an essential mediator of Dll1 expression. Gain- and loss-of-function experiments revealed that MyoD activates the expression of the Notch ligand gene Dll1, resulting in the activation of the Notch pathway and enhanced myogenic differentiation. However, MyoD autonomously inhibits the Notch pathway within cells that express Dll1, promoting a myogenic program and facilitating differentiation [49]. Notch1 can enhance SRY-related high-mobility-group box 2 (SOX2) expression by enhancing the transcription of SOX2 and promoting the invasion of glioma stem cells (GSCs). A positivefeedback loop of the Notch1-SOX2 axis has been found to control GSC invasion [50]. The expression of Notch and Sox2 serve as promising markers for evaluating the differentiation status of NSCs [51]. Moreover, hypoxia-inducible factor 1α (HIF-1α) activates Jagged1/Notch signaling and enhance scardiac differentiation of cardiac stem cells [52]. Moreover, Notch1 promotes the differentiation of MSCs to promote the differentiation of MSCs into ECs. Both Jagged1 and HIF-1α levels are promoted following Notch1 overexpression. increased the expression of MSCs [53]. Hence, there is a positive feedback loop, namely, the Notch1-Jagged1-HIF-1α axis, that mediates stem cell differentiation.

2.3 Cytokines Mediate Stem Cell Differentiation via the Notch Signaling Pathway

Cytokines assume a pivotal function in mediating stem cell differentiation, partly by mediating the Notch signaling pathway [54,55]. For example, IL-6-mediated Stat3 signaling participates inbasal stem cell differentiation and enhances ciliogenesis by promoting the expression of the multicilin gene and the forkhead box protein J1. Thus, the Notch pathway is inhibited after IL-6/Stat3 pathway activation [56]. Shear stress promotes the endothelial differentiation of stem cells from human exfoliated deciduous teeth, which is accompanied by elevated levels of VEGF, VEGFR2, EphrinB2, DLL4, Notch1, Hey1, and Hey2 [57], suggesting that VEGF is potentially activated. Moreover, VEGF can induce DLL4 expression and activate the Notch/DLL4 signaling pathway. VEGF may exert a positive regulatory influence on the Notch/DLL4 signaling pathway [58]. Transforming growth factor β1 (TGF-β1) accelerates the myofibroblast transformation of hepatic stellate cells and promotes the expression of Jagged1 and cholangiocyte markers (CK19, SOX9, Hes1), suggesting that it dominates the differentiation fate of hepatic stellate cells via regulation of the Jagged1/Notch pathway [59]. Fibroblast growth factors (FGFs) constitute a cluster of signaling proteins with multifaceted functions in stem cell differentiation, such as induction of pluripotency [60], promotion of mesoderm differentiation [61], and neural differentiation [62]. Treatment with the FGF receptor inhibitor SU5402 promoted the commitment of human pluripotent stem cells (hPSCs) to the neural crest (NC) cell fate. SU5402 enhanced NICD expression and activated Notch signaling upon blockade of ERK1/2 phosphorylation. The results indicated that FGF controls human NC cell differentiation from hPSCs by mediating the Notch signaling pathway [63].

3. Notch Signaling Pathway Mediators in hUCMSC Differentiation

3.1 The Notch Signaling Pathway in hUCMSC Differentiation

hUCMSC differentiation involves complex processes. Many transcription factors are expressed at specific levels and are involved in the directional differentiation of hUCMSCs, encompassing the Notch signaling pathway [64]. Positive signals for Notch receptors (Notch 1 and Notch 2) and ligands (Jagged 1 and Delta-like 1) were detected in hUCMSCs. Hu YH *et al*. [65] reported that overexpression of Notch signaling contributed to reduced insulin gene expression, proinsulin protein expression, and insulin-positive cell percentage in hUCMSCs. Thus, Notch signaling plays a crucial role in governing the differentiation of insulin-producing cells (IPCs) from hUCMSCs, and further suppressing Notch signaling could be an effective strategy for augmenting the quantity of IPCs. However, Venkatesh K and his colleagues reported that the Notch inhibitor DAPT suppressed NSC differentiation from hUCMSCs, accompanied by repression of the Notch intracellular domain [NICD], HES, and HES1. This study suggested that the Notch signaling pathway has an essential role in the derivation of NSCs and their subsequent lineage commitment from hUCMSCs [66]. Therefore, the Notch signaling pathway has dual roles in mediating the differentiation of hUCMSCs.

The Notch signaling pathway can regulate hUCMSC differentiation via a noncanonical mechanism. For example, the overexpression of transcription factor genes (*GATA-4* and *Nkx 2.5*) promotes hUCMSC differentiation into the cardiomyogenic lineage. Within the cardiomyogenic-induced cohort, there was notable overexpression of cardiac-specific genes, including *GATA-4*, *Nkx-2.5*, *MHC*, *cTnI*, α*-actinin*, and *Wnt2* [67]. Moreover, Altarche-Xifró W *et al*. [68] reported that the transcription factors Gata-4, Nkx-2.5, and Notch-2 were upregulated during cardiogenic differentiation in response to ischemic injury. Transplanted hUCMSCs for endometrial reconstruction can transdifferentiate into endometrial cells (including epithelial cells, fibroblasts, and macrophages). During this process, NF-κB signaling has a crucial function in modulating the Th17/Treg balance and inhibiting the immune response in damaged endometrial tissue [69]. With the help of Notch-

HES1 signaling, the stemness of human adipose-derived mesenchymal stromal cells (hASCs) was sustained through alterations in p53, HIF-1α, and NF-κB [70]. In rheumatoid arthritis (RA), a reduction in mesenchymal stem cell osteoblast differentiation was found, and the expression of genes encoding Notch pathway members and members of the noncanonical NF-κB pathway was notably increased. Treatment with Notch inhibitors prevents bone loss and osteoblast inhibition in RA mice. In addition, overexpression of p52 and RELB, two noncanonical NF-κB subunits, enhanced the activation of an RBPjκ reporter and the levels of the transcription factor HES1, which are dependent on the Notch intracellular domain (NICD) [71].

Recently, several transcription factors, such as SOX2 [50], SOX9 [72] and HIF-1α [52], have been found to activate Notch signaling and mediate stem cell differentiation. The functions of these genes in hUCMSC differentiation have also been identified. For example, SOX2 is a pluripotent stem cell marker that is overexpressed during the instantaneous neuronal differentiation of hUCMSCs [73]. Upregulating SOX9 enhanced aggrecan and type II collagen in hUCMSCs, suggesting that SOX9 promotes the chondrogenic differentiation of the latter [74]. *In vivo* experiments also confirmed that Sox9 upregulation helps hUCMSCs form cartilage-like tissue [75]. Moreover, miR-21 can enhance the proliferation, migration, and angiogenesis of hUCMSCs by activating HIF-1α [76]. These studies indicate that the functions of the Notch signaling pathway in hUCMSC differentiation might be altered under different pathological conditions. Elucidation of the regulation of vital transcription factors in the Notch signaling pathway can aid in the directional differentiation of hUCMSCs.

3.2 Notch Signaling Pathway-Targeting Drugs/Agents in hUCMSC Differentiation

Novel mediators that affect the Notch signaling pathway have been found to regulate hUCMSC differentiation. Sera from severe burn patients (BPS) can significantly promote hUCMSC activation and proliferation through the enhancement of Notch-1 and Hes-1 expression [77]. 5-azacytidine (5-azac) is a cytidine nucleoside analog with the specific capacity to impede DNA methylation. It is widely used for inducing stem cell differentiation, such as cardiogenic differentiation of murine bone marrow-derived mesenchymal stem cells [78] and chondrogenic differentiation of metabolic syndrome-derived mesenchymal stem cells [79]. hUCMSCs that are treated with 5-AzaC can be differentiated into myocardial cells. Both Notch1 and DLL1 are promoted by 5-azac, suggesting that the DLL4-Notch signaling pathway may be pivotal for 5-azac-induced cardiomyocyte differentiation of hUCMSCs [78]. Valproic acid is an orally active HDAC inhibitor and can activate Notch1 signaling [80]. Valproic acid treatment facilitates myotube formation and cardiomyocyte differentiation of human-elicited pluripotent stem cell-derived mesodermal progenitors (cdMiPs) by activating the Notch signaling pathway [81]. Notably, valproic acid increased hepatic differentiation at the expense of adipogenic differentiation in hUCMSCs by mediating AKT and ERK activation [82], suggesting that valproic acid might affect the differentiation of hUCMSCs via the Notch signaling pathway. Melatonin is a hormone generated by the pineal gland that can activate the melatonin receptor. In an intervertebral disc degeneration model, melatonin enhanced Sirt1 expression and inhibited Notch expression, thus inhibiting M1-type macrophage polarization, oxidative stress, and proinflammatory reactions. Sirt1 can interact with NICD. Therefore, the SIRT1/Notch signaling pathway may be involved in the role of melatonin in mediating Mφ polarization [83]. Interestingly, melatonin administration led to enhanced hUCMSC proliferation, migration, and differentiation [84]. Hence, exploring novel drugs/agents that target the Notch signaling pathway in hUCMSC differentiation

3.3 The Role of Cytokines in Mediating hUCMSC Differentiation via the Notch Signaling Pathway

TGF-β and its related signaling pathways play vital roles in mediating the differentiation of stem cells (such as prostate basal stem/progenitor cells, human pluripotent stem cells, and bone marrow mesenchymal stem cells) by regulating Notch signaling [54,85,86]. Notably, TGF-β plays a crucial role in improving the differentiation of hUCMSCs. For example, exosomes isolated from gastric cancer cells enhance the expression of carcinoma-associated fibroblast (CAF) markers through TGF-β transfer and activation of the TGF-β/Smad pathway [87]. Another study revealed that a low dose of TGF-β1 increased fibronectin production by hUCMSCs, while the immunophenotype and differentiation capacity of the latter were not significantly altered [88]. Therefore, TGF-β might affect hUCMSC differentiation through mediating Notch signaling. In addition, basic fibroblast growth factor (bFGF) can reduce scarring by inhibiting the differentiation of epidermal stem cells (ESCs) to myofibroblasts (MFBs) by activating the Notch1/Jagged1 pathway [89]. bFGF-overexpressing hUCMSCs exhibit enhanced proliferation and cell cycle progression and reduced apoptosis [90]. Moreover, the combination of FGF-2 and TGF-β1 promotes the differentiation of human umbilical cord perivascular cells (HUCPVCs) into osteoblast-like cells [91]. bFGF supplementation improved the growth capacity and adipogenic differentiation of unrestricted somatic stem cells isolated from cord blood (CB-USSCs) [92]. Interferon-gamma (IFN-γ), a proinflammatory cytokine, has been found to restrain the differentiation of mouse adult liver and BMHSCs by suppressing the Notch signaling pathway [93]. In addition, a recent study revealed that IFN-γ treatment inhibits chondrogenesis in hUCMSCs. Instead, IFN-γ maintains the potential for multilineage differentiation and immunomodulatory properties of hUCMSCs [94]. Considering the crucial roles of cytokines in stem cells, further ap-

plication of these cytokines for the controlled or directional differentiation of hUCMSCs via the mediation of Notch signaling is warranted.

3.4 Noncoding RNAs Affect hUCMSC Differentiation by Targeting the Notch Signaling Pathway

Several microRNAs (miRNAs) have been found to affect hUCMSC differentiation. For example, the cotransfection of miR-106a, miR-574-3p, and miR-451 collectively prompts the differentiation of hUCMSCs into fully functional hepatocytes [95]. Notch1 has been found to negatively regulate miR-451 expression by regulating the transcription factor AP-1 in lung adenocarcinoma [96]. In another study, miR-451 was shown to act as a potent suppressor of oncogenesis in patients with Notch1-induced T-cell acute lymphoblastic leukemia [97]. MiR-410 has inhibitory effects on the direct retinal pigment epithelium differentiation of hUCMSCs [98], and upregulation of miR-410 by tangeretin was shown to lead to decreased Notch-1, Jagged1/2, Hey-1, and Hes-1 expression [99]. Therefore, miR-451 and miR-410 potentially have opposite effects on hUCMSC differentiation by regulating the Notch1 signaling pathway. Additionally, the miR-18b profile was attenuated within the placental tissues of patients with preeclampsia (PE). EV-derived miR-18b boosted the trophoblast proliferation and migration of hUCMSCs by targeting Notch2 [100]. These studies suggest that miRNAs play vital roles in hUCMSC differentiation by targeting the Notch signaling pathway, and additional miRNAs need to be identified in the future.

4. The Notch Signaling Pathway is Implicated in hUCMSC Therapy

Notch signaling pathway dysregulation is associated with several diseases and disorders. Aberrant Notch signaling can contribute to cancer development and progression, cardiovascular diseases, neurological disorders, autoimmune diseases, and developmental abnormalities [101,102]. Interestingly, hUCMSC therapy affects both the canonical and noncanonical Notch signaling pathways (Fig. 3). For example, hUCMSC transplantation improves liver functions in rats with acute-on-chronic liver failure through the inhibition of Notch and Stat1/Stat3 signaling [103]. Activating Toll-like receptor-3 (TLR3) with poly (I:C) (a ligand of TLR3) enhanced the therapeutic efficacy of hUCMSCs against colitis. Poly (I:C) treatment promoted the expression of prostaglandin E_2 (PGE_2) and Jagged-1, while the immunosuppressive activity of hUCMSCs was reversed by the inhibition of Notch-1 [104]. hUCMSCs enhanced the profile of miR-148a-5p in initiated LX-2 cells and inhibited hepatic fibrosis development. Notch2 is a target gene of miR-148a-5p and is suppressed by hUCMSC treatment [105]. Thus, hUCMSC therapy may exert its effects through a mechanism dependent on the balance of the Notch signaling pathway.

4.1 Cytokine Secretion by hUCMSCs Mediates Notch Signaling

MSCs extracted from adipose, umbilical cord, and placental tissues exhibit differences in the secretion of specific factors. The three MSCs abundantly secreted insulin-like growth factor-binding protein (IGFBP)-4, IGFBP-3, tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-2, IGFBP-6, monocyte chemoattractant protein-1, and granulocyte colony-stimulating factor. Vis-à-vis A-MSCs and P-MSCs, hUCMSCs secrete elevated cytokines, such as HGF, TNF-IR, and TGF-β3 [106]. Therefore, it is postulated that UCMSCs exhibit a more pronounced cytokine secretion profile [23]. The cytokines produced by UCMSCs play essential roles in UCMSC therapy. Bajetto A *et al*. [107] recently reported that metformin inhibits the production of IL-6, MCP-1, and COX-2 by hUCMSCs. Conditioned medium from metformin-treated hUCMSCs markedly mitigated $CD3^+$ T lymphocyte growth and the $CD8^+$ T-cell population in stimulated PBMCs. Monocyte differentiation into DCs and directing monocytes toward other cell types partly depend on the secretion of IL-6 and HGF [108]. Another study revealed a positive feedback loop between Notch signaling and the Janus kinase (JAK)/STAT3 pathway in human blood-derived monocytes. The γ-secretase inhibitor DAPT leads to the inhibition of Notch signaling and suppresses TLR4-stimulated IL-6 production [109]. HGF has also been proven to elicit the osteogenic differentiation of vascular smooth muscle cells by way of c-Met/Akt/Notch3 signaling [110]. Insulin-like growth factor-1 (IGF-1) is a potent mediator of the neural differentiation of hUCMSCs [111], and IGF-1-modified hUCMSCs can contribute to enhanced protection against gentamicin-induced acute kidney injury [112]. hUCMSC injection promoted the expression of IGF-1, VEGF, and HGF in the myocardium, suggesting that these cytokines are involved in hUCMSC-mediated cardioprotective effects [113]. Interestingly, noggin/Dkk-1/IGF-1 induction enhances retinal transdifferentiation of human adipose-derived stem cells, and this effect is further promoted by Notch signaling activation [114]. Recently, C-X-C chemokine receptor type 7 (CXCR7) overexpression was shown to enhance the therapeutic efficacy of hUCMSCs in an acute lung injury mouse model. CXCR7 overexpression augmented SDF-1-induced proliferation and migration of lung epithelial cells (Base-2b cells) and impeded pulmonary fibrosis by attenuating the Notch/Jag1 pathway [115]. Consequently, the Notch pathway, which is stimulated by secreted cytokines in hUCMSCs, potentially involves hUCMSC-mediated therapeutic effects.

4.2 hUCMSC-Derived Microvesicles/Exosomes Regulate Notch Signaling

Stem cell-derived conditioned medium (CM) treatment has been regarded as a practicable approach to overcome limitations such as tumorigenic potential and low

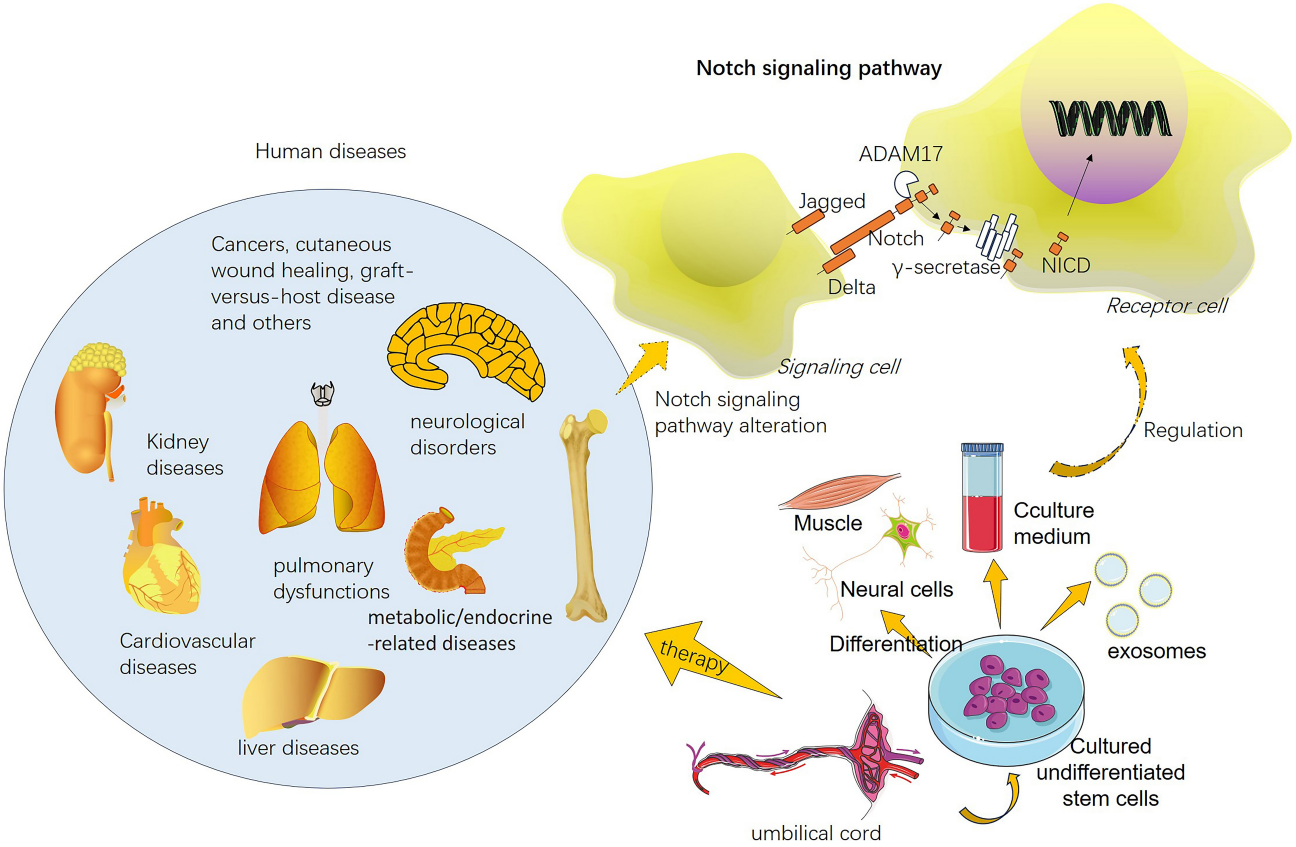

Fig. 3. Human umbilical cord mesenchymal stem cells (hUCMSCs) exert therapeutic effects on both the canonical and noncanonical Notch signaling pathways. hUCMSCs have been applied in the treatment of multiple human diseases, such as kidney diseases, cardiovascular diseases, pulmonary diseases, liver diseases, metabolic/endocrine-related diseases, neurological disorders, and cancers. hUCMSC therapy can mediate the activation of Notch signaling in receptor cells via canonical or noncanonical pathways.

survival rates of transplanted cells [116]. CMs consist of soluble proteins such as cytokines, chemokines, enzymes, cell adhesion molecules, signaling and signal transduction proteins, and growth factors; nucleic acids such as DNA, RNA fragments, and microRNAs; and lipids and extracellular vesicles such as apoptotic bodies, exosomes, and microvesicles. The complex combination of soluble components and vesicular elements is known [117,118]. hUCMSC-derived microvesicles/exosomes show promising therapeutic effects against diseases [119,120]. Notch signaling has been found to play vital roles in hUCMSC-derived microvesicle/exosome therapy (Fig. 4). For example, exosomes derived from hypoxia-preconditioned MSCs (HP-MSC-DEs) heightened the self-renewal capacity and long-term clonogenic potential of hUCMSCs. Jagged-1 shuttled by HP-MSC-DE stimulates the Notch pathway in hUCMSCs [121]. Extracellular vesicles derived from hUCMSCs can alleviate silica-induced epithelial–mesenchymal transition (EMT) in fibrosis miR-26a-5p from hUCMSC-derived extracellular vesicles attenuated the activation of the Adam17/Notch signaling pathway, thus mitigating EMT in silica-elicited pulmonary fibrosis [122]. Chen X *et al.* [123] reported that miR-146a-5p delivered by hUCMSC-derived exosomes significantly relieved bleeding and inflammation and diminished M1 macrophage polarization in a diffuse alveolar hemorrhage (DAH) mouse model. Notch1 was targeted and negatively regulated by miR-146a-5p. Thus, hUCMSC-derived exosomes reversed Notch1 signaling pathway-induced DAH.

5. Conclusions and Perspectives

In this context, we have outlined the latest developments pertaining to the role of the Notch signaling pathway in preserving homeostasis and dictating the cellular fate of hUCMSCs, the factors influencing hUCMSC differentiation through the Notch signaling pathway, and the changes and mechanisms of the Notch signaling pathway in hUCMSC therapy. In the future, there is a need for a more extensive understanding of the biological role of Notch signaling in hUCMSC therapy.

Stem cell-based therapies offer new prospects for addressing a range of challenging-to-treat ailments. Compared with other stem cell sources, hUCMSC therapy is considered relatively safe and ethical. Nonetheless, comprehensive research and carefully structured clinical trials are imperative to gain a comprehensive grasp of the thera-

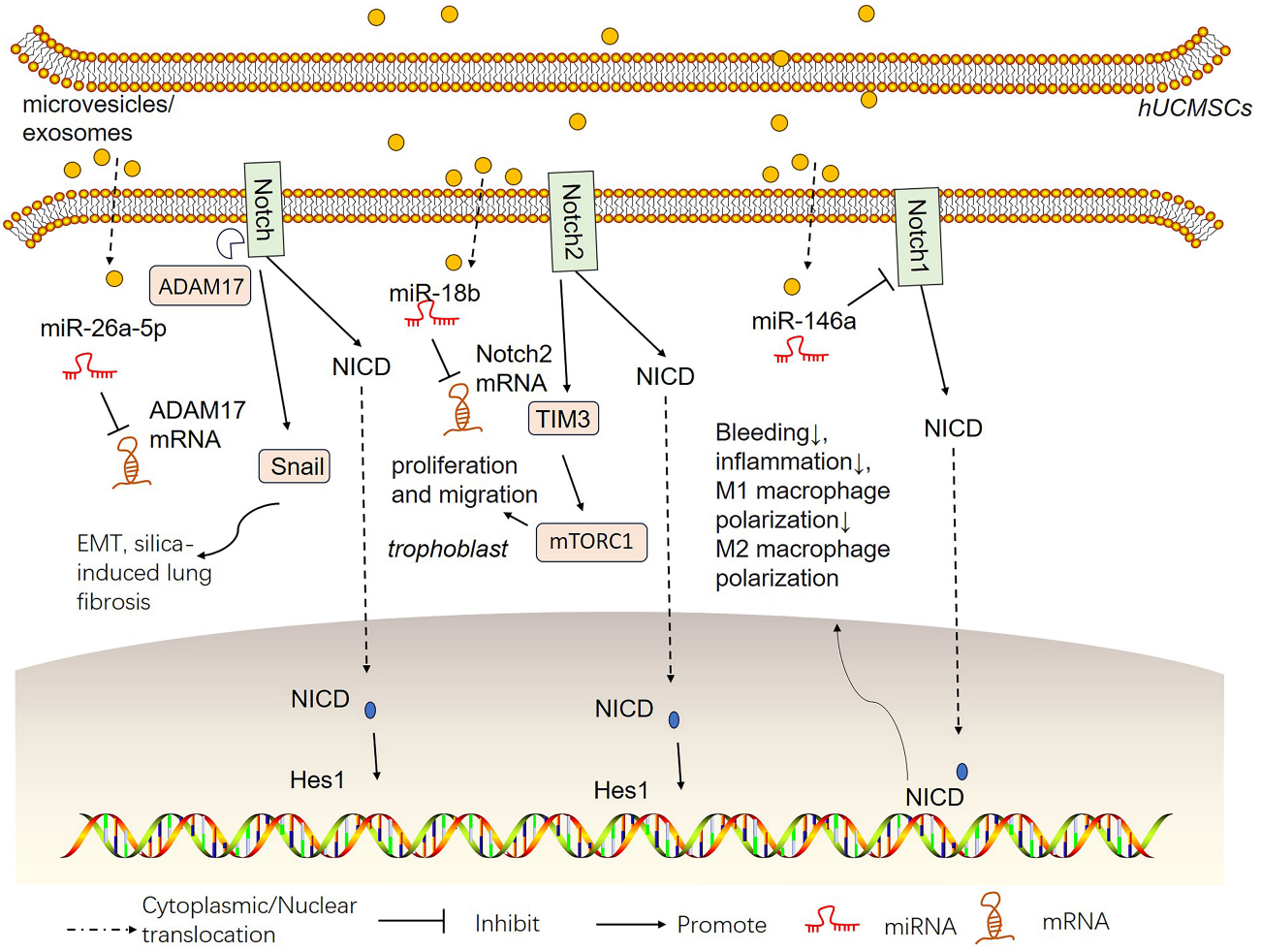

Fig. 4. hUCMSC-derived microvesicles/exosomes affect the Notch signaling pathway in different pathological environments. hUCMSCs can produce microvesicles or exosomes. microRNAs (miRNAs) shuttled by these proteins have therapeutic effects on different pathological environments.

peutic efficacy, ideal dosage, long-term safety, and effectiveness of these agents [124,125]. Moreover, there is a need to maintain cell viability and function during continuous cell passaging [126]. Efficient and controlled differentiation of hUCMSCs into specific cell lineages is challenging. The optimization of differentiation protocols and identification of factors that stimulate lineage-specific differentiation need to be further investigated to improve therapeutic outcomes. Therefore, the differentiation of hUCMSCs holds great promise for future advancements in regenerative medicine and tissue engineering. Further advancements in the differentiation of hUCMSCs are expected, which require continued research, optimization of protocols, and exploration of novel applications.

The Notch signaling pathway is critical for deciphering the intricate mechanisms underlying its functions in cell fate decisions, development, and disease. The interplay among these mediators determines that cellular responses and disease outcomes are influenced by Notch signaling. Mediating the Notch signaling pathway through pharmacological targeting and genetic targeting holds the potential for manipulating hUCMSC behavior and directing their differentiation for various applications in regenerative medicine and disease modeling. However, challenges remain since therapeutic interventions targeting Notch signaling might lead to significant toxicity *in vivo*. Additionally, nonspecific intervention via Notch signaling can yield contrasting outcomes in terms of disease management due to the presence of various Notch mediators within hUCMSCs. For instance, poly (I:C) treatment activates TLR3 and enhances the adipogenic differentiation capability [127] and immunosuppressive properties [104] of UCMSCs by activating the Notch1 signaling pathway. The transcription factor NF-κB can be activated by TLR3 stimulation and promote Notch signaling pathway activation. However, the suppression of NF-κB by honokiol enhances the survival and chondrogenesis of hUCMSCs [128]. Thus, future studies should also address the sophisticated modulation of the Notch signaling pathway in hUCMSC therapy.

Author Contributions

WHX, JXY, LY and CY conceptualized this review. WHX drafted the manuscript. YZ, YQC, XP, and CY generated the figures. WHX reviewed and edited the language. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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