

## Stem Cell Physiology and Cellular Differentiation: Molecular Insights and Therapeutic Potential

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### Abstract

Stem cells are unique cells capable of maintaining self-renewal and differentiating into various specialized cell types. These properties make them crucial components in tissue physiology, homeostasis, and regeneration. Stem cell differentiation is regulated through a complex interplay of molecular signaling pathways, transcription factors, microRNAs, and epigenetic mechanisms. Key pathways such as Wnt, Notch, Hedgehog, and BMP/TGF- $\beta$  play a role in determining cell fate, regulating proliferation, and balancing self-renewal and specialization. Proper regulation ensures that stem cells transition to specific phenotypes in a directed, stable, and physiologically appropriate manner, while

disruption of regulation can lead to tissue degeneration or tumors. Understanding these molecular mechanisms paves the way for innovative regenerative therapies. Pluripotent and multipotent cells are used for cell replacement strategies in degenerative diseases, organ injuries, and hematopoietic disorders. iPSCs allow the engineering of a patient's own cells to minimize the risk of immunoreactions, while MSCs possess immunomodulatory capabilities and secrete paracrine factors that support endogenous tissue repair. Technologies such as 3D organoid culture, biomaterial scaffolds, and gene editing improve control over differentiation, tissue integration, and therapeutic safety before clinical application. Stem cells and cellular differentiation mechanisms form a solid scientific foundation for the development of modern regenerative therapies. Ongoing research in this area is expected to open new opportunities for tissue function restoration, the treatment of degenerative diseases, and the implementation of safe, effective, and precision therapeutic strategies.

**Keywords:** Stem cells, Cellular differentiation, Disease, Molecular signaling, Regenerative medicine

## Introduction

Stem cells are specialized cells with the ability to maintain self-renewal while simultaneously differentiating into various specific cell types [1]. This property makes them a crucial component in tissue physiology, maintaining homeostasis, and supporting regeneration after injury [2]. Based on their differentiation potential, stem cells are grouped into several categories. Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), are capable of forming all somatic cell types [3]. Meanwhile, multipotent stem cells, such as mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs), can only generate a few cell types within a specific tissue lineage [4]. Furthermore, adult stem cells reside in specific tissues and play a role in maintaining homeostasis and local regeneration [5]. In contrast, ESCs isolated from blastocysts have broader differentiation capabilities, but their use faces ethical issues and the risk of tumorigenesis [6].

The ability of stem cells to differentiate in a directed manner is a crucial foundation for cellular physiology and tissue regeneration [7]. This differentiation process is a complex mechanism regulated by molecular signaling pathways, transcription factors, microRNAs, and epigenetic mechanisms such as Deoxyribonucleic Acid (DNA) methylation and histone modifications [8]. Key pathways, including Wingless-related integration site (Wnt), Notch, Hedgehog, and Bone Morphogenetic Protein/Transforming Growth Factor-beta (BMP/TGF- $\beta$ ), influence cell fate determination, triggering the transition from self-renewal to specialization, and

maintaining the balance between proliferation and apoptosis [9]. These mechanisms are not only crucial during embryonic development but also play a role in adult tissue regeneration and the maintenance of organ function [10]. Disruption of the regulation of stem cell differentiation can trigger various pathological conditions, including regeneration failure, organ degeneration, and tumorigenesis [11].

In the realm of regenerative therapy, understanding the physiology and mechanisms of stem cell differentiation has significant clinical implications [12]. Stem cells are being utilized to design therapeutic strategies for a variety of conditions, including cardiovascular disease, neurodegenerative disorders, organ injury, and hematopoietic disorders [13]. iPSCs and ESCs allow for the engineering of a patient's own cells, minimizing the risk of immune reactions, while MSCs possess immunomodulatory capabilities and produce paracrine factors that support endogenous tissue regeneration [14]. Furthermore, technological advances such as 3D organoid culture, biomaterial scaffolds, and gene editing facilitate the regulation of differentiation, control of cell integration, and functional testing prior to *in vivo* transplantation, thereby improving the safety and effectiveness of clinical therapies [15].

Alongside these developments, research also highlights the importance of using *in vitro* and *in vivo* experimental models to understand stem cell physiology and differentiation mechanisms [16]. 2D monolayer culture systems, 3D organoids, co-culture systems, and xenotransplantation animal models enable the analysis of molecular pathways, niche interactions, and regenerative capacity of stem cells under

conditions more closely resembling natural physiology [17]. This approach is crucial for assessing efficacy, tissue integration, and potential risks of tumorigenesis before clinical application [18].

This review aims to provide a comprehensive overview of stem cell physiology and cellular differentiation mechanisms, focusing on molecular pathways, epigenetic regulation, and interactions with the microenvironment. It also assesses their therapeutic potential and clinical challenges. This approach is expected to provide a comprehensive understanding of the role of stem cells in maintaining tissue homeostasis, organ regeneration, and the development of modern regenerative therapies. It also identifies opportunities for future research and clinical applications.

### Data collection method

A narrative, theory-anchored review was conducted to synthesize advances in stem cell physiology, differentiation mechanisms, and therapeutic applications. Literature was identified via PubMed/MEDLINE, Scopus, Web of Science, and targeted Google Scholar searches for peer-reviewed articles published from January 2016 to November 2025. Boolean combinations of keywords/MeSH terms covered signaling pathways (Wnt/Notch/Hedgehog/BMP-TGF- $\beta$ ), core factors (OCT4/SOX2/NANOG), epigenetic regulation, experimental models (2D, organoids, animal, clinical), bioengineering (biomaterials, bioprinting, CRISPR), and target organs (heart, CNS, liver). Inclusion encompassed primary studies, reviews, meta-analyses, guidelines, and methods relevant to stem cell identity, signaling/epigenetics, niche-mechanics-metabolism, and translational safety; exclusions were non-peer-reviewed preprints (unless corroborated), editorial pieces without data, and off-topic studies. Two-stage screening with reference snowballing was applied; overlapping datasets prioritized the most comprehensive or recent source. Data were extracted on design, model/system, key pathways, epigenetic/metabolic readouts, and translational endpoints and synthesized across five axes (pluripotency control, signaling crosstalk, niche-metabolism coupling, experimental models, efficacy/safety). Bias was mitigated by emphasizing high-quality evidence and cross-validation, while topic

breadth and model heterogeneity limited meta-analysis and leave rapidly evolving areas potentially undercovered.

### Stem cell physiology

Stem cell physiology reflects the unique ability of cells to maintain their identity through self-renewal, while maintaining pluripotent or multipotent potential, which is governed by cell cycle regulation, transcriptional signaling pathways, and complex epigenetic mechanisms.

#### Basic characteristics of stem cells

Stem cells are characterized by two key properties that underlie their role in tissue physiology and regenerative therapies: Self-renewal and differentiation potential [19]. Self-renewal allows stem cells to divide symmetrically or asymmetrically, thus maintaining the stem cell population without losing its identity [20]. This process is regulated by a complex network of molecular signals, including the Wnt/ $\beta$ -catenin, Notch, and Phosphoinositide 3-kinase/Protein Kinase B (PI3K/AKT) pathways, which control cell proliferation while preventing premature differentiation [21]. Furthermore, epigenetic regulation plays a crucial role through histone modifications, DNA methylation, and microRNAs, ensuring that the expression of key genes that determine stem cell identity is maintained according to physiological needs [22].

Differentiation potential reflects the ability of stem cells to produce offspring with specific phenotypes [23]. Based on their level of potency, stem cells are classified as: Pluripotent, which can form almost all types of somatic cells; multipotent, which is limited to a few specific cell lineages; and unipotent, which produces only one specialized cell type [24]. For example, embryonic stem cells (ESCs) are pluripotent, while MSCs are multipotent and can differentiate into osteoblasts, chondrocytes, and adipocytes [25]. Furthermore, interactions with the microenvironmental niche and external signals also determine the direction of differentiation, so that phenotypic changes occur in a coordinated and adaptive manner according to tissue needs [26].

The balance between self-renewal and differentiation is crucial for maintaining tissue homeostasis [27]. Imbalances in this regulation can

trigger excessive cell proliferation or reduced regenerative capacity, potentially leading to degenerative diseases and tumorigenesis [28].

### **Cell cycle regulation and homeostasis mechanisms**

The cell cycle is a coordinated series of stages that allow stem cells to maintain self-renewal while preparing for differentiation when needed [29]. In stem cells, cell cycle regulation not only controls proliferation but also maintains genome integrity and the balance between symmetric and asymmetric division [30]. The major stages of the cell cycle—Gap 1 phase (G1), Synthesis phase (S), Gap 2 phase (G2), and Mitosis phase (M)—are regulated by complex interactions between cyclin-dependent kinases (CDKs), cyclins, and CDK inhibitors (CKIs), which act as checkpoints to prevent the proliferation of cells experiencing DNA damage or mitotic errors [31].

Stem cells have a relatively short G1 phase, allowing rapid proliferation while maintaining differentiation capacity [32]. Molecular signaling pathways, such as PI3K/AKT, Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase (MAPK/ERK), and Notch, play a role in regulating CDK and cyclin activity, allowing stem cells to balance proliferation and maintain their identity [33]. DNA checkpoint mechanisms, including Tumor protein p53 (p53) and Ataxia Telangiectasia Mutated/ATM and Rad3-related (ATM/ATR) activation, ensure that cells with severe genetic damage undergo apoptosis or premature differentiation, thus minimizing the risk of mutation and tumorigenesis [34].

Stem cell homeostasis is also influenced by interactions with the microenvironmental niche, which provides autocrine and paracrine signals to maintain the stem cell population in a quiescent or active state [35]. In the quiescent state, stem cells are in the Gap 0 phase (G0), minimizing excessive division while maintaining regenerative capacity [36]. Activation of stem cells from the quiescent state occurs in response to physiological needs or tissue injury, controlled by a combination of growth factor signals, extracellular matrix, and mechanotransduction [37].

In addition to cell cycle regulation, stem cell homeostasis also relies on epigenetic and metabolic mechanisms [38]. Histone modifications and DNA

methylation regulate the expression of genes associated with proliferation and differentiation, while metabolic pathways, such as glucose oxidation and oxidative phosphorylation, adjust energy availability to the cell's specific conditions [39]. The combination of cell cycle regulation, niche signals, and epigenetic control ensures that stem cells maintain their identity, genetic integrity, and regenerative capacity, which underpins stem cell physiology and stem cell-based therapeutic applications [40].

### **Transcription factors and signaling networks that maintain stem cell identity**

Stem cell identity is controlled by tight transcriptional regulation, with nuclear transcription factors acting as master regulators in maintaining pluripotency and self-renewal [41]. In pluripotent stem cells, factors such as Octamer-binding transcription factor 4 (OCT4), SRY-related HMG-box gene 2 (SOX2), and NANOG form a transcriptional network that interacts both autoregulatory and cross-regulatory [42]. OCT4 maintains pluripotent status by activating genes that promote self-renewal and suppressing premature differentiation pathways [43]. SOX2 works synergistically with OCT4 to maintain the expression of key target genes, while NANOG strengthens the stability of the transcriptional network and increases resistance to external differentiation signals [44]. **Table 1** summarizes the nuclear transcription factors and the molecular signaling networks that play a role in maintaining pluripotent stem cell identity.

In addition to nuclear transcription factors, molecular signaling networks from the external environment also play a crucial role in modulating transcriptional activity and maintaining stem cell homeostasis [45]. In mouse ESCs, the Leukemia Inhibitory Factor/Signal Transducer and Activator of Transcription 3 (LIF/STAT3) pathway, and in human ESCs, the Fibroblast Growth Factor/Extracellular Signal-Regulated Kinase (FGF/ERK) pathway, interact with nuclear transcription factors to balance self-renewal and differentiation readiness [46]. The Wnt/ $\beta$ -catenin pathway supports proliferation and maintains stem cell identity by enhancing the expression of pluripotency genes, while the Notch and Hedgehog pathways play a role in maintaining the stem cell niche

and modulating responses to environmental signals [47].

The interactions between transcription factors and signaling pathways are not linear, but rather form a dynamic and redundant network [48]. For example, activation of  $\beta$ -catenin through the Wnt pathway can increase the expression of OCT4 and NANOG, while the Notch pathway helps stabilize SOX2 expression in the quiescent state [49]. Epigenetic mechanisms are also integrated into this network; histone modifications and DNA methylation facilitate the access of

transcription factors to chromatin and maintain the gene expression patterns characteristic of stem cells [50].

Coordination between transcription factors and signaling networks is crucial for stem cells to remain pluripotent and respond to physiological needs [3]. Disruption of these networks, whether due to genetic mutations or dysregulated signals from the microenvironment, can result in loss of stem cell identity, premature differentiation, or neoplastic transformation [51].

**Table 1** Transcription factors and signaling networks in the maintenance of stem cell identity.

Component	Main function	Related signal paths	Interaction mechanism
OCT4	Maintaining pluripotent status and self-renewal	Wnt/ $\beta$ -catenin and LIF/STAT3	Activates self-renewal genes and suppresses premature differentiation pathways and expression can be enhanced by $\beta$ -catenin
SOX2	Supports pluripotency and expression of critical target genes	Notch	Works synergistically with OCT4 and stabilization of expression by Notch in quiescent conditions
NANOG	Strengthening the stability of the transcription network	Wnt/ $\beta$ -catenin	Supports resistance to external differentiation signals and expression can be enhanced by $\beta$ -catenin
LIF/STAT3	Maintain self-renewal on mouse ESC	Core transcription factors	Interacts with OCT4/SOX2/NANOG to balance proliferation and differentiation readiness
FGF/ERK	Maintaining self-renewal in human ESC	Core transcription factors	Modulating OCT4, SOX2, and NANOG activity for self-renewal-differentiation balance
Wnt/ $\beta$ -catenin	Supports stem cell proliferation and identity	OCT4 and NANOG	$\beta$ -catenin activation increases OCT4 and NANOG expression
Notch	Maintaining niche and cell response to the environment	SOX2	Stabilizing SOX2 expression under quiescent conditions
Hedgehog	Modulating cell response to niche	Core transcription factors	Supports homeostasis and readiness for stem cell differentiation
Epigenetics (DNA methylation and histone modifications)	Facilitates access of transcription factors to chromatin	OCT4, SOX2, and NANOG	Ensures stem cell-specific gene expression profiles; supports transcription and signaling networks

### **Epigenetic mechanisms that influence stem cell stability**

Stem cell stability and the ability to maintain their identity are largely determined by epigenetic regulation, which regulates gene expression without altering the DNA sequence [52]. Key epigenetic mechanisms include histone modification, DNA methylation, and regulation by non-coding ribonucleic acid (RNA), which work synergistically to maintain self-renewal and pluripotency [53].

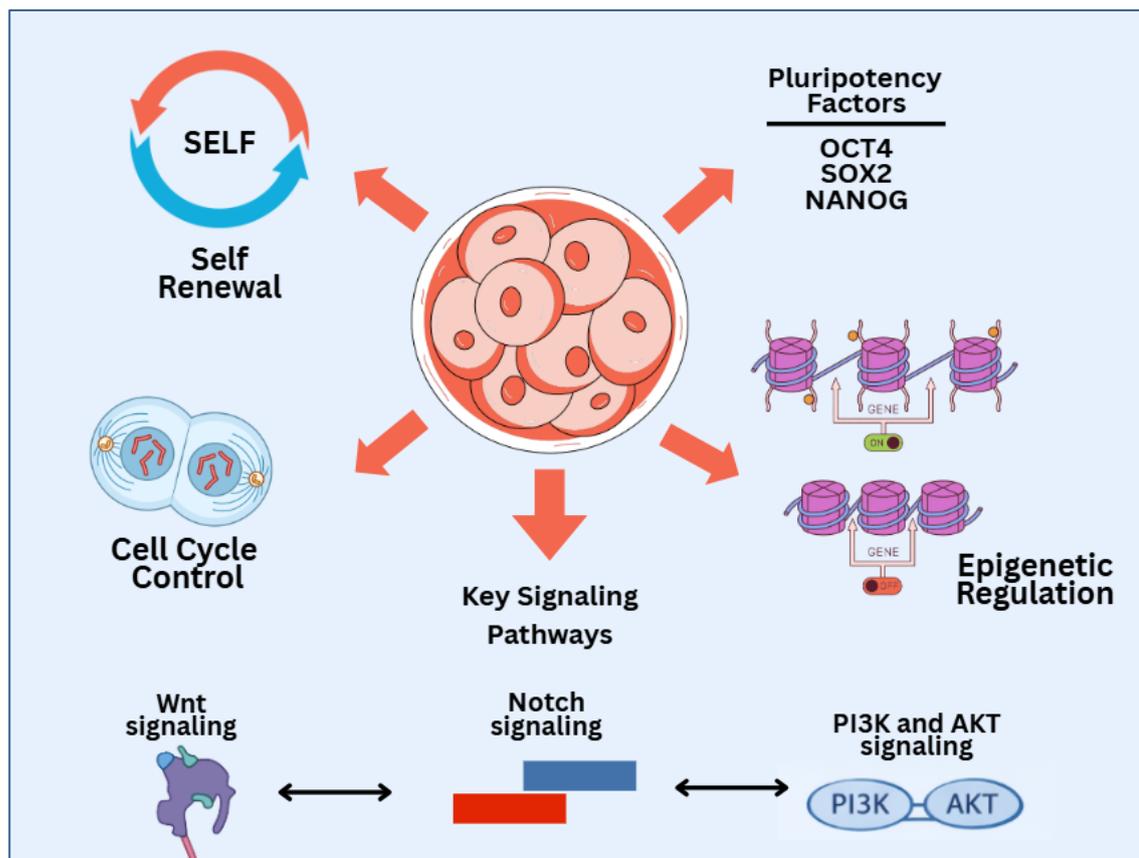
Histone modifications play a crucial role in regulating chromatin accessibility and gene transcriptional status [54]. For example, H3 lysine 4 methylation (H3K4me3) is associated with active genes that support pluripotency, while H3 lysine 27 trimethylation (H3K27me3) marks repressed differentiation genes, forming bivalent domains [55]. This bivalent configuration allows stem cells to remain flexible, ready to differentiate rapidly upon receiving specific signals, while maintaining their basic cellular identity [56].

DNA methylation in gene promoter regions is also crucial for stabilizing stem cell phenotypes [57]. Genes that support pluripotency generally remain hypomethylated, maintaining their expression, while genes that promote differentiation remain hypermethylated to prevent premature activation [58]. These methylation dynamics are regulated by DNA methyltransferase (DNMT) enzymes and Ten-Eleven Translocation (TET) dioxygenases, which interact with

nuclear transcription factors such as OCT4, SOX2, and NANOG, thus maintaining a balance between self-renewal and differentiation readiness [59].

In addition, microRNAs and long non-coding RNAs (lncRNAs) play a role in maintaining stem cell stability by targeting specific genes involved in the cell cycle, apoptosis, and differentiation pathways [60]. For example, microRNAs from the microRNA (miR)-290/295 family in mouse ESCs or miR-302 in human ESCs suppress the expression of pro-differentiation factors while supporting proliferation [61]. Some lncRNAs also function as scaffolds to recruit chromatin remodeling complexes, thereby enhancing the expression of pluripotency genes and suppressing differentiation genes [62].

The integration of these various epigenetic mechanisms creates a dynamic chromatin landscape, allowing stem cells to maintain their identity while remaining responsive to external signals [63]. Epigenetic disruption, whether due to mutations in chromatin-modifying enzymes or exposure to damaging environments, can lead to loss of pluripotency, premature differentiation, or neoplastic transformation [64]. Stem cell identity and physiological homeostasis are maintained through the coordinated regulation of self-renewal capacity, cell cycle control, pluripotency transcription factors, epigenetic mechanisms, and key signaling pathways, as illustrated in **Figure 1**.



**Figure 1** Regulation of stem cell identity and physiology.

### Molecular mechanisms of cellular differentiation

Cell differentiation is a coordinated process regulated by key molecular signaling pathways, specific transcriptional regulation, microRNAs, and epigenetic dynamics, which together determine cell fate and its interaction with the microenvironmental niche.

#### Main signal path

Cell differentiation is controlled by evolutionarily conserved molecular signaling pathways that play a role in cell fate determination, proliferation, and interactions with the microenvironment [65]. Pathways such as Wnt, Notch, Hedgehog, and Bone Morphogenetic Protein/Transforming Growth Factor-beta (BMP/TGF- $\beta$ ) form complex regulatory networks, enabling the phenotypic transition of stem cells into specific cells in a coordinated and timely manner [9]. **Table 2** summarizes the major molecular signaling pathways that regulate stem cell differentiation and maintenance.

The Wnt pathway acts as a key regulator of pluripotency and early differentiation [10]. Wnt activation results in the accumulation of  $\beta$ -catenin in the cytoplasm and its translocation to the nucleus, where it interacts with T-cell factor/Lymphoid enhancer-binding factor (TCF/LEF) transcription factors to activate the expression of genes supporting pluripotency [47]. This pathway also plays a role in determining the mesoderm and endoderm lineages in embryonic stem cells and balancing proliferation and differentiation in adult tissues [66]. Dysregulation of the Wnt pathway can lead to excessive proliferation or failure of differentiation, potentially leading to tumorigenesis [67].

Notch is an intercellular communication pathway that relies on ligand-membrane receptor interactions [68]. Notch activation leads to proteolytic cleavage of the receptor, generating intracellular fragments (NICDs) that enter the nucleus to modulate target gene expression [69]. This pathway plays a crucial role in maintaining stem cells in a quiescent state, determining the direction of progenitor cell differentiation, and

regulating tissue proliferation [70]. Notch effects are contextual, strongly influenced by cell type, signal strength, and interactions with other pathways such as Wnt and Hedgehog [71].

The Hedgehog pathway controls cell proliferation, tissue patterning, and specialization [72]. Activation of this pathway through the ligand Sonic Hedgehog (Shh) inactivates the protein Patched, allowing Smoothened to activate the transcription factor Glioma-associated oncogene (Gli) [73]. Gli then regulates gene expression that supports stem cell proliferation and specific differentiation [74]. The Hedgehog pathway plays a crucial role in organ formation during embryogenesis and in maintaining homeostasis of adult tissues, including the nervous and hematopoietic systems [75].

Bone Morphogenetic Protein (BMP) and Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) are cytokine ligands that regulate differentiation through activation

of serine/threonine kinase receptors and phosphorylation of Smads [76]. These pathways determine mesodermal and endodermal cell lineage specialization and interact with the Wnt and Notch pathways to direct differentiation spatiotemporally [77]. BMPs tend to promote mesenchymal cell differentiation, while TGF- $\beta$  can induce epithelial differentiation while suppressing excessive proliferation, thus playing a crucial role in maintaining tissue homeostasis [78].

Coordination between the Wnt, Notch, Hedgehog, and BMP/TGF- $\beta$  pathways forms a dynamic signaling network, enabling stem cells to respond to both internal and external stimuli [9]. Cross-reactions between these pathways ensure directed, stable, and physiologically appropriate differentiation, while maintaining a stem cell population ready for tissue regeneration [79].

**Table 2** Key signaling pathways in stem cell differentiation and maintenance.

Signal path	Activation mechanism	Main function	Interaction/Coordination
Wnt/ $\beta$ -catenin	Wnt activation $\rightarrow$ accumulation of $\beta$ -catenin $\rightarrow$ translocation to the nucleus $\rightarrow$ interaction with TCF/LEF	Maintaining pluripotency, specifying mesoderm and endoderm lineages, and regulating proliferation vs differentiation	Interacting with Notch, BMP/TGF- $\beta$ and dysregulation can lead to excessive proliferation or failure of differentiation
Notch	Ligand-membrane receptor $\rightarrow$ receptor cleavage $\rightarrow$ NICD enters nucleus $\rightarrow$ target gene modulation	Maintains stem cell quiescence, determines the direction of progenitor cell differentiation, and regulates tissue proliferation	Contextual effects and interactions with Wnt and Hedgehog
Hedgehog (Hh)	Sonic Hedgehog (Shh) ligand inactivates Patched $\rightarrow$ Smoothened activates $\rightarrow$ Gli regulates gene expression	Regulates proliferation, tissue patterning, and cell specialization and is important during embryogenesis and adult tissue homeostasis	Coordination with Notch and Wnt for organ formation and homeostasis
BMP/TGF- $\beta$	Ligand activates serine/threonine kinase receptors $\rightarrow$ Smad phosphorylation $\rightarrow$ modulation of gene expression	Determines mesoderm and endoderm specialization, promotes mesenchymal (BMP) or epithelial (TGF- $\beta$ ) differentiation, and regulates tissue homeostasis	Interacts with Wnt and Notch to direct spatiotemporal differentiation

### Specific transcriptional regulation and microRNAs

Stem cell differentiation relies heavily on specific transcriptional regulation, where transcription factors regulate the expression of genes that determine cell fate [80]. Factors such as GATA-binding transcription factor (GATA), PU box-binding protein 1 (PU.1),

Runt-related transcription factor (RUNX), and Paired box (PAX) direct differentiation pathways toward specific lineages, such as hematopoietic, mesenchymal, or neural [81]. The interactions between these transcription factors form a coordinated regulatory network, enabling directed cellular transitions while maintaining phenotypic integrity and stability [82]. For

example, the combination of GATA1 and PU.1 determines the specialization of hematopoietic progenitor cells toward erythroid or myeloid, while RUNX2 promotes osteogenic differentiation in mesenchymal stem cells [83].

In addition to transcriptional regulation, miRNAs act as post-transcriptional regulators, stabilizing differentiation pathways and preventing the expression of genes inconsistent with the desired cell fate [84]. miRNAs target specific mRNAs for degradation or translational inhibition, thereby reinforcing differentiation decisions driven by transcription factors [85]. For example, miR-290/295 in mouse ESCs and miR-302/367 in human ESCs repress pro-differentiation genes while promoting self-renewal, while miR-124 and miR-9 direct neural progenitors toward a mature neuronal phenotype [61]. miRNAs also serve as buffers against fluctuations in extrinsic signals, maintaining consistent gene expression during differentiation and enabling adaptive responses to microenvironmental conditions [86].

The interaction between specific transcription factors and microRNAs forms a multilevel regulatory network that ensures proper cellular differentiation [85]. Transcription factors can regulate miRNA expression, while miRNAs can modulate the levels of specific transcription factors, creating a feedback loop that reinforces the direction of differentiation [87]. This dynamic allows stem cells to balance self-renewal and specialization, ensuring that differentiation occurs only when both internal and external signals are adequate [88].

Dysregulation of transcription factors or microRNAs can trigger premature differentiation, loss of stem cell identity, or neoplastic transformation, emphasizing the importance of maintaining the integrity of these regulatory networks [89].

#### **Epigenetic dynamics during differentiation**

The process of cell differentiation in stem cells involves complex and coordinated epigenetic changes, enabling cells to adopt specific phenotypes while maintaining genetic integrity [90]. These epigenetic dynamics include histone modifications, DNA methylation and demethylation, and regulation by non-coding RNA, which collectively orchestrate specific

gene expression programs for specific cell lineages [91].

During differentiation, histone modifications play a role in opening or closing chromatin to allow transcription factors to access it [92]. For example, trimethylation of H3 lysin 27 (H3K27me3) marks repressed differentiation genes in stem cells, while acetylation of H3 lysin 27 (H3K27ac) activates specific target genes as cells begin to differentiate [93]. Bivalent domains, which are combinations of H3K4me3 and H3K27me3 at specific genes, allow cells to remain flexible, allowing differentiation genes to be activated immediately upon receiving specific signals [56].

DNA methylation also exhibits significant dynamics [94]. Genes that support pluripotency tend to be hypomethylated to maintain active expression, while differentiation genes remain hypermethylated until the differentiation pathway is activated [95]. DNMT enzymes and TET dioxygenases play a role in regulating this methylation, enabling the proper transition between self-renewal and specialization states [59].

Additionally, non-coding RNAs, including microRNAs and lncRNAs, play a crucial role in epigenetic modulation during differentiation [96]. MicroRNAs target pro-differentiation factor mRNAs for degradation or translational inhibition, thereby facilitating the linear transition from stem cell phenotype to programmed cell phenotype [97]. LncRNAs can serve as scaffolds to recruit chromatin remodeling complexes or histone modification enzymes, which modulate chromatin status at key differentiation genes [98].

These epigenetic dynamics are flexible yet controlled, allowing stem cells to respond adaptively to both internal and external signals [99]. Dysregulation of these mechanisms, whether due to mutations in epigenetic enzymes or exposure to environmental stress, can lead to premature differentiation, loss of cell identity, or neoplastic transformation [100].

#### **Interaction with micro-environmental niches**

Stem cells do not function autonomously but are constantly influenced by their microenvironmental niche—a cellular and molecular network that regulates stem cell behavior, proliferation, and differentiation

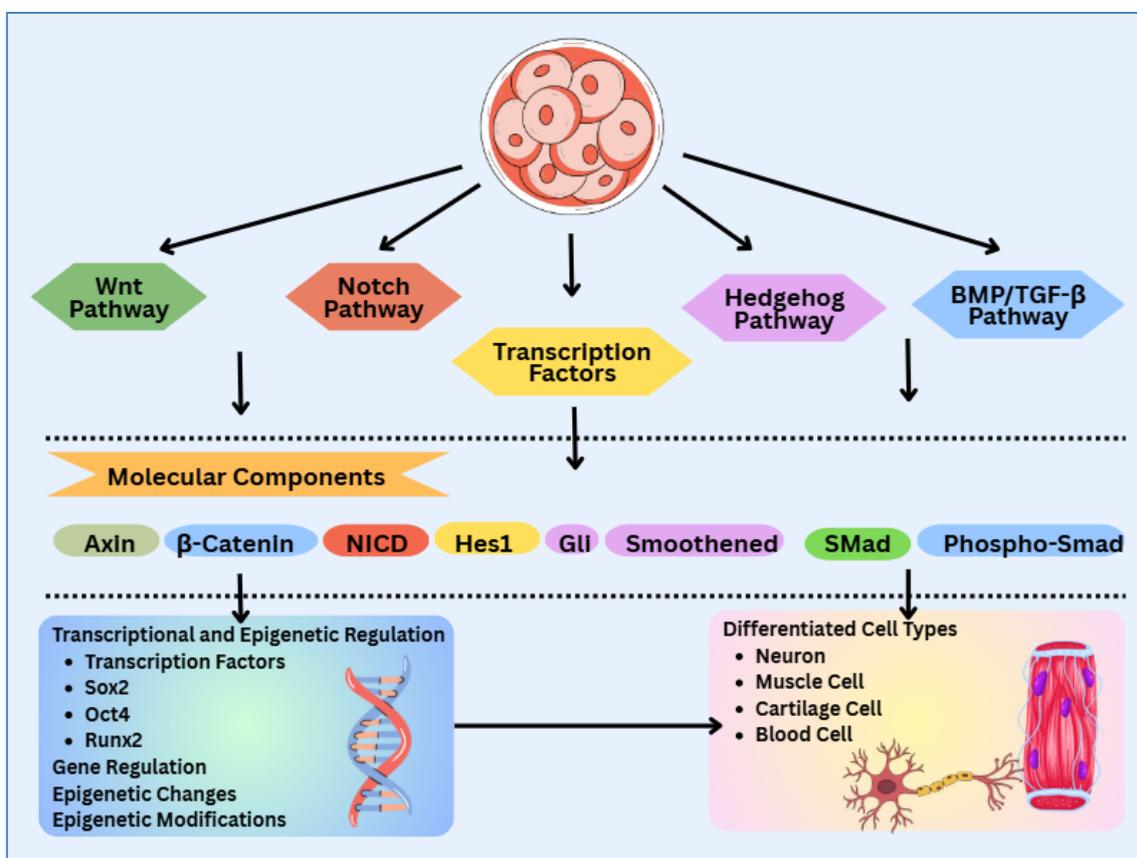
[101]. This niche consists of stromal cells, extracellular matrix (ECM), growth factors, cytokines, and mechanical and metabolic components that form a dynamic and adaptive microecosystem [102]. These interactions ensure a balance between self-renewal and differentiation in the stem cell pool according to the physiological needs of the tissue [103].

Communication between stem cells and their niche occurs through several mechanisms. First, paracrine and autocrine signals, such as Leukemia Inhibitory Factor (LIF), Wnt, Notch ligands, and BMP/TGF- $\beta$ , convey molecular instructions that determine cell fate [104]. These pathways not only influence proliferation and differentiation but also maintain the quiescent state of stem cells during homeostasis [105]. Second, physical interactions with the ECM and cell adhesion via integrins or cadherins transmit mechanotransduction signals, which modulate gene expression, transcription factor activity, and cytoskeleton reorganization [106].

Furthermore, the microenvironmental niche also regulates the availability of metabolites and oxygen,

which influence the metabolic profile of stem cells [107]. Hypoxic conditions in certain niches, such as the bone marrow, increase the expression of Hypoxia Inducible Factor 1 Alpha (HIF-1 $\alpha$ ), which supports self-renewal while preventing premature differentiation [108]. Niches also provide protective mechanisms, including anti-apoptotic factors and antioxidant enzymes, to maintain genome integrity and prevent the accumulation of mutations [109].

These interactions are dynamic and context-dependent; changes in the niche due to injury, inflammation, or aging can trigger stem cell activation, modulate differentiation pathways, or even dysregulate proliferation [110]. Disruption of the relationship between stem cells and their niche can result in loss of tissue homeostasis, decreased regenerative capacity, or neoplastic transformation [111]. The molecular mechanisms of stem cell differentiation are illustrated in **Figure 2**, showing how major signaling pathways converge on transcriptional regulation and lineage commitment.



**Figure 2** Stem cell differentiation pathways.

## **Interaction between stem cell physiology and differentiation**

The interplay between stem cell physiology and cellular differentiation exhibits a dynamic balance between self-renewal, proliferation, and specialization, which is regulated by molecular signaling pathways, transcriptional regulation, and microenvironmental factors, and can be studied using both *in vitro* and *in vivo* experimental models.

### **How stem cell physiology determines differentiation capacity**

Stem cell physiology directly influences differentiation capacity through a complex integration of cell cycle regulation, molecular signaling pathways, transcription factors, and epigenetic mechanisms [112]. Effective self-renewal, optimal metabolic conditions, and interactions with the microenvironmental niche form the basis of stem cells' ability to differentiate appropriately [113].

First, cell cycle status influences differentiation readiness. Stem cells with a longer G1 phase are more ready to respond to differentiation signals, while cells undergoing rapid proliferation tend to maintain self-renewal [32]. Checkpoint regulation, CDK/cyclin activity, and pathways such as PI3K/AKT and MAPK/ERK balance proliferation and differentiation readiness, allowing cellular transitions to occur in a controlled manner [33].

Second, the metabolic profile of stem cells plays a crucial role. Pluripotent stem cells generally rely on glycolytic metabolism, supporting rapid proliferation while maintaining pluripotency [114]. As differentiation begins, a shift toward oxidative metabolism occurs, providing energy and metabolites essential for the development of specific cell phenotypes [115]. Thus, the physiological status of stem cells determines their ability to effectively respond to differentiation signals [38].

Third, nuclear transcription factors such as OCT4, SOX2, and NANOG, along with epigenetic regulation (histone modification, DNA methylation, and microRNA), form the genetic and chromatin basis for differentiation [116]. Transcription factors maintain the expression of pluripotency genes while suppressing premature differentiation pathways [117]. When cells

receive specific signals, epigenetic changes unlock differentiation genes, allowing stem cells to execute a linear program toward their targeted phenotype [118].

Fourth, interactions with the microenvironmental niche provide an external context that directs differentiation pathways [119]. Paracrine factors, ECM adhesion, mechanotransduction, and hypoxic conditions can modulate the activity of the Wnt, Notch, Hedgehog, or BMP/TGF- $\beta$  pathways, allowing stem cells to adjust proliferation and differentiation according to the physiological needs of the tissue [51].

### **The relationship between self-renewal, proliferation, and specialization**

Stem cells maintain tissue homeostasis through a complex coordination of self-renewal, proliferation, and differentiation [120]. Self-renewal allows stem cells to maintain a population with pluripotent or multipotent identities, while proliferation ensures sufficient cell numbers for tissue regeneration [121]. Differentiation allows stem cells to produce phenotypically programmed progeny, forming functional tissues [122]. These three aspects are dynamically interconnected, allowing cellular differentiation to proceed without depleting the stem cell reservoir [123].

Self-renewal and proliferation are controlled by intrinsic and extrinsic signaling pathways, including Wnt/ $\beta$ -catenin, Notch, Hedgehog, and PI3K/AKT [124]. These pathways balance symmetric division, which increases the number of stem cells, with asymmetric division, which produces one stem cell and one progenitor cell for differentiation [125]. Symmetric division supports the expansion of the stem cell population during growth or regeneration, while asymmetric division allows integration between self-renewal and specialization [126]. Cell cycle checkpoint mechanisms and epigenetic control ensure proliferation occurs without loss of cell identity or the introduction of potentially pathological genetic mutations [127].

Cellular specialization relies on the integration of signals from specific transcription pathways and epigenetic mechanisms [128]. Core transcription factors such as OCT4, SOX2, and NANOG maintain the expression of pluripotency genes, while activation of lineage-specific factors such as GATA, RUNX, or

PAX drives stem cells to transition to a specific phenotype [129]. Epigenetic dynamics, including histone modifications and DNA methylation, stabilize differentiation decisions, so that progenitor cell proliferation remains aligned with the desired specialization [91].

The interplay between self-renewal, proliferation, and specialization allows stem cells to adaptively respond to the physiological needs of tissues [130]. For example, tissue injury triggers stem cell proliferation through the activation of signaling pathways, followed by specific differentiation to replace lost cells while maintaining the self-renewing reservoir [131]. Conversely, dysregulation of this relationship can lead to loss of the stem cell reservoir, excessive proliferation, or premature differentiation, potentially leading to regeneration failure or tumorigenesis [132].

#### Examples of *in vitro* and *in vivo* experimental models

A deep understanding of stem cell physiology and cellular differentiation mechanisms has largely been achieved through *in vitro* and *in vivo* experimental models, which allow for controlled observation of molecular and phenotypic processes [133]. These models have become essential tools for elucidating signaling pathways, transcriptional regulation, epigenetic interactions, and the therapeutic potential of stem cells [1]. **Table 3** summarizes the *in vitro* and *in vivo* experimental models used to study stem cell physiology and differentiation mechanisms.

*In vitro* models allow precise manipulation of the cell environment and direct observation of proliferation, self-renewal, and differentiation [134]. ESC culture is a classic model, in which mouse or human ESCs are maintained in media containing growth factors such as LIF or basic Fibroblast Growth Factor (bFGF) to maintain pluripotency [135]. Differentiation is directed using a combination of extrinsic factors, such as BMP4 for the mesodermal pathway or retinoic acid for the neuroectodermal pathway [136].

iPSCs are also an important model for studying linear differentiation in human cells, allowing the evaluation of tissue-specific phenotypes without the need for embryonic material [137]. 2D monolayer culture protocols or 3D organoids are used to mimic

the native tissue environment, including brain, liver, pancreas, and heart organoids, which replicate intercellular interactions, polarity, and three-dimensional differentiation [138].

Co-culture systems and biomaterial scaffolds are also used to mimic microenvironmental niches [139]. For example, co-culture of MSCs with endothelial cells or osteoblasts allows studies of paracrine signaling and intercellular interactions in the regulation of self-renewal and differentiation [140]. Synthetic polymer matrices or hydrogels support proliferation and linear differentiation by providing mechanical and adhesive signals that mimic native tissue [141].

Reporter genes and live-cell imaging are often used to monitor the expression of transcription factors or signaling pathways in real time [142]. For example, using Green Fluorescent Protein (GFP) reporters for OCT4 or SOX2 in ESCs allows observation of pluripotency status when the Wnt or Notch pathways are manipulated [143].

Meanwhile, *in vivo* models provide a complete physiological context for evaluating cell integration, specialization, and tissue function [144]. Mouse chimeras and transplantation are classic models, in which ESCs or iPSCs are introduced into embryonic or adult tissues to assess the contribution of stem cells to organ formation and tissue homeostasis [145]. Another example is the xenotransplantation of human stem cells into immunodeficiency models (e.g., NOD/SCID mice) to evaluate linear differentiation and therapeutic potential [146].

*In vivo* lineage tracing using Cyclization recombinase-Locus of crossover in P1 (Cre-LoxP) or fluorescent reporter systems allows tracking the origin and fate of progenitor cells in complex tissues, as well as identifying cell populations involved in regeneration or pathology [147]. Furthermore, injury-induced regeneration models, such as those involving the heart, brain, or bone marrow, allow evaluation of how stem cells proliferate, differentiate, and interact with their microenvironmental niches in both physiological and pathological contexts [148].

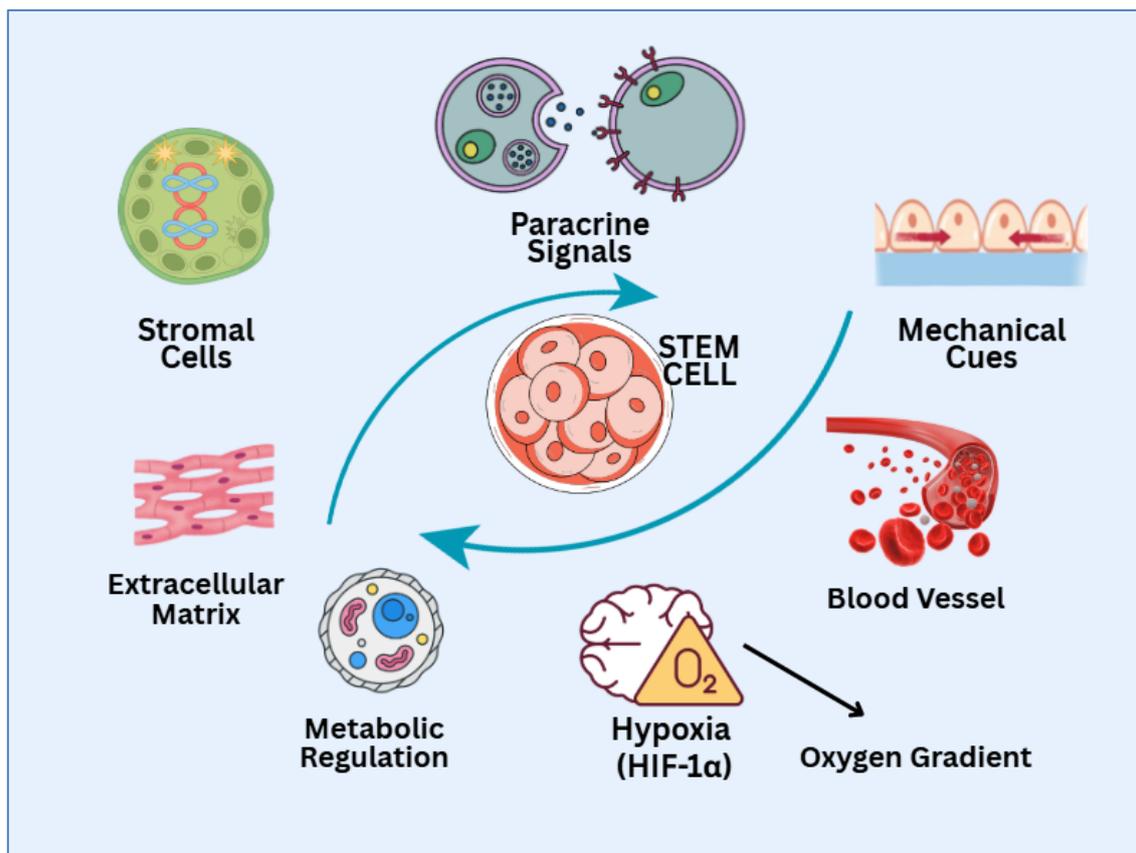
Combined *in vitro* and *in vivo* approaches are also being applied, for example by transplanting organoids into experimental animals to assess their integration and functional capabilities, or by pre-conditioning stem cells *in vitro* before use in *in vivo* regenerative

therapies [149]. As illustrated in **Figure 3**, stem cell fate is dynamically regulated by reciprocal interactions with the surrounding microenvironmental niche,

including extracellular matrix components, stromal cells, vascular-derived signals, oxygen gradients, and biochemical as well as mechanical cues.

**Table 3** Examples of *in vitro* and *in vivo* experimental models for stem cell studies.

Experimental model	Stem cell types	Purpose/Application	Key methods/Techniques	Reference
ESC culture	Embryonic Stem Cell (mouse/human)	Maintaining pluripotency and studying linear differentiation	Media with growth factors (LIF, bFGF) and directed differentiation with BMP4 and retinoic acid	[134-136]
iPSC culture	Induced pluripotent stem cell	Assessing specific differentiation of human tissues without the use of embryos	2D monolayer or 3D organoid culture (brain, liver, pancreas, and heart)	[137,138]
Co-culture System	MSC, ESC, and iPSC	Understanding paracrine interactions, self-renewal, and differentiation	Co-culture with endothelial cells or osteoblasts and scaffold biomaterials and hydrogels	[139-141]
Reporter gene and live-cell imaging	ESC and iPSC	Tracking the expression of transcription factors or signaling pathways in real-time	GFP reporter for OCT4/SOX2 and monitoring of the Wnt or Notch pathway	[142,143]
Chimera / Transplant	ESC and iPSC	Evaluation of cell integration and contribution to organ formation and homeostasis	Transplantation into embryos or adult tissues and xenotransplantation into NOD/SCID mice	[144-146]
Lineage tracing <i>in vivo</i>	ESC, iPSC, and progenitor cells	Tracing the origin and fate of cells and identifying regenerative populations	Cre-LoxP or fluorescent reporter system	[147]
Injury-induced regeneration model	ESC, iPSC, and MSC	Assessing proliferation, differentiation, and interaction with the post-injury niche	Heart, brain, and bone marrow injuries and evaluation of stem cell responses	[148]
Organoid transplantation	iPSC-derived organoid	Assess network integration and functionality	<i>In vitro</i> pre-conditioning followed by <i>in vivo</i> transplantation	[149]



**Figure 3** Stem cell–niche interactions.

### Therapeutic potential and clinical applications

Stem cells have great therapeutic potential in tissue regeneration and the treatment of degenerative diseases and cancer, with strategies that include regenerative therapy of vital organs, microenvironmental modulation, and the use of programmed cells such as iPSCs, ESCs, and MSCs.

#### Regenerative therapy

Stem cell-based regenerative therapies aim to restore damaged tissue function through cell integration, linear differentiation, and stimulation of endogenous tissue repair [131]. Both pluripotent and multipotent stem cells provide promising platforms for therapies in the heart, nervous system, and liver, each with specific challenges and approaches [150]. **Table 4** summarizes the applications of regenerative therapies using stem cells in various organs and body systems.

Myocardial injury from cardiac infarction causes massive loss of cardiomyocytes, which are difficult to regenerate effectively [151]. Stem cells, including human iPSC-derived cardiomyocytes and MSCs, are used to replace lost cells and restore cardiac contractile

function [152]. Preclinical studies in mouse and pig models have shown that stem cell transplantation can increase ventricular ejection fraction and reduce fibrogenesis through paracrine mechanisms, including the secretion of angiogenic factors such as VEGF and FGF2, as well as anti-inflammatory effects [153]. Furthermore, *in vitro* cardiac organoids are used to evaluate electrical integration and cell contractility prior to *in vivo* transplantation [154].

Central nervous system injuries, such as spinal cord injury or neurodegenerative diseases, present significant challenges due to the limited regeneration capacity of adult neurons [155]. Neural progenitor cells (NPCs) and iPSC-derived neurons are used to replace lost neurons while supporting remyelination by oligodendrocytes [156]. In mouse models of spinal cord injury, NPC transplantation has been shown to enhance axon regeneration, reduce gliosis, and improve motor function [157]. Therapeutic mechanisms include direct differentiation, modulation of the inflammatory microenvironment, and secretion of neurotrophic factors such as Brain-Derived Neurotrophic Factor (BDNF) and Glial Cell Line-Derived Neurotrophic

Factor (GDNF) [158]. Furthermore, 3D brain organoids are used to model neurodegenerative diseases and evaluate the potential for neuronal differentiation and tissue integration [159].

The liver has natural regenerative abilities, but extensive damage from cirrhosis or chronic hepatitis often requires additional intervention [160]. Stem cells, including hepatic progenitor cells, iPSC-derived hepatocyte-like cells, and MSCs, are used to improve hepatocyte function while modulating the inflammatory microenvironment [161]. In a mouse model of chemical liver injury, stem cell transplantation demonstrated restoration of liver function, increased expression of metabolic enzymes, and reduced fibrosis through directed differentiation and secretion of anti-fibrotic factors [162].

Furthermore, liver organoids are used to assess metabolic function and toxicology prior to clinical application [163].

The success of regenerative therapy is determined not only by cell transplantation alone, but also by the stem cells' ability to respond to local signaling pathways such as Wnt, Notch, and BMP/TGF- $\beta$ , as well as interact with the microenvironmental niche [164]. Factors such as oxygen levels, cell growth, and the extracellular matrix influence cell proliferation, differentiation, and integration, resulting in coordinated tissue repair [131]. *In vitro* preconditioning of stem cells using specific factors or biomaterial scaffolds can improve cell viability and integration after *in vivo* transplantation [165].

**Table 4** Examples of regenerative therapy using stem cells in specific organs and systems.

Organ/System	Stem cell types	Goals of therapy	Mechanism/Strategy	Experimental model	Reference
Heart	Human derived cardiomyocytes and MSCs	Replacing lost cardiomyocyte cells and improving contractile function	Cell integration, linear differentiation, secretion of paracrine factors (VEGF and FGF2), and modulation of fibrogenesis	Mouse and pig models and <i>in vitro</i> cardiac organoids	[151-154]
Central nervous system	Neural progenitor cells (NPC) and iPSC-derived neurons	Replacing lost neurons, supporting oligodendrocyte myelination, and improving motor function	Direct differentiation, modulation of the inflammatory microenvironment, and secretion of neurotrophic factors (BDNF and GDNF)	Spinal cord injury mouse model and 3D brain organoid	[155-159]
Liver	Hepatic progenitor cells, iPSC-derived hepatocyte-like cells, and MSC	Improve hepatocyte function, reduce fibrosis, and modulate inflammation	Direct differentiation, secretion of anti-fibrotic factors, and increased expression of metabolic enzymes	Chemical liver injury mouse model and <i>in vitro</i> liver organoids	[160-163]

### Implications in the treatment of degenerative diseases and cancer

Stem cells have significant therapeutic potential in the treatment of degenerative diseases and cancer, thanks to their unique ability to self-renewal, linear differentiation, and modulate the tissue microenvironment [166]. Stem cell-based therapies aim to repair damaged tissues, regenerate specific cells, and normalize disrupted molecular pathways, opening up opportunities for precision and regenerative medical approaches [167].

In degenerative diseases, including neurodegenerative, cardiovascular, and musculoskeletal disorders, tissue damage is often irreversible or has limited natural regenerative capacity [168]. NPCs and iPSC-derived neurons are used in Parkinson's and Alzheimer's models to replace lost neurons, promote myelination by oligodendrocytes, and increase the secretion of neurotrophic factors such as BDNF and GDNF [169]. Preclinical studies in mice have shown improvements in motor and cognitive function, confirming that the therapeutic mechanism

involves direct differentiation and modulation of the inflammatory microenvironment [170].

In cardiovascular therapy, iPSC-derived cardiomyocytes and MSCs are used to replace cardiomyocytes lost due to myocardial infarction [171]. In addition to differentiating into contractile cells, stem cells also stimulate angiogenesis through the secretion of VEGF and FGF2, and suppress the local inflammatory response, thus improving cardiac function recovery [172]. *In vitro* approaches, such as cardiac organoids or 3D cardiac tissue culture, allow for evaluation of electrophysiological integration before transplantation, improving the safety and effectiveness of therapy [173].

In musculoskeletal tissue regeneration, MSCs and bone progenitors play a role in osteogenic differentiation and the secretion of anti-inflammatory factors, which help reduce bone and cartilage degradation and accelerate tissue recovery [174]. The use of 3D scaffolds and co-culture with stromal cells allows cellular interactions that mimic the native niche, thereby increasing differentiation efficiency [175].

In cancer therapy, stem cells play a dual role, serving both as therapeutic targets and as research models [176]. The study of cancer stem cells (CSCs) helps explain tumor heterogeneity, resistance to chemotherapy, and metastatic potential [177]. CSCs maintain an aggressive tumor cell population through self-renewal and limited differentiation, making conventional therapies often incapable of eradicating all tumor cells [178].

Stem cell-based therapies offer strategies for targeting CSCs specifically or as therapeutic vectors [179]. For example, MSCs modified to express antitumor factors or prodrug systems can influence the tumor microenvironment, inhibit CSC proliferation, and minimize damage to normal tissue [180]. Furthermore, cancer organoid models and iPSC-derived tumor models enable mapping of resistance pathways, drug screening, and the development of precision therapies tailored to a patient's molecular profile [181].

The use of stem cells in degenerative diseases and cancer emphasizes the need for controlled differentiation, cell integration, and clinical safety [182]. Precisely modified cells are capable of balancing self-renewal, specialization, and niche interaction, thus

supporting tissue regeneration or effective tumor cell elimination [51]. This approach opens up opportunities for the development of regenerative therapies and precision oncology, which combine molecular strategies, bioengineering, and cellular transplantation [183].

#### **Cell integration, safety, risk of tumorigenesis**

Stem cell-based therapies have significant regenerative potential, but their implementation faces significant challenges related to cell integration, regulation of differentiation, and the risk of tumorigenesis [184]. The success of these approaches depends heavily on the cells' ability to interact functionally with target tissues, ensuring patient safety, and preventing uncontrolled cell proliferation [13].

Effective cell integration is essential for tissue function recovery [185]. Transplanted stem cells must be able to migrate to the injured area, interact with their microenvironmental niche, and establish physiological connections with surrounding cells [131]. For example, in cardiovascular therapy, transplanted cardiomyocytes must integrate electrophysiologically with native cardiomyocytes to ensure synchronous heart contractions [186]. Meanwhile, in the nervous system, transplanted neurons must form stable, functional synapses to maintain normal impulse transmission [187]. Disturbed microenvironmental conditions, such as those caused by inflammation or fibrosis, can inhibit cell migration and differentiation, therefore the use of biomaterial scaffolds and *in vitro* preconditioning are applied to enhance cell integration [139].

Pluripotent stem cells have a vast differentiation capacity, placing them at risk of remaining undifferentiated or undergoing inappropriate differentiation after transplantation [188]. Cells that retain pluripotency or differentiate uncontrollably can disrupt tissue homeostasis, reduce therapeutic effectiveness, and lead to clinical complications [189]. To minimize this risk, strategies such as *in vitro* pre-differentiation, the use of growth factors or specialized matrices, and the selection of differentiated cell populations prior to transplantation are employed [190]. Furthermore, monitoring differentiation markers and assessing genomic integrity are essential to ensure therapeutic safety [191].

The risk of tumor formation is a major challenge, especially when using pluripotent stem cells [192]. Cells that are not fully differentiated or have genetic mutations have the potential to form teratomas or neoplasms after transplantation [193]. This risk is further increased by long-term *in vitro* expansion and dysregulation of proliferation pathways such as Wnt, PI3K/AKT, and Myelocytomatosis oncogene (MYC) [194]. To minimize this risk, strategies such as strict selection of differentiated cells, the implementation of suicide gene systems, and long-term monitoring with molecular imaging and tumor biomarkers are employed [195]. This approach is crucial for detecting abnormal proliferation or tumorigenesis at an early stage [196].

Addressing these challenges requires a multidimensional approach that integrates an understanding of stem cell physiology, differentiation pathways, niche interactions, and epigenetic regulation [197]. Strategies such as *in vitro* preconditioning, the use of biomaterial scaffolds, regulation of signaling pathways, and the selection of pre-differentiated cell populations can support optimal cell integration, reduce uncontrolled proliferation, and enhance clinical safety [198]. The combined application of these strategies ensures that stem cell therapy effectively and safely balances self-renewal, differentiation, and restoration of tissue function [199].

### **Programmed cell differentiation-based therapy**

Programmed stem cell-based therapies, such as iPSCs, ESCs, and MSCs, have emerged as innovative strategies for tissue regeneration and the treatment of degenerative diseases [200]. These cells' abilities for self-renewal, directed differentiation, and microenvironmental modulation make them promising candidates for precision therapy and individualized treatments [1].

ESCs possess high pluripotency, allowing them to differentiate into all somatic cell types [137]. In therapeutic applications, ESCs are used to replace cells lost due to injury or degenerative diseases, such as cardiomyocytes in myocardial infarction, neurons in neurodegenerative disorders, and hepatocytes in chronic liver damage [201]. The ESC differentiation process is regulated by a combination of growth factors and molecular signals, including Bone Morphogenetic

Protein (BMP), Wnt, and Fibroblast Growth Factor (FGF), which determine the cell's lineage-specific pathways [202]. Major challenges in ESC therapy include the risk of tumorigenesis and immunological rejection, making *in vitro* pre-differentiation strategies and the use of biomaterial scaffolds crucial to ensure safe integration *in vivo* [203].

iPSCs are obtained by reprogramming somatic cells through the expression of nuclear transcription factors such as OCT4, SOX2, Kruppel-Like Factor 4 (KLF4), and cellular Myelocytomatosis oncogene (c-MYC), thus achieving pluripotency similar to ESCs [204]. The advantage of iPSCs lies in their ability to generate autologous cells, which reduces the risk of immune reactions. iPSCs are used in regenerative therapies for the heart, nervous system, and liver, as well as as disease models for drug screening and molecular mechanism research [131]. *In vitro* models, including 2D cultures, 3D organoids, and co-culture systems, allow for controlled differentiation, monitoring of lineage markers, and optimization of interactions with the microenvironmental niche before *in vivo* transplantation [205].

MSCs are multipotent cells capable of differentiating into osteoblasts, chondrocytes, and adipocytes, and possess significant immunomodulatory properties [206]. MSCs are widely used in regenerative therapies for bone, cartilage, heart, and inflamed tissues [207]. In addition to their linear differentiation capabilities, MSCs act through paracrine mechanisms by secreting growth factors, anti-inflammatory cytokines, and angiogenic molecules that support endogenous tissue regeneration [208]. The advantages of MSCs include ease of harvest from bone marrow, adipose tissue, or the placenta, and a relatively low risk of tumorigenesis compared to pluripotent cells [209].

Programmed cell therapy faces challenges related to differentiation control, integration into target tissues, and the potential for abnormal proliferation [12]. Mitigation strategies include *in vitro* pre-differentiation, the use of biomaterial scaffolds, regulation of specific signaling pathways, and monitoring marker expression and cell viability before transplantation [198]. The success of therapy depends heavily on optimal cell integration with host tissue and modulation of the microenvironment, allowing precise and safe restoration of tissue function [207].

### Challenges and future perspectives

Although stem cell-based therapies hold significant potential for tissue regeneration and the treatment of degenerative diseases, their implementation still faces numerous scientific, technical, and clinical challenges [164]. Limited understanding of stem cell physiology and the limitations of current experimental models are major obstacles to realizing safe and effective therapies [210].

Key limitations include heterogeneity within stem cell populations, the inability to fully control differentiation consistently, and a limited understanding of stem cell interactions with the *in vivo* microenvironmental niche [211]. *In vitro* models often fail to fully mimic the physiological conditions of intact tissues, while *in vivo* models face challenges such as biological variability and limitations in scale and long-term monitoring [212]. Furthermore, the risk of tumorigenesis in pluripotent stem cells and the potential for immune reactions to transplanted cells remain critical issues requiring more effective mitigation strategies [213].

Technological advances present promising opportunities to overcome the limitations of stem cell therapy [19]. Gene editing techniques, such as Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated protein 9 (CRISPR-Cas9), enable targeted gene modification to correct mutations, enhance linear differentiation, or reduce the risk of tumorigenesis [214]. 3D organoid cultures enable the creation of more accurate miniature human tissues to assess cell function, differentiation, and interactions [138]. Furthermore, bioprinting and advanced biomaterial scaffolds provide platforms for constructing three-dimensional tissues that support more physiological integration of transplanted cells [215]. The combined application of these technologies enhances control over cell proliferation, differentiation, and integration with high precision [216].

The safety and effectiveness of stem cell therapy can be enhanced through a multidimensional approach [196]. This approach includes *in vitro* pre-differentiation, selection of differentiated cell populations, monitoring of genomic integrity and lineage marker expression, and the use of biomaterial scaffolds to support homing and integration with target tissues [12]. Long-term monitoring using molecular

imaging and stem cell biomarkers allows early identification of abnormal proliferation or tumorigenic risk [217]. Furthermore, combination with growth factors, signaling pathway modulation, or cell-microenvironment therapy strategies enhances the ability of stem cells to adapt to the physiological needs of the tissue [218].

### Kesimpulan

Stem cell physiology and cellular differentiation processes are controlled by complex interactions between molecular signaling pathways, transcription factors, epigenetic mechanisms, and the tissue microenvironment. A deeper understanding of these mechanisms opens up opportunities for designing safer and more effective regenerative therapy strategies, including for degenerative diseases, tissue injury, and cancer. Future research should focus on optimizing differentiation control, cell integration, reducing the risk of tumorigenesis, and utilizing technologies such as organoids, bioprinting, and gene editing to improve the precision and efficacy of clinical therapies.

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### Declaration of Generative AI in Scientific Writing

The authors declare that no generative AI tools were used in the writing or preparation of this manuscript.

### CRedit Author Statement

**Aswin Rafif Khairullah:** Conceptualization, Literature review, and Writing – original draft. **Saifur Rehman:** Literature review, Data curation, and Writing – original draft. **Imam Mustofa:** Literature review, Data curation, and Writing – original draft. **Bima Putra Pratama:** Reference management, Data curation, and Validation. **Eny Martindah:** Conceptualization, Supervision, Funding acquisition, and Writing – review and editing. **Arif Nur Muhammad Ansori:** Writing – review and editing, Validation, and Supervision. **Mohammad Sukmanadi:** Manuscript preparation, Critical review, and Validation. **Ilma Fauziah Ma'ruf:** Manuscript preparation, Critical review, and Validation. **Analinasari Analinasari:** Manuscript preparation, Critical review, and Validation. **Riza Zainuddin Ahmad:** Methodological review, Critical revision, and Validation. **Sri Suryatmiati Prihandani:** Manuscript preparation, Critical review, and Validation. **Bantari Wisynu Kusuma Wardhani:** Writing – review and editing, Supervision, and Validation. **Siti Hamidatul Aliyah:** Manuscript preparation, Critical review, and Validation. **Wita Yulianti:** Reference management, Resources, and Validation. **Dea Anita Ariani Kurniasih:** Writing – review and editing, Visualization, and Validation. **Fadhila Utari:** Project administration, Supervision, Writing – review and editing, and Validation.

### Conflict of Interest Statement

The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest in the conduct and publication of this work.

### Data Availability Statement

All data generated or analyzed in this study are included in this published article. No additional datasets were generated or analyzed during the current study.

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