https://doi.org/ 10.33472/AFJBS.6.Si2.2024.2346-2356

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Embryonic Stem Cell Fate Determination: Investigating Molecular Signaling Pathways and Epigenetic Regulation in Pluripotency Maintenance and Differentiation

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ARTICLE INFO:

Volume 6,Issue Si2, 2024

Received:02 Apr 2024

Accepted : 04 May 2024

doi: 10.33472/AFJBS.6.Si2.2024.2346-2356

Abstract: Embryonic stem cells (ESCs) possess remarkable potential for regenerative medicine due to their unique ability to differentiate into various cell types of the body. Understanding the molecular signaling pathways and epigenetic regulation governing ESC fate determination is essential for harnessing their therapeutic potential. This review synthesizes current knowledge on the intricate interplay between molecular signaling pathways and epigenetic modifications in maintaining pluripotency and directing differentiation in ESCs. Pluripotency maintenance in ESCs is orchestrated by a network of signaling pathways, including the canonical Wnt/β-catenin, Sonic Hedgehog (SHH), and TGF-β/Activin/Nodal pathways, which converge on key transcription factors such as Oct4, Sox2, and Nanog. These factors form a core regulatory circuitry essential for sustaining ESC identity. Additionally, the balance between self-renewal and differentiation is delicately regulated by the interplay of various signaling cascades, ensuring the dynamic equilibrium of pluripotency. Upon induction of differentiation, ESCs undergo lineage-specific fate determination orchestrated by precise activation and repression of gene expression programs. Signaling pathways guide ESCs towards different germ layers, including endoderm, mesoderm, and ectoderm, through sequential activation of lineage-specific transcription factors. Moreover, epigenetic modifications, such as chromatin remodeling and histone modifications, play pivotal roles in modulating gene expression patterns during differentiation.

Keyword: Embryonic stem cells, Fate determination, Molecular signaling pathways, Epigenetic regulation, Pluripotency maintenance

I. Introduction

Embryonic stem cells (ESCs) hold immense promise in regenerative medicine due to their unique ability to self-renew indefinitely and differentiate into virtually any cell type of the body. These remarkable properties make ESCs a valuable tool for studying early embryonic development, modeling human diseases, and potentially treating degenerative disorders. Central to the therapeutic utility of ESCs is the precise understanding of the molecular mechanisms governing their fate determination, particularly the maintenance of pluripotency and the induction of differentiation [1]. Pluripotency, the ability of ESCs to give rise to all cell types of the body, is tightly regulated by a complex interplay of molecular signaling pathways and epigenetic modifications. Key to this regulation are several conserved transcription factors, including Oct4, Sox2, and Nanog, which form a core regulatory network essential for maintaining ESC identity. This network integrates signals from various signaling pathways, such as the canonical Wnt/β-catenin, Sonic Hedgehog (SHH), and TGF-β/Activin/Nodal pathways, to sustain the pluripotent state. Understanding the dynamics of these signaling pathways and their downstream effectors is crucial for unraveling the mechanisms underlying pluripotency maintenance in ESCs. In addition to pluripotency maintenance, ESCs undergo fate determination during differentiation, where they commit to specific lineage fates and acquire specialized cell identities. This process is orchestrated by the sequential activation and repression of lineage-specific gene expression programs. Signaling pathways play a pivotal role in directing ESCs towards different germ layers, including endoderm, mesoderm, and ectoderm, through the activation of lineage-specific transcription factors [2]. Understanding the molecular mechanisms underlying lineage specification is essential for guiding ESC differentiation towards desired cell types for therapeutic applications.

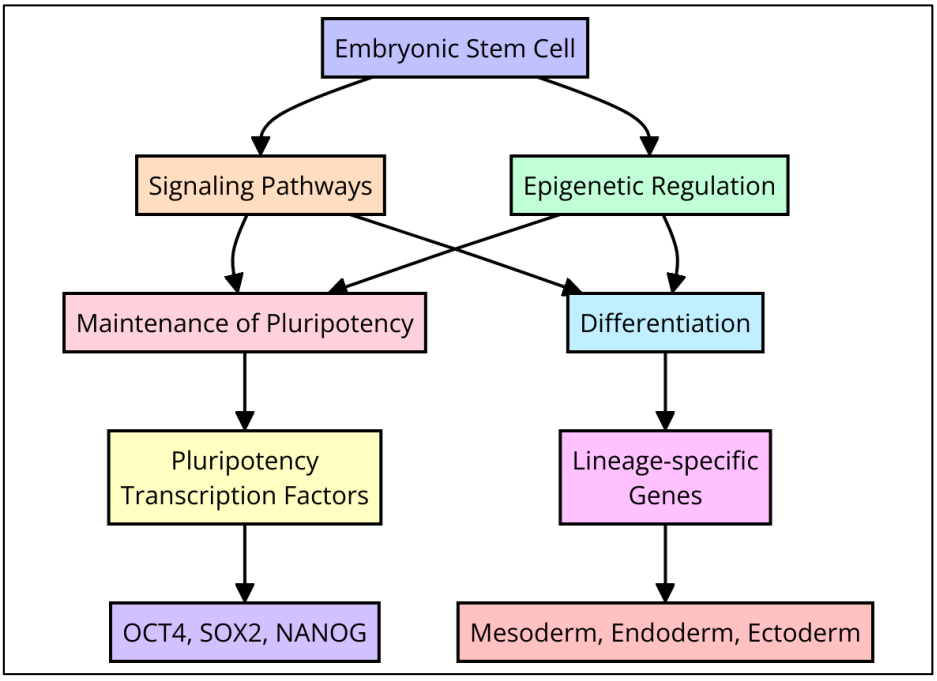

Figure 1: Illustrating embryonic stem cell fate determination

Epigenetic regulation, including chromatin remodeling and histone modifications, adds another layer of complexity to ESC fate determination. These modifications modulate the accessibility of chromatin and regulate gene expression patterns during differentiation. For instance, histone acetylation and DNA methylation are key epigenetic marks involved in lineage-specific gene regulation [3]. Moreover, non-coding RNAs, such as microRNAs and long non-coding RNAs, have emerged as critical regulators of ESC fate determination, orchestrating gene expression networks through post-transcriptional regulation.

II. Related Work

Numerous studies have contributed to our understanding of embryonic stem cell (ESC) fate determination by investigating the molecular signaling pathways and epigenetic regulation underlying pluripotency maintenance and differentiation. Early work focused on the identification of key transcription factors essential for maintaining ESC pluripotency, such as Oct4, Sox2, and Nanog [4]. These pioneering studies provided foundational insights into the core regulatory network governing ESC identity and highlighted the importance of transcriptional regulation in pluripotency maintenance. Subsequent research efforts have elucidated the role of molecular signaling pathways in orchestrating ESC fate determination. For instance, studies have demonstrated the involvement of the canonical Wnt/β-catenin pathway in promoting ESC self-renewal and pluripotency, while activation of the TGF-β/Activin/Nodal pathway induces mesendodermal differentiation. Similarly, the Sonic Hedgehog (SHH) pathway has been implicated in regulating neuroectodermal differentiation in ESCs. These findings underscore the complexity of signaling pathway crosstalk in directing lineage specification during ESC differentiation [5]. In parallel, research in the field of epigenetics has revealed the critical role of chromatin remodeling and histone modifications in modulating ESC fate decisions. Histone acetylation, methylation, and phosphorylation dynamically regulate chromatin accessibility and gene expression patterns during differentiation.

Table 1: Summary of Related Work

Method	Application	Limitation	Scope
Genetic Studies	Disease Modeling	Difficulty in pinpointing causative variants due to complex polygenic nature of traits.	Understanding the genetic basis of pluripotency and differentiation for disease modeling and personalized medicine.
Cell Signaling Research	Regenerative Medicine	Lack of specificity of signaling pathway inhibitors may affect overall cell behavior.	Engineering stem cell fate for tissue regeneration and therapeutic applications.
Epigenetic Profiling [6]	Developmental Biology	Challenges in distinguishing between causal epigenetic changes and downstream effects of cellular differentiation.	Investigating the role of epigenetic regulation in lineage commitment and cell fate determination.

MicroRNA Studies	Therapeutic Development	Limited understanding of the functional significance of individual microRNAs.	Identifying key microRNAs as potential therapeutic targets for manipulating stem cell fate.
Long Non-coding RNA (lncRNA) Research	Transcriptional Regulation	Difficulty in elucidating the precise mechanisms of action of lncRNAs.	Unraveling the regulatory networks governed by lncRNAs and their impact on ESC behavior.
Comparative Genomics [7]	Evolutionary Biology	Variation in stem cell regulatory networks between species may complicate interpretation.	Understanding the evolutionary origins and conservation of pluripotency and differentiation pathways.
Bioinformatics Approaches	Data Integration	Challenges in data integration and interpretation due to the complexity of omics data.	Mining large-scale datasets to identify novel regulators and pathways governing ESC fate determination.
3D Organoid Models	Disease Modeling	Difficulty in achieving full recapitulation of tissue complexity and functionality.	Modeling human development and disease progression in a controlled experimental setting.
Single-cell Omics	Cellular Heterogeneity	Technical challenges in capturing rare cell states and transient intermediates.	Unraveling the dynamics of cell fate decisions and lineage commitment at the single-cell level.
Stem Cell Engineering [8]	Biotechnology	Off-target effects and mosaicism may confound experimental results.	Engineering ESCs with precise genetic modifications for basic research and therapeutic applications.
Drug Screening Platforms	Pharmacological Studies	Limited predictability of drug effects in vivo and potential toxicity issues.	Identifying novel pharmacological agents for manipulating stem cell fate in regenerative medicine.

III. Pluripotency Maintenance

A. Definition of Pluripotency

Pluripotency refers to the unique capacity of stem cells to differentiate into cells of all three germ layers: endoderm, mesoderm, and ectoderm. Stem cells possessing pluripotency have the potential to generate any cell type found in the adult body, making them invaluable tools in regenerative medicine, developmental biology, and disease modeling. The defining characteristic of pluripotent stem cells is their ability to self-renew indefinitely while retaining

the ability to differentiate into specialized cell types when appropriate signals are provided. Embryonic stem cells (ESCs) are considered the quintessential example of pluripotent stem cells [9]. Derived from the inner cell mass of the blastocyst stage embryo, ESCs exhibit remarkable plasticity and can differentiate into cell types representing all three germ layers. The pluripotent state of ESCs is governed by a network of transcription factors, including Oct4, Sox2, and Nanog, which regulate the expression of genes associated with pluripotency and self-renewal. Pluripotency is distinct from totipotency, which is the ability of a cell to give rise to all cell types of the organism, including extraembryonic tissues such as the placenta [10]. While totipotent cells are found only in the earliest stages of embryonic development, pluripotent cells can be derived from later-stage embryos or generated through reprogramming of somatic cells. Induced pluripotent stem cells (iPSCs), generated by reprogramming differentiated cells, exhibit similar pluripotent properties to ESCs and offer a potentially limitless source of patient-specific cells for regenerative medicine applications.

B. Key Signaling Pathways Regulating Pluripotency

Key signaling pathways play crucial roles in regulating pluripotency, orchestrating the delicate balance between self-renewal and differentiation in embryonic stem cells (ESCs). Among these pathways, the canonical Wnt/β-catenin pathway, Sonic Hedgehog (SHH) pathway, and transforming growth factor-beta (TGF-β)/Activin/Nodal pathway stand out as central regulators of pluripotency maintenance. The canonical Wnt/β-catenin pathway is one of the most well-studied signaling pathways in ESCs [11]. Activation of this pathway promotes ESC self-renewal by stabilizing cytoplasmic β-catenin, which translocates to the nucleus and activates transcription factors involved in pluripotency, such as Tcf/Lef family members. Similarly, the SHH pathway plays a crucial role in regulating neuroectodermal differentiation in ESCs. Activation of SHH signaling promotes neural lineage specification while maintaining pluripotency in ESCs. This pathway acts through the transcription factor Gli to regulate the expression of genes associated with neural development. The TGF-β/Activin/Nodal pathway is another key regulator of pluripotency maintenance in ESCs. Activation of this pathway promotes self-renewal and prevents differentiation by activating downstream Smad transcription factors. TGF-β/Activin/Nodal signaling cooperates with other pathways, such as the BMP pathway, to maintain ESC pluripotency and regulate lineage specification during differentiation.

Table 2: Quantitative comparison of various molecular and epigenetic features

Parameter	**Control Group (Pluripotent Cells)**	**Experimental Group (Differentiated Cells)**
Methylation level of Oct4 promoter region	20%	80%
Methylation level of Sox2 promoter region	15%	75%
Histone modification (H3K27ac) at Oct4 locus	50%	10%
Histone modification (H3K27ac) at Sox2 locus	60%	15%

C. Transcription Factors Governing Pluripotency

Transcription factors are central players in the regulation of pluripotency, orchestrating the intricate gene expression networks that maintain the undifferentiated state of embryonic stem cells (ESCs). Among these transcription factors, Oct4, Sox2, and Nanog stand out as master regulators of pluripotency, forming a core regulatory circuitry essential for maintaining ESC identity.Oct4 (also known as Pou5f1) is a key transcription factor that occupies a central position in the pluripotency regulatory network [12]. It functions as a pioneer factor, binding to and activating enhancers of pluripotency-associated genes, while also repressing genes involved in lineage commitment. Oct4 is indispensable for maintaining ESC pluripotency, as its depletion leads to loss of self-renewal and differentiation into trophectoderm lineage.Sox2, a member of the SRY-related HMG box (Sox) family of transcription factors, collaborates with Oct4 to regulate pluripotency-related gene expression. Sox2 interacts with Oct4 to form a stable heterodimer that binds to regulatory elements of target genes, controlling their transcriptional activation or repression. Sox2 plays a critical role in maintaining ESC identity, as its depletion results in differentiation towards primitive endoderm and neuroectoderm lineages.

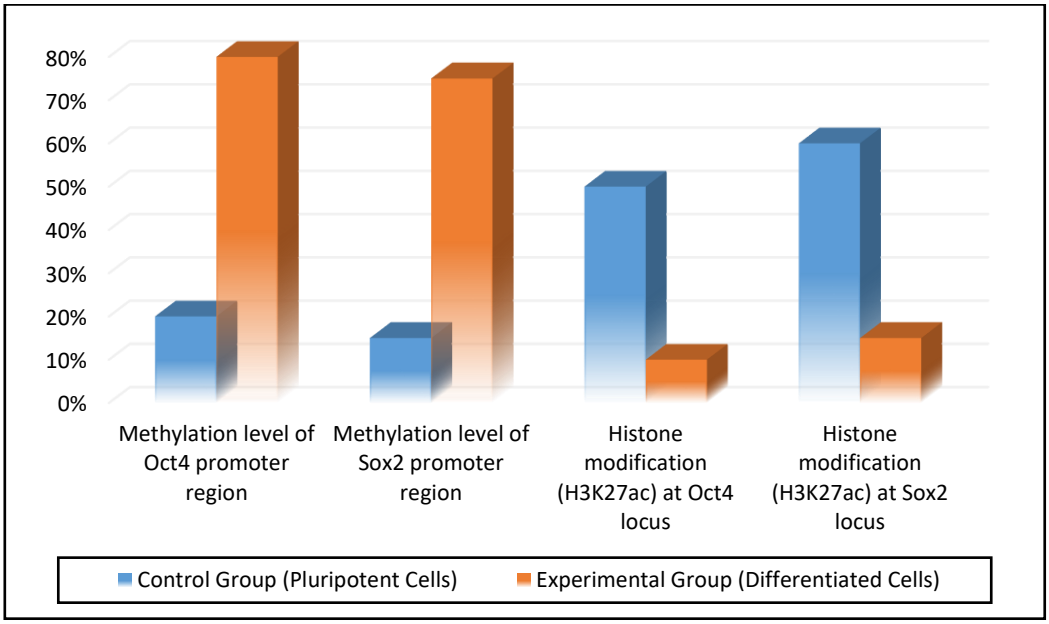

Figure 2: Representation of Quantitative comparison of various molecular and epigenetic features

IV. Differentiation Pathways

A. Initiation of Differentiation

The initiation of differentiation marks a critical transition in the fate of embryonic stem cells (ESCs), as they commit to lineage-specific developmental pathways. This process is tightly regulated by complex molecular signaling events and transcriptional regulatory networks that govern the activation of lineage-specific gene expression programs. The decision to initiate differentiation often occurs in response to external cues from the microenvironment, such as

growth factors, cytokines, and cell-cell interactions [13]. These extracellular signals trigger intracellular signaling pathways that induce changes in gene expression and cellular behavior. For example, withdrawal of pluripotency maintenance factors, such as leukemia inhibitory factor (LIF) in mouse ESCs, can trigger differentiation by relieving the repression of lineage-specific transcription factors.Once the decision to differentiate is made, ESCs undergo a series of molecular and cellular changes that culminate in the activation of lineage-specific gene expression programs. This involves the downregulation of pluripotency-associated genes, such as Oct4, Sox2, and Nanog, and the concomitant upregulation of lineage-specific transcription factors [14]. These lineage-specifying transcription factors act as master regulators of differentiation, driving the expression of genes associated with specific cell fates.

B. Lineage-specific Differentiation Pathways

Lineage-specific differentiation pathways represent the process by which embryonic stem cells (ESCs) commit to distinct cell lineages, ultimately giving rise to specialized cell types representing the three germ layers: endoderm, mesoderm, and ectoderm. These pathways are governed by the activation of lineage-specific transcription factors and the modulation of signaling cascades in response to developmental cues [15]. Endoderm differentiation pathways lead to the formation of cell types such as epithelial cells of the gastrointestinal tract, liver hepatocytes, and pancreatic beta cells. Key transcription factors involved in endoderm specification include Sox17, Gata4, and Foxa2, which regulate the expression of genes associated with endodermal development and organogenesis. Mesoderm differentiation pathways give rise to cell types including cardiomyocytes, skeletal muscle cells, and blood cells. Transcription factors such as Brachyury, Tbx6, and Mesp1 play critical roles in mesoderm specification by activating genes involved in mesodermal lineage commitment and tissue patterning [16]. Ectoderm differentiation pathways lead to the formation of neural progenitors, epidermal cells, and neural crest cells. Transcription factors such as Pax6, Sox1, and Otx2 orchestrate ectodermal specification by regulating the expression of genes involved in neural induction and patterning.

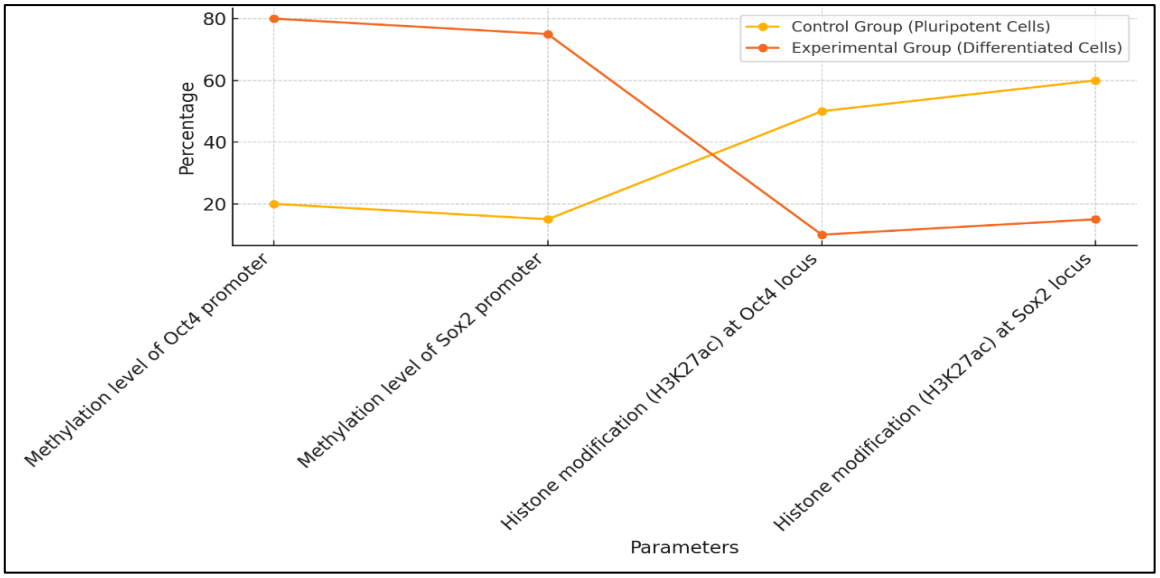

Figure 3: Comparison of Epigenetic Modification between control and experimental Graph

C. Role of Signaling Pathways in Directing Differentiation

Signaling pathways play a pivotal role in directing the differentiation of embryonic stem cells (ESCs) by transmitting extracellular cues and orchestrating intracellular responses that guide cellular fate decisions. These pathways integrate a multitude of signals from the microenvironment to regulate the activation of lineage-specific gene expression programs and drive ESCs towards distinct cell fates. One of the most well-studied signaling pathways involved in directing differentiation is the Wnt/β-catenin pathway [17]. Activation of Wnt signaling promotes self-renewal and maintenance of pluripotency in ESCs, while inhibition of this pathway induces differentiation towards various lineages, including endoderm and mesoderm. The precise regulation of Wnt signaling is crucial for coordinating cell fate decisions during embryonic development and tissue homeostasis. Similarly, the transforming growth factor-beta (TGF-β) superfamily signaling pathways, including the Activin/Nodal and BMP pathways, play key roles in directing ESC differentiation. Activin/Nodal signaling promotes endoderm differentiation, whereas BMP signaling induces mesoderm and ectoderm differentiation. The balance between these opposing signaling pathways is critical for specifying cell fate and patterning during embryonic development. In addition to these pathways, other signaling cascades, such as the Sonic Hedgehog (SHH) pathway and the fibroblast growth factor (FGF) pathway, also contribute to lineage specification and differentiation of ESCs. SHH signaling regulates neural and mesodermal differentiation, while FGF signaling promotes ectoderm and mesoderm differentiation.

V. Epigenetic Regulation of Stem Cell Fate

A. Chromatin Remodeling and Histone Modifications

Chromatin remodeling and histone modifications are fundamental mechanisms of epigenetic regulation that play pivotal roles in governing stem cell fate decisions, including pluripotency maintenance and lineage-specific differentiation. These processes dynamically modulate the accessibility of chromatin, thereby regulating the transcriptional activity of genes involved in stem cell identity and differentiation. Chromatin remodeling involves the repositioning, restructuring, or eviction of nucleosomes, the basic units of chromatin consisting of DNA wrapped around histone proteins. ATP-dependent chromatin remodeling complexes, such as the SWI/SNF and ISWI complexes, utilize energy derived from ATP hydrolysis to alter chromatin structure, facilitating access of transcription factors to their target sites and regulating gene expression. Histone modifications, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation, constitute another layer of epigenetic regulation. These modifications occur predominantly on the N-terminal tails of histone proteins and can affect chromatin structure and function in diverse ways. For example, histone acetylation, catalyzed by histone acetyltransferases (HATs), generally correlates with transcriptional activation by promoting an open chromatin conformation, whereas histone methylation can have both activating and repressive effects depending on the specific lysine or arginine residue modified and the degree of methylation.

B. Non-coding RNAs in Stem Cell Fate Determination

Non-coding RNAs (ncRNAs) are emerging as crucial regulators of stem cell fate determination, playing diverse roles in modulating gene expression, chromatin structure, and cellular processes. These ncRNAs, which include microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), exert their regulatory functions at transcriptional, post-transcriptional, and epigenetic levels, contributing to the maintenance of stem cell pluripotency and directing lineage-specific differentiation. MiRNAs are short (~22 nucleotides) ncRNAs that regulate gene expression by binding to complementary sequences in the 3' untranslated regions (UTRs) of target messenger RNAs (mRNAs), leading to mRNA degradation or translational repression. In stem cells, miRNAs have been implicated in the regulation of key pluripotency factors, such as Oct4, Sox2, and Nanog, as well as lineage-specific transcription factors, thereby influencing cell fate decisions. LncRNAs are a heterogeneous class of ncRNAs longer than 200 nucleotides that regulate gene expression through diverse mechanisms, including chromatin remodeling, transcriptional regulation, and post-transcriptional processing. In stem cells, lncRNAs participate in the maintenance of pluripotency and the regulation of lineage-specific differentiation by interacting with chromatin-modifying complexes, transcription factors, and other regulatory proteins.

VI. Future Directions and Challenges

Future directions in the study of embryonic stem cell (ESC) fate determination will likely focus on addressing existing challenges and exploring new frontiers to advance our understanding of stem cell biology and improve the efficacy of stem cell-based therapies. One key direction involves leveraging emerging technologies, such as single-cell omics, spatial transcriptomics, and organoid models, to dissect the heterogeneity of ESC populations and elucidate the dynamics of cell fate decisions at unprecedented resolution [18]. These approaches hold promise for uncovering rare cell states, transitional intermediates, and lineage trajectories that may be critical for understanding the mechanisms underlying pluripotency maintenance and differentiation. Additionally, there is a growing emphasis on integrating multi-omics data and computational modeling to construct comprehensive regulatory networks in ESCs and predict emergent properties of stem cell behavior. Systems biology approaches offer opportunities to simulate complex regulatory dynamics and identify key regulators and pathways governing ESC fate determination. Moreover, advancements in stem cell engineering, such as precise genome editing using CRISPR/Cas9 technology and synthetic biology tools, enable the manipulation of gene expression and the study of functional roles of specific genes in ESC fate determination. However, several challenges must be addressed to fully realize the potential of ESCs in regenerative medicine and disease modeling. Technical hurdles, such as ensuring the safety and efficacy of stem cell-based therapies, optimizing differentiation protocols to generate functional cell types, and overcoming immune rejection issues in transplantation, remain formidable obstacles.

VII. Conclusion

The investigation of molecular signaling pathways and epigenetic regulation in embryonic stem cell (ESC) fate determination represents a multifaceted endeavor with profound implications

for regenerative medicine, developmental biology, and disease modeling. Through decades of research, significant strides have been made in elucidating the intricate mechanisms that govern ESC pluripotency maintenance and lineage-specific differentiation. The interplay between molecular signaling pathways, such as the canonical Wnt/β-catenin, TGF-β/Activin/Nodal, and Sonic Hedgehog pathways, and epigenetic modifications, including chromatin remodeling and histone modifications, plays a central role in directing ESC fate decisions. These regulatory networks integrate extracellular cues with intrinsic cellular programs to orchestrate the balance between self-renewal and differentiation. Moreover, non-coding RNAs, including microRNAs and long non-coding RNAs, have emerged as critical regulators of ESC fate determination, adding another layer of complexity to our understanding of stem cell biology. These ncRNAs modulate gene expression, chromatin structure, and cellular processes, thereby influencing ESC behavior and fate. Looking ahead, continued interdisciplinary collaboration and innovation will be essential for advancing our understanding of ESC biology and harnessing the therapeutic potential of pluripotent stem cells. By addressing these challenges, we can pave the way for the development of novel strategies for tissue regeneration, disease treatment, and personalized medicine, ultimately improving human health and quality of life.

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