GMR
The Original

# FUNCTIONAL IMPACT OF EPIGENETIC MODIFICATIONS ON STEM CELL FATE DETERMINATION

**Dr. Vidhya Lakshmi[1], Dr. Yamuna V[2], Dr. B. Anu[3], Simran Kalra[4], Anvesha Garg[5], Ms. Rucha N. Acharya[6]**

[1]Assistant Professor, Pharmacology, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research, Enathur, Kanchipuram, Tamil Nadu – 631552, India, Email: vidhyalakshmi@maher.ac.in
[2]Lecturer, Pharmaceutical Chemistry, Meenakshi College of Pharmacy, Meenakshi Academy of Higher Education and Research
Email: yamuna@maher.ac.in
[3]Assistant Professor, Forensic Medicine, ORCID: https://orcid.org/0009-0003-9162-1063
[4]Centre of Research Impact and Outcome, Chitkara University, Rajpura – 140417, Punjab, India
Email: simran.kalra.orp@chitkara.edu.in, ORCID: https://orcid.org/0009-0005-9008-8118
[5]Quantum University Research Center, Quantum University, Roorkee, Uttarakhand – 247667, India,
Email: anvesha.garg@quantumeducation.in, ORCID: https://orcid.org/0009-0006-2621-8763
[6]Assistant Professor, Faculty of Allied and Healthcare, Gokul Global University, Sidhpur, Gujarat, India,
Email: rnacharya.gpc@gokuluniversity.ac.in, ORCID: https://orcid.org/0009-0008-1720-3660

## ABSTRACT

Stem cells are highly plastic similar to their ability to self-renew and develop into a variety of more specialized cell types, and thus they are the key to development, tissue homeostasis, and regenerative medicine. An accumulating body of evidence suggests that heritable yet reversible alterations that do not affect the DNA sequence, called epigenetic regulation, is a key to the process of stem cell fate determination. The combination of critical epigenetic events such as DNA methylation, histone alterations, chromatin remodeling or non-coding RNA-mediated regulation, orchestrate gene expression programs that result in pluripotency or lineage-specific differentiation of a stem cell. These processes work in a very dynamic and coordinated way, which provides a sufficient level of control over the transcriptional activity in terms of time and space. This review summarizes recent progress in the field of epigenetic landscape regulation of stem cell self-renewal and differentiation, the balance between activating and repressive states of the chromatin, the role of bivalent domains, and the effects of the epigenetic reprogramming in induced pluripotent stem cells. Moreover, non-coding RNAs integration into epigenetic networks has been argued to be a crucial level of regulatory complexity shaping cellular identity. The clinical utility of epigenetic regulation is becoming apparent and there is much promise in the regenerative therapies, disease modeling and the treatment of diseases that have aberrant epigenetic regulation, such as cancer where there is a disruption of normal stem cell activity. Although significant advances have been achieved, there are still issues with deciphering the complexity and context-dependence of the epigenetic regulation process. Future studies that incorporates multi-omics techniques and new powerful computational systems are likely to advance our knowledge and allow an accurate manipulation of stem cell fate to treat diseases. Altogether, epigenetic processes are inherent controllers of stem cell life, and potential actions in next-generation biomedical procedures.

**KEYWORDS:** Stem cell fate determination, Epigenetic regulation, DNA methylation, Histone modifications, Chromatin remodelling, Pluripotency and differentiation, Induced pluripotent stem cells (iPSCs), Non-coding RNAs.

## 1. INTRODUCTION

The major characteristic of stem cells is their self-renewal and differentiation into tissue-specific cell types, and this makes them essential in embryonic development, tissue repair, and regenerative medicine. Stem cells are most generally categorized into embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells, based on their origin and potency. ESCs are pluripotent, which means they have the capacity to form all cell lineages of the body, whereas adult stem cells are usually restricted to a limited number of lineages and are multipotent. The development of the iPSC technology has transformed the sector to allow the reprogramming of somatic cells into a pluripotent condition using specific transcription factors to offer a strong model of disease and customized treatments (Yamanaka, 2020). The tightly controlled process through which stem cells can either retain their undifferentiated status or differentiate into certain developmental fates is known as stem cell fate determination. The complex regulatory networks involving transcription factors, signaling pathways, and chromatin dynamics are in charge of this process. The key to these mechanisms is the accurate regulation of the gene expression patterns that determine cell identity. The transition between pluripotency and differentiation consists of the coordinated processes of gene activation and repression of lineage-specific genes that is facilitated by dynamic alterations of chromatin structure and accessibility (Atlasi & Stunnenberg, 2017).

Regulation of epigenetics has become a key factor of stem cell fate, existing outside of the fixed genetic code, but, within a reversible and context-dependent manner. Epigenetic processes such as DNA-methylation, histone-modifications and chromatin-remodelling are molecular switches that can either activate or silence transcriptional activity without changing the underlying DNA sequence. These changes create permissive or repressive chromatin conditions, thus dictating the activation or repression of particular genes in the process of stem cell maintenance and differentiation (Allis and Jenuwein, 2016; Smith and Meissner, 2013). Moreover, the epigenetic landscapes are very dynamic, and as such, the stem cells can react to both developmental signals and environmental cues with great plasticity (Cavalli & Heard, 2019). Over the past years, the advent of high-throughput sequencing and single-cell technologies has presented more information than ever about the epigenetic architecture of stem cells. These methods have shown the existence of bivalent chromatin domains, poised regulatory elements and lineage-specific epigenetic signatures that work jointly to direct cell fate choices. Besides, non-coding RNAs have been identified as notable regulators that engage chromatin-modifying complexes to adjust the expression program of genes (Nicetto & Zaret, 2019). In spite of these progresses, the context-dependency and complexity of epigenetic regulation is a significant challenge to the complete comprehension of stem cell biology.

The purpose of the article is to give the overall picture of the functional role of the epigenetic alterations on stem cell fate determination. It combines existing information on important epigenetic pathways, the functions of these pathways in pluripotency and differentiation, and their disease and regenerative medicine implications. This review points to the feedback relationship between cellular identity and epigenetic regulation as dynamically interplaying based on the recent studies findings.

This review is the first to summarize a wide range of epigenetic processes -such as DNA methylation, histone modification, and chromatin remodelling and non-coding RNA regulation- into a single model that elucidates their collective contribution to stem cell fate determination. This work also stresses the integrative and dynamic quality of epigenetic control as opposed to other reviews, which focus on isolated mechanisms, which is critical to offer a global view of epigenetic control spanning the basics of biology into the emerging clinical uses of regenerative medicine and therapeutic interventions.

## 2. Overview of Epigenetic Mechanisms

Epigenetic processes are involved in a complicated regulatory system that regulates the expression of genes without changing the underlying DNA sequence. These processes are crucial in preserving pluripotency, facilitating lineage commitment, and stabilizing cellular identities in stem cells. Several interlinked mechanisms influence the epigenetic landscape, such as DNA-methylation and histone-modifications, chromatin remodeling, and regulation by non-coding RNAs. These mechanisms are dynamic regulators of chromatin structure and transcriptional accessibility and together, control stem cell fate choices.

### 2.1 DNA Methylation

One of the most thoroughly examined examples of epigenetic modifications is DNA methylation which is critically important in the regulation of gene expression in development and differentiation. This is mainly achieved by the addition of a methyl group to the 5-position of cytosine bases in the form of CpG dinucleotides found in clusters (CpG islands) in the promoter regions of genes. Transcriptional repression is supposed to be related to the methylation of these areas, because the process disrupts the binding of transcription factors and the recruitment of repressive chromatin-associated proteins. DNA methyltransferase (DNMT) is involved in the establishment and maintenance of DNA methylation patterns via DNA methyltransferases (DNMTs), such as DNMT1, DNMT3A and DNMT3B. DNMT1 mainly helps to preserve the pattern of methylation during DNA replication to ensure that epigenetic inheritance takes place, but DNMT3A and DNMT3B act as de novo methyltransferases, creating new methylations patterns in the course of development and differentiation. In stem cell context, DNA methylation is vital in an act of lineage restriction which silences pluripotency-associated transcripts and promotes differentiation-specific gene programs. Global hypomethylation, in contrast, tends to be linked with the pluripotent states, which have more flexibility in transcription.

### 2.2 Histone Modifications

Another basic layer of epigenetic regulation is histone modifications, which is the post-translational modification of histone proteins on which the DNA is wound. Those alterations are acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and all of them play their role in controlling chromatin structure and expression. Histone acetylation and methylation among others are key in the area of stem cell biology. Histone acetylation, which is largely linked with transcriptional activation, decreases interaction between histones and DNA leading to a more open chromatin structure. Conversely, histone methylation may either activate or suppress gene expression based on the particular residue and level of methylation. Actually, H3K4me3 (methylation of histone H3 at the lysine 4 position) has been linked with active promoters, and H3K27me3 (methylation of histone H3 at the lysine position 27) with transcriptional repression. Moreover, histone marks like H3K9ac also aid in active chromatin statuses. The three classes of proteins regulating these modifications are: writers which add chemical groups (e.g., histone acetyltransferases and methyltransferases); erasers which remove them (e.g., histone deacetylases and demethylases); and readers which bind to a particular histone mark to mediate downstream effects. The occurrence of bivalent domains, i.e., regions that are simultaneously stamped by activating (H3K4me3) and repressive (H3K27me3) marks, in the context of stem cells prepares developmental genes to be rapidly activated or silenced, and hence enables plasticity in lineage commitment.

### 2.3 Chromatin Remodelling

Chromatin remodeling is the dynamic rearrangement of chromatin structure to control access of the DNA to transcriptional machinery. There are two main types of chromatin namely euchromatin, transcriptionally active, and loosely packed, and heterochromatin, transcriptionally silent and very condensed. The process of these transitions is essential to regulating gene expression in stem cell maintenance and differentiation. SWI/SNF and ISWI are ATP-dependent chromatin remodeling complexes that are key to regulating nucleosome positioning and chromatin accessibility. These complexes use the energy of ATP hydrolysis to reposition, eject or restructure the nucleosomes, thus revealing or covering regulatory DNA elements. Chromatin remodeling in stem cells is necessary to keep the genes needed to maintain pluripotency in cells open, and genes associated with differentiation are either switched on or silenced. A break in chromatin remodeling processes may result in abnormal gene expression and cell fate choice.

### 2.4 Non-Coding RNA in Regulation of Epigenetics

Non-coding RNAs (ncRNAs) are other major participants in the regulation of epigenetic processes that provide yet another dimension of complexity to gene regulation. They are long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs), and each has a different way in which it regulates gene expression. Of interest are lncRNAs that are capable of controlling gene expression in a manner that is cis (influencing other genes nearby) and trans (influencing other genomic loci that are far away). They are able to serve as molecular scaffolds, and direct chromatin-regulating complexes like Polycomb repressive complexes to particular genomic locations, and thus attenuate chromatin states. Instead, miRNAs are post-transcriptional in that they often act indirectly by instigating epigenetic interactions by targeting messenger RNAs to be degraded or translatedally repressed. CircRNAs can be molecular sponges of miRNAs, or interact with RNA-binding proteins, further controlling gene regulatory pathways. The interaction of the ncRNA and chromatin-modifying machineries plays a key role in stem cells with the close regulation of gene expression being the major factors that dictate whether cells remain pluripotent or differentiate. These RNA-mediated processes work together with DNA methylation and histone modifications to create a very well-coordinated regulatory network that determines stem cell fate.

## 3. Epigenetic Regulation of Stem Cell Self-Renewal

A tightly-regulated epigenetic regulatory network controls the self-renewal capacity of stem cells as well as their pluripotency. The heart of this process is the concomitant expression of all the vital pluripotency transcription factors such as OCT4, SOX2 and NANOG which are master regulators of the stem cell identity. Such factors create a transcriptional circuitry which facilitates the expression of genes necessary to maintain an undifferentiated state and at the same time suppress differentiation-related pathways. This transcriptional network is maintained by the epigenetic processes that regulate chromatin accessibility and provide accurate spatial and temporal regulation of gene expression. An open and highly-accessible chromatin structure is one of the characteristic features of pluripotent stem cells. Stem cells have a globally permissive state of chromatin compared to that of differentiated cells with decreased methylation of the DNA and increased histone acetylation. The open chromatin structure makes it easier to bind pluripotency transcriptional factors to regulatory DNA binding specific sites, thus maintaining active transcriptional programs. Heterochromatin (closed chromatin) and open chromatin (euchromatin) are connected, as shown in Fig 1, with the activation marks (H3K4me3 and H3K9ac), and the repressive marks (H3K27me3 and H3K9me3), respectively. These two states undergo a dynamic transition that is necessary to keep the stem cell identity.

Fig 1 also gives a combined perspective of the action of various epigenetic processes on stem cell self-renewal. The figure illustrates that CpG transcriptional silencing is caused by the DNA methylation of CpGs that recruit repressive protein complexes, and inhibit lineage-specific genes in pluripotent cells. Simultaneously, histone modifications serve as a reversible switch that can either activate or silence the expression of a gene, based on the type of chemical marks that occur. Histone writers, erasers and readers work together to keep chromatin states in check and this allows stem cells to retain transcriptional plasticity. Moreover, chromatin remodelling complexes including SWI/SNF and ISWI, which are of interest in Fig 1 actively reposition nucleosomes in ATP-dependent processes. This remodelling controls accessibility of transcription factors and RNA polymerase to DNA hence gene expression programs that are vital during self-renewal. The figure is a good representation of how open and closed chromatin states are intermediated by chromatin remodeling, which supports the dynamicism of epigenetic regulation in the case of stem cells.

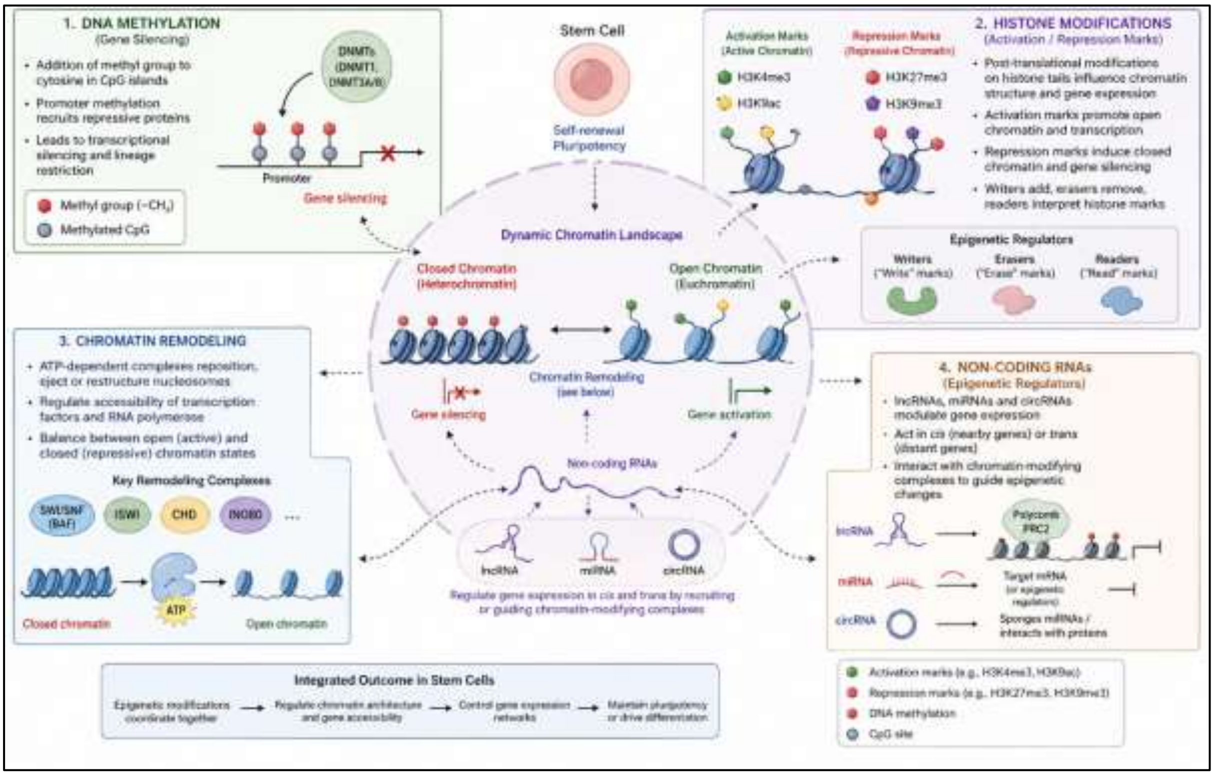

**Fig 1. Integrated Epigenetic Regulation in Stem Cells.**

The other element that is very vital as represented in Fig 1 is the part of non-coding RNAs such as lncRNAs, miRNAs and circRNAs in regulating epigenetic processes. These molecules play a role as important modulators through interactions with chromatin-modifying complexes and directing them to particular genomic loci. Non-coding RNAs regulate gene expression, using cis and trans mechanisms, by either supporting pluripotency-associated genes or silencing differentiation pathways. This extra layer of regulation adds to the complexity and accuracy of epigenetic regulation in the stem cells. The hallmark characteristic of the pluripotent stem cells is the occurrence of bivalent chromatin domains, which have both activating (H3K4me3) and repressive (H3K27me3) marks. These domains keep developmental genes in a poised state, up to their rapid activation or repression in response to differentiation signals. This dual epigenetic mark is to ensure that the stem cells have the flexibility to commit to lineage and not prematurely differentiate.

Epigenetic stability and plasticity is another important factor in stem cell self-renewal. It remains stable in the context of epigenetics, which maintains the fundamental identity of stem cells throughout the cell divisions, maintaining stable expression of the pluripotency-related genes. Plasticity on the other hand, allows the stem cells to dynamically respond to environmental and developmental signals by changing their epigenetic profile. This equilibrium is obtained by reversible modifications like DNA methylations and histone acetylation as shown in Fig 1 and this can be quickly adjusted to control the expression of a gene. In general, Fig 1 highlights the idea that self-renewal of stem cells cannot happen through one particular mechanism but through a combination of various layers of epigenetics, such as DNA methylation, histone modification, chromatin remodeling, and non-coding RNA interactions. These processes act in a well-coordinated and dynamic way to retain pluripotency and retain differentiation potential, which emphasizes the sophistication and flexibility of epigenetic control in stem cell biology.

## 4. Epigenetic Control of Stem Cell Differentiation

A highly controlled process is the differentiation of stem cell in which pluripotent or multipotent cells undergo differentiation into specialized cell types that possess functional identities. Activation and repression of certain gene expression programs are highly regulated by epigenetic processes that coordinate this process. In contrast to self-renewal, which has an open and permissive state of chromatin, differentiation is a progressive limitation of development potential through coordinated epigenetic remodeling. The alterations maintain cellular identity with lineage-specific genes being turned on and pluripotency-linked genes switched off forever.

### 4.1 Lineage Commitment Mechanisms

Lineage commitment is another important process in stem cell differentiation, and calls upon cells to permanently lose their ability to develop in any other way. It is mainly due to the silencing of key pluripotency genes (e.g., OCT4, SOX2, and NANOG) through epigenetic mechanisms, as well as the activation of lineage-specific transcriptional programs. DNA methylation and repressive histone modifications are at the centre of what occurs to stop the reversion to an undifferentiated state via the reactivation of pluripotency networks. At the same time, lineage-specific genes are also induced by the formation of permissive chromatin states in which histone acetylation and activating methylation marks like the H3K4me3 are set. These epigenetic modifications are defined by transcription factors that define lineage and usually are recruited to particular genomic loci by chromatin-modifying complexes. This stepwise and coordinated silencing of pluripotency genes and activation of differentiation pathways allows a stable and unidirectional shift to specialized cell types.

### 4.2 Dynamics of DNA Methylation in Differentiation

The processes of DNA methylation are dynamically regulated during stem cell differentiation, and are an essential part of the regulation of gene expression. New patterns of DNA methylation, which is catalyzed by DNMT3A and DNMT3B, help to silence the pluripotency-associated genes and to prevent the alternative lineage potentials. This mechanism helps to stabilize variousiated states enhancing transcriptional repression. On the other hand, DNA demethylation is also crucial in the activation of lineage specific genes. Ten-Eleven Translocation (TET) enzymes mediate this process and catalyze the oxidation of 5-methylcytosine, which results in its removal in the end. The previously silenced genes may then be reactivated with the help of active and passive forms of demethylation and the ability to differentiate into a particular lineage. The homeostasis of methylation and demethylation is thus obligatory to normal lineage specification, since a lack of control in the homeostasis may result in abnormal differentiation or developmental maladaptations.

### 4.3 Histone Code Reprogramming

The modifications of the histone are highly reprogrammed during stem cell differentiation and indicate the changes in the chromatin structure and transcriptional activity. The shift between active and repressive histone marks, including H3K4me3 and histone acetylation, and the H3K27me3 and H3K9me3, respectively, is noted as one of the major aspects of this process at the loci of pluripotency genes. This switch helps in stable silencing of genes and it also avoids reactivation of pluripotency programs. Meanwhile, the lineage-specific genes obtain activating histone marks resulting in a higher chromatin accessibility and transcriptional activation. Mediators of histone modification redistribution include concerted activities of histone-modifying enzymes, such as methyltransferases, demethyltransferases, acetyltransferases, and deacetyltransferases. These enzymes are dynamically responding to developmental cues by dynamically remodelling the epigenetic landscape. Chromatin patterns of condensation are altered as well as histone marks during differentiation. Differentiated cells generally have a more compact chromatin organization than pluripotent stem cells, which involves a decrease in transcriptional plasticity. This augmented chromatin condensation supports lineage commitment by restricting access to regulatory DNA components, to guarantee stability of the differentiated condition.

## 5. Epigenetic Reprogramming and Induced Pluripotency

Restoration of differentiated somatic cells to a pluripotent state, similar to embryonic stem cells, is called epigenetic reprogramming. The Yamanaka factors, consisting of OCT4, SOX2, KLF4, and c-MYC are also typical inducers of this process, where they activate transcriptional networks of pluripotency and inhibit somatic lineage-specific programs. In the process of iPSC production, the somatic epigenetic environment has to be significantly reshaped in order to restore the previous silenced pluripotency genes, and down-regulate the differentiated cell identity genes. The epigenetic switch controlling stem cell fate is a coordinated event as indicated in Fig 2. The active genes in the pluripotent state include OCT4, SOX2, and NANOG, which is open chromatin, low levels of DNA methylation, and activating histone proteins like H3K4me3 and H3K9ac. On the contrary, lineage-specific genes are either silenced or in an inactive state until differentiation signals are transduced. It demonstrates that pluripotency is not a quiescent state, and is a dynamically regulated epigenetic state.

The pattern is reversed in Fig 2 during differentiation. The pluripotency genes are silenced by means of elevated levels of DNA methylation, repressive histone marks like H3K27me3 and H3K9me3, as well as chromatin condensation. Meanwhile, lineage-specific genes are activated due to DNA demethylation, opening of chromatin, and enrichment of activating histone marks. This change is what makes stem cells lose pluripotency and develop fixed specialized identities, like Neuronal, muscle or blood cell lineage. This has to be done in the reverse with regard to iPSC reprogramming. Differentiated cell would have to clear away restrictive epigenetic marks and re-establish a pluripotent chromatin condition. This resetting however is not always complete. As highlighted in Table 1, variousiated cells have generally more DNA methylation, more repressive histone marks, condensed chromatin, and loss of epigenetic plasticity. To overcome these features, reprogramming will have to undo them: by reducing the degree of methylation of promoters of pluripotency genes, by reinstating activating histone marks, and by reopening previously silenced regions of the chromatin necessary to express pluripotent genes.

Residual epigenetic memory is a great obstacle to successful reprogramming. Other iPSCs can maintain methylation or histone marks of their native somatic tissue, and this can be biased and bias their subsequent differentiation. As an example, blood or skin cells might have some remnant of molecular memory of the cell type they are derived by, and be more prone to redifferentiating that cell type. This partial recombination minimizes the standardization and predictability of populations of iPSC.

The efficiency of reprogramming is also low, typically in the range of 0.01 per cent -1 per cent depending on the type of cell, delivery conditions, culture conditions and reprogramming strategy. This reduces efficiency indicates a potent epigenetic resistance in differentiated cells, such as compact heterochromatin, stable DNA methylation, and stable repressive histone marks. Thus, the release of transcription factors is not sufficient to produce successful iPSCs but a profound chromatin remodeling is also needed. On the whole, both Fig 2 and Table 1 indicate that the process of induced pluripotency requires the reversal of the epigenetic program associated with normal differentiation. The process of differentiation transforms open and flexible pluripotent chromatin into lineage-specific chromatin and reprogramming seeks to rejuvenate the open and plastic state. The key issue is that the full, stable and safe epigenetic reconstitution should be attained without any memory, abnormal gene expression, or genomic instability.

**Table 1. Epigenetic Changes during Stem Cell Differentiation**

| Feature | Pluripotent State | Differentiated State | Functional Outcome |
|---|---|---|---|
| DNA Methylation | Low (hypomethylation) | High (hypermethylation) | Silencing of pluripotency genes |
| Histone Marks | H3K4me3 (active), acetylation ↑ | H3K27me3, H3K9me3 (repressive) | Lineage-specific gene regulation |
| Chromatin State | Open (euchromatin) | Closed (heterochromatin) | Reduced transcriptional plasticity |
| Gene Expression | OCT4, SOX2, NANOG active | Lineage genes activated | Stable cell identity |
| Epigenetic Flexibility | High | Low | Commitment to specific lineage |

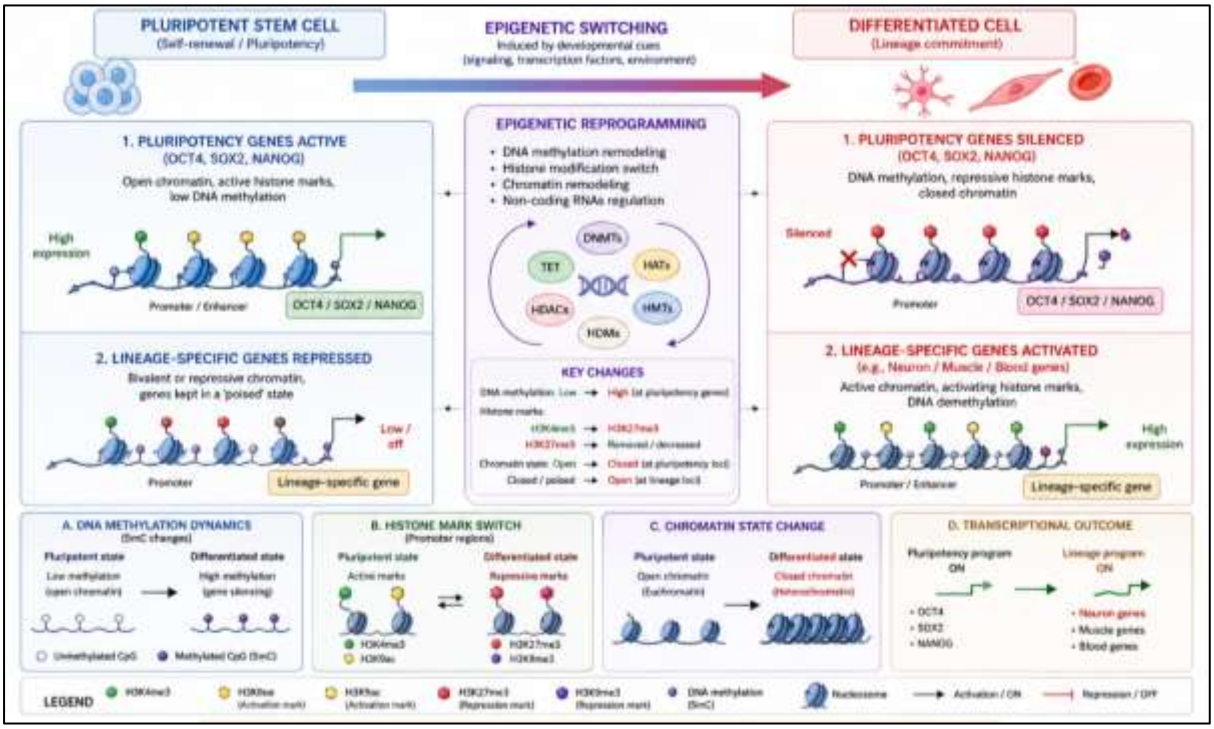

**Fig 2. Epigenetic Switching in Stem Cell Fate Determination.**

## 6. Role of Non-Coding RNAs in Stem Cell Fate Determination

Non-coding RNAs (ncRNAs) have become important controllers of stem cell fate determination and play the role of important modulators of gene expression on the transcriptional and post-transcriptional regulation. In contrast to protein-coding genes, ncRNAs have their regulatory effect on the interface with DNA, RNA and proteins, thus becoming a part of the overall epigenetic environment. These molecules play a role in the fine-tuning of pluripotency maintenance and lineage-specific differentiation as coordinating with the classical epigenetic pathways involving DNA methylation and histone modification, and chromatin remodeling. Long non-coding RNAs (lncRNAs) especially are very relevant in the regulation of chromatin. They serve as molecular scaffolds or guides recruiting chromatin-modifying complexes to particular loci in the genome thus modulating local chromatin states. LncRNAs can control adjacent genes via cis-acting mechanisms, but trans-acting lncRNAs are able to control distal genomic locations. Figure 3 shows that ncRNA-mediated regulation is coordinately coupled with epigenetic reprogramming pathways, with RNA molecules directed by important enzymatic regulators including DNMTs, TET enzymes, histone acetyltransferases (HATs) and histone methyltransferases (HMTs) to loci. This specific recruitment allows an accurate activation and repression of genes, which eventually determines stem cell identity.

Fig 3 gives a detailed overview of a role of non-coding RNAs in the process of epigenetic reprogramming. The figure depicts the conversion of a highly methylated, transcriptionally repressed genome in a somatic cell to induced pluripotent stem cell (iPSC) with open chromatin and active expression of genes in an induced state. In the process, the ncRNAs mediate binding and activity of the Yamanaka factors (OCT4, SOX2, KLF4 and c-MYC), which augments the activation of pluripotency genes and repression of somatic gene programs. This emphasizes the importance of ncRNAs as mediators linking transcription factors and chromatin-modifying machineries. Moreover, Figure 3 shows the progressive stages of the epigenetic reprogramming, such as initiating, epigenetic resetting, establishment, and maturation. Included in the epigenetic resetting, ncRNAs take part in global DNA de-methylation by engaging with TET enzymes, which convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). At the same time, ncRNAs can help in the process of chromatin remodeling through the repositioning of nucleosomes and the promotion of open chromatin states. These well-coordinated activities facilitate the reactivation of pluripotency related genes.

Another significant group of ncRNAs that play a role in the regulation of stem cells are the microRNAs (miRNAs). The main mechanism of action of these small RNA molecules is to bind to complementary sequences of target messenger RNAs, triggering their degradation or inhibition of translation. miRNAs in stem cells modulate the important differentiation-related transcription factors and signaling pathways. Fig 3 demonstrates that miRNAs have the capability to silence somatic gene expression programs during reprogramming, thus facilitating the shift to pluripotency. They have a mode of action that is rapid and reversible, and thus can be tightly controlled in terms of when genes are expressed during developmental transitions. Also, the significance of RNA-protein and RNA-DNA interactions in epigenetic regulation is emphasized in Fig 3. The ncRNAs establish a ribonucleoprotein complex with RNA-binding proteins, which allow them to guide the chromatin-modifying enzymes to particular regions of the genome. Additionally, in other scenarios, ncRNAs have the ability to get directly into contact with DNA to establish RNA-DNA hybrid structures, which affect chromatin accessibility and transcriptional behaviors. These interactions offer another level of specificity and regulation of genes.

Notably, Fig 3 also highlights the obstacles to full reprogramming, including residual DNA methylation, long-term repressive histone structures, and heterochromatin areas, which are not easy to open. The role of ncRNAs in overcoming and facilitating these barriers is through modulation of epigenetic activities of enzymes and chromatin structure. Nevertheless, partial regulation of ncRNA may play a role in epigenetic memory, inefficiency of reprogramming, and instability in population of iPSCs. Altogether, incorporation of ncRNAs into epigenetic networks is an essential degree of regulatory complexity in stem cells biology. The ncRNAs, as shown in Fig 3, act as the central coordinators of interconnecting these three processes DNA methylation, histone modification and chromatin remodeling into a single regulatory system. This combined control allows tight control of the pluripotency-related genes and allows lineage-specific programming to be controlled. The knowledge of these RNA-mediated processes further informs the processes of stem cell fate determination as well as creating new opportunities in therapeutic intervention in regenerative medicine.

## 7. EXPERIMENTAL AND COMPUTATIONAL METHODS

The experimental and computational technologies have made tremendous progress, which has increased our knowledge about epigenetic regulation in stem cell fate determination. Current high throughput sequencing methods, paired with integrative bioinformatics methods allow independent profiling of chromatin states, gene expression patterns and regulatory networks on large and single cell levels. Such strategies offer vital information about the roles of dynamic epigenetic processes in regulating self-renewal, differentiation, and reprogramming of stem cells. Chromatin immunoprecipitation sequencing (ChIP-seq) is a very popular method of genome-wide mapping histone modifications and transcription factor binding sites. ChIP-seq can be used to obtain important data about the state of chromatin related to pluripotency and differentiation by determining the distribution of activating and repressive histone marks, including H3K4me3 and H3K27me3. In complement to this, the transposase-accessible chromatin using sequencing (ATAC-seq) assay allows one to identify open chromatin regions, which discloses accessible regulatory machinery like promoters and enhancers. These techniques can be used together to reconstruct the epigenetic landscapes that control the identity of the stem cells. Otherwise, the RNA sequencing (RNA-seq) also gives quantitative data on the gene expression patterns, providing connections between changes in the epigenome and the actual functional transcriptional activity.

Recent advances in single-cell epigenomics have transformed stem cell biology by allowing the study of heterogeneity in epigenetics at the level of single cells. Single-cell RNA-seq (scRNA-seq), single-cell ATAC-seq (scATAC-seq), and single-cell DNA methylation mapping (scMethylation) methods can enable researchers to capture dynamic cellular changes and reveal rare subpopulations in differentiation and reprogramming. Such methods are especially helpful to learn about stochastic processes and lineage directions that are often obscured in bulk measurements. Epigenome editing technologies, especially CRISPR/dCas9-based genome editing has proven to be a large tool to directly manipulate epigenetic states. Catalytically inactive dCas9 can be fused with epigenetic modifiers including DNA methyltransferases, demethylases or histone-modifying enzymes in contrast to traditional CRISPR systems which induce DNA breakages. This allows activation or repression of specific genes to be targeted but does not change the DNA sequence. This type of strict control of the epigenetic mark offers a therapeutic method to uncover the causal functions of epigenetic alteration in stem cell fate determination and has great potential in the treatment of disorders.

The capability to combine various data types with multi-omic methods has broadened the research agenda of epigenetics. Multi-omics analyses are a combination of transcriptomics, epigenomics, proteomics and metabolomics, which provide a more comprehensive picture of cellular regulation. These integrative structures in stem cells facilitate the discovery of multifaceted regulatory networks and interactions among various layers of molecules that provide insights into the role of epigenetic processes in scaffolding gene expression and cell behaviors. Bioinformatics tools and computational models are critical to handle and interpret the tremendous data produced using these approaches. Sequence alignment, statistical analysis of the differences between two samples, peak calling, and network inference are performed using advanced algorithms. Regulatory elements, epigenetic signatures, and stem cell differentiation trajectories are some of the areas where machine learning and artificial intelligence methods are gaining use to make predictions. Such computational techniques not only improve the interpretation of data, but also can be used to predictively model the situation of epigenetic regulation under various biological circumstances. Altogether, high-throughput experimental methods and advanced computational methods offer an attractive platform to unravel the complexity of epigenetic regulation in stem cells. The tools have revolutionized the field beyond descriptive studies to predictive and mechanistic science, which can now understand stem cell fate determination more and have created new possibilities in precision medicine and regenerative therapies.

## 8. Biological and Clinical Implications

The growing knowledge of epigenetic control in stem cell fate specification has had far-reaching biological and clinical implications, especially in the area of regenerative medicine, disease modeling, and targeted therapeutics. In addition to regulating normal development and tissue homeostasis, epigenetic processes can also play a role in the pathogenesis of many diseases upon dysregulation. These reversible modifications are highly manipulable and offer tremendous possibilities to manipulate cell identity and functionality to treat diseases.

Regenerative medicine is one of the most promising areas of use of stem cells, where they are used to fix or replace damaged tissues and organs. Epigenetics is also essential in preserving the pluripotency of stem cells and their differentiation into certain cell types that are needed to regenerate tissues. With epigenetic state modulations, one can boost stem cell differentiation and enhance the production of functional cell types: neurons, cardio-myocytes, and 2-cell pancreatic beta cells. This has a great implication in terms of treatment of conditions like the spinal cord injury, heart diseases, and diabetes.

Intimately near this would be the development of stem cell therapy, where epigenetically re-engineered cells, in this case, induced pluripotent stem cells (iPSCs), would be utilized in customized treatment approaches. Patient specific iPSC derivation means that the chance of immune rejection is minimized and it can be transplanted as an autologous. The success of these therapies, however, is sensitive to the attainment of stable and accurate epigenetic reprogramming since incomplete resetting may result in abnormal differentiation or tumorigenesis. Hence, to ensure the safety and effectiveness of stem cell-based interventions, it is crucial to understand and regulate epigenetic processes. Another marker of cancer stem cells (CSCs), a subpopulation of tumor cells, is epigenetic dysregulation, and these cells have the capacity to self-renew and differentiate like normal stem cells do. Aberrant patterns of DNA methylation, histone alterations, and chromatin remodeling help to keep CSCs viable and facilitate tumor growth, development and therapy resistance. The idea to attack epigenetic regulators of CSCs presents a tremendous opportunity to get rid of these cells and enhance the efficacy of cancer therapy. In cancer treatment, epigenetic drugs like DNA methyltransferase and histone deacetylase inhibitors are already under clinical investigation in order to revert abnormal gene expression patterns.

Besides cancer, epigenetic regulation is an important aspect in modeling disease, especially complex diseases like neurodegenerative diseases and metabolic diseases. Models that are based on the use of stem cells, particularly the iPSCs, enable one to manipulate the epigenetic changes in the disease-related conditions within a controlled setting. As an example, patient-derived iPSCs may be differentiated to neurons to learn how epigenetic modifications related to Alzheimer or Parkinson disease, or to metabolic tissues to learn about diabetes and obesity. These models are useful in the understanding of disease pathophysiology and development of specific treatment. The introduction of epigenetic understanding in stem cell biology, overall, has created new possibilities in translation research and clinical applications. The reversible character of epigenetic changes can be used to develop new approaches to regenerating tissues, treating diseases, and personalized medicine. Nevertheless, issues concerning epigenetic stability, safety, and long-term effectiveness should be tackled to maximize the therapeutic potential of the stem cell-based therapies.

## 9. Challenges and Limitations

Although there have been remarkable improvements in epigenetic regulation in stem cell fate determination, there are a few challenges and limitations that still hinder the basic research and clinical translation. These issues are due to the complexity nature of epigenetic mechanisms, their dynamics, and dependence on context. Complexity of the epigenetic networks is one of the main problems. Epigenetic regulation is not dependent on any one mechanism but it consists of the coordinated action of DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs. These elements constitute very inter-linked regulatory networks that have feedback and pathway cross-talk elements. Consequently, it is still a challenge to distinguish individual epigenetic factors in determining stem cell fate. This and the associated complexity are frequently associated with context-dependent effects, whereby the same manipulation can result in varied effects depending upon the cellular condition or environmental cues.

The other outstanding weakness is that epigenetic alterations are temporally and spatially diverse. The epigenetic states are very dynamic and they can vary quickly through development, differentiation and re programming. Furthermore, these modifications tend to be cell-type specific as well as disparate even between cells within a specific population. This heterogeneity complicates the ability to have precise and repeatable epigenetic states, especially when bulk analysis methods are involved. Despite advances in resolution of single-cell technologies, there are limitations to coverage, sensitivity and cost. The growing popularity of high-throughput technologies has produced huge volumes of data, and now data integration problems arise with multi-omics strategies. Synthesis of genomics, epigenomics, transcriptomics, and proteomics involves complex computational analyses and standardized pipelines. Biases and inconsistencies in the interpretation of results may arise due to the differences in data formats, conditions of the experiment, and the techniques used to analyze the results. Moreover, the absence of standardized models of multi-omics integration restricts the possibility of obtaining insights in a comprehensive and biologically meaningful way.

One of the major shortcomings in the field is the absence of adequate validation in vivo. Numerous epigenetic experiments are performed in vitro on cell lines or stem cell models, which are not necessarily as complex as the living organisms. Though these models yield useful mechanistic information, they may have limited physiological applicability. In vivo experiments are necessary to confirm the results and comprehend the mechanism of epigenetic regulation in the conditions of entire tissues and organ systems. Very often, however, such studies are difficult technically, time-consuming, and ethically limited. Lastly, the clinical use of epigenetic and stem cell-based therapies is limited by significant hurdles, which are generally connected to translation. Creating a stable and specific epigenetic changes in clinical environment remains challenging and unwanted off-target effects can result in negative consequences like tumor formation or aberrant differentiation. Also, reprogramming efficiency, epigenetic memory, and long-term stability of induced pluripotent stem cells vary, making it difficult to use them therapeutically. The translation of laboratory findings to clinical practice is complicated by additional regulatory, ethical, and manufacturing considerations. On the whole, although the studies of epigenetics have contributed a great deal to the current knowledge about the stem cell biology, addressing these issues is crucial to achieving success in transferring this information to practical and safe therapeutic usage.

## 10. Future Trends and Future

Epigenetic control of stem cell life is a fast developing area of research, which is facilitated by technological progress and cross-disciplinary convergences. The new tendencies aimed at bettering resolution, accuracy and prediction in the comprehension of epigenetic procedures that control stem cell fate determination is set to gain momentum. This is predicted to revolutionize the basic research and clinical studies in regenerative medicine. Single-cell epigenetic profiling is one of the most revolutionary developments that make it possible to analyze the state of epigenetics on the level of a single cell. Single-cell ATAC-seq, single-cell RNA-seq and single-cell DNA methylation sequencing techniques can be used to capture cellular heterogeneity and discover rare subpopulations, which are important in differentiation and reprogramming. This method offers a more realistic picture of the dynamism in cellular transitions and lineages and is not constrained by bulk analyses.

Intimately connected is the development of spatial epigenomics that combines epigenetic information with space in tissues. In comparison to the classic sequencing technologies that also cause the loss of positional information, spatial technologies enable researchers to map chromatin states, gene expression, and epigenetic changes in intact tissue structures. This is especially significant in comprehending the effects of micro environmental cues on stem cell behavior, and the role of spatial organization in regulating tissue development and regeneration. The other potential avenue is the use of artificial intelligence (AI) and machine learning in the modeling of epigenetics. Multi-omics data are analyzed using AI-based solutions to determine regulatory patterns and predict the result of gene expression depending on epigenetic characteristics. Such models have the potential to recapitulate the stem cell differentiation pathways, reveal hidden regulatory interactions, and give predictive information on the effect of certain epigenetic mutations on the fate of a cell. These computational resources are crucial to handle the ever-increasing complexity of biological data and to facilitate discoveries through data.

The creation of epigenetic editing technologies will be a great move in the direction of therapeutic use. Systems consisting of CRISPR/dCas9 plus epigenetic modifiers enable specific and accurate manipulation of DNA methylation and histone marks without modifying the genetic sequence. In this method, the activation or inhibition of certain genes that activate or suppress stem cell differentiation and disease pathways is achieved. The Epigenetic editing has immense possibilities in remedying abnormal patterns of gene expression in pathologies like cancer, genetic disorders and degenerative diseases. Lastly, the combination of these breakthroughs is ushering in the practice of individualized regenerative medicine. With the integration of patient-specific iPSCs and comprehensive epigenomic profiling, customized therapeutic approaches addressing individual genetic and epigenomic differences can be developed. Such a personalized method increases the effectiveness of treatment and reduces side effects, providing new opportunities in the field of precision medicine. On the whole, these new trends demonstrate a movement in the direction of more specific, integrative, and application-oriented studies in epigenetics. Further developments of experimental methods and computational models will continue to enhance our insights into the regulation of stem cells and will enable the rapid application of epigenetic knowledge to clinical practice.

## 11. CONCLUSION

Epigenetic changes are the key regulators of stem cell fate determination as they dynamically regulate the expression of genes without changing the DNA sequence. The reversible and context-dependent property of stem cells allows them to balance between self-renewal and differentiation, and to retain cellular plasticity but with a stable lineage commitment. Transcriptional controls, including DNA methylation, histone modifications, chromatin remodelling and interactions of non-coding RNAs act in a concerted fashion, to form and reconfigure the epigenetic landscape, in response to developmental and environmental signals. Combination of hi-tech multi-omics with artificial intelligence-driven analysis systems will greatly improve our capacity to decipher complicated epigenetic circuits and forecast cell behavior. Beyond enhancing our knowledge of basic stem cell biology, these advances have tremendous potential in precision medicine, allowing development of targeted regenerative therapies and new treatment plans to a broad array of diseases.

## REFERENCES

1. Allis, C. D., & Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. Nature reviews genetics, 17(8), 487-500.
2. Atlasi, Y., & Stunnenberg, H. G. (2017). The interplay of epigenetic marks during stem cell differentiation and development. Nature Reviews Genetics, 18(11), 643-658.
3. Cavalli, G., & Heard, E. (2019). Advances in epigenetics link genetics to the environment and disease. Nature, 571(7766), 489-499.
4. Chen, T., & Dent, S. Y. (2014). Chromatin modifiers and remodellers: regulators of cellular differentiation. Nature Reviews Genetics, 15(2), 93-106.
5. Di Croce, L., & Helin, K. (2013). Transcriptional regulation by Polycomb group proteins. Nature structural & molecular biology, 20(10), 1147-1155.
6. Goldberg, A. D., Allis, C. D., & Bernstein, E. (2007). Epigenetics: a landscape takes shape. Cell, 128(4), 635-638.
7. Meshorer, E., & Misteli, T. (2006). Chromatin in pluripotent embryonic stem cells and differentiation. Nature reviews Molecular cell biology, 7(7), 540-546.
8. Nicetto, D., & Zaret, K. S. (2019). Role of H3K9me3 heterochromatin in cell identity establishment and maintenance. Current opinion in genetics & development, 55, 1-10.
9. Pearce, C. L., Van Den Berg, D. J., Makridakis, N., Reichardt, J. K., Ross, R. K., Pike, M. C., ... & Henderson, B. E. (2008). No association between the SRD5A2 gene A49T missense variant and prostate cancer risk: lessons learned. Human molecular genetics, 17(16), 2456-2461.
10. Smith, Z. D., & Meissner, A. (2013). DNA methylation: roles in mammalian development. Nature Reviews Genetics, 14(3), 204-220.
11. Wu, H., & Zhang, Y. (2014). Reversing DNA methylation: mechanisms, genomics, and biological functions. Cell, 156(1), 45-68.
12. Yamanaka, S. (2020). Pluripotent stem cell-based cell therapy—promise and challenges. Cell stem cell, 27(4), 523-531.