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MOLECULAR REGULATION OF STEM CELL DIFFERENTIATION THROUGH EPIGENOMIC AND TRANSCRIPTOMIC CHANGES

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ABSTRACT

Stem cell differentiation is a highly controlled biological mechanism, through which pluripotent and multipotent cells can differentiate into the specific cell types to play an essential role in development, tissue repair and in regenerative medicine. It is regulated by complicated molecular processes, and epigenomic and transcriptomic regulation can be considered some of the determinants of cell fate choice. Epigenomic changes such as DNA methylation, histone changes, and chromatin remodeling, have an effect on chromatin structure and accessibility of genes. Simultaneously, transcriptomic dynamics including mRNA, non-coding RNA and alternative splicing expression regulate the pattern of gene expression that is critical to specification of lineage. The emergence of high-throughput sequencing and multi-omics technologies recently has made it possible to analyze these regulatory layers in a comprehensive way and has shown that these interactions between chromatin states and transcriptional programs are complex. Combined epigenomic-transcriptomic studies have made a great contribution to our knowledge on the differentiation of stem cells and complexity of regulatory networks. Nevertheless, there are still difficulties related to data integration, interpretation and provision of causal relations between regulation mechanisms. The paper is an overview of what is known today regarding the molecular basis of stem cell differentiation, with a particular focus on epigenomic and transcriptomic interactions. It also outlines the emerging multi-omics approaches and talks about future trends that one should look, such as advanced computational tools and epigenome editing, to ensure specificity in the regenerative medicine and therapeutic fields.

KEYWORDS: Stem cells, Epigenomics, Transcriptomics, Cell differentiation, Gene regulation, Multi-omics

1. INTRODUCTION

Stem cells are a special category of undifferentiated cells that have a property to self-renew and differentiate to specialized cells (Orkin & Hochedlinger, 2011; Lessard & Crabtree, 2010). Stem cells can be universally divided into pluripotent, multipotent and unipotent stems based on their developmental potential. Pluripotent stem cells, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are capable of differentiating into all cell lineages and are therefore useful in developmental biology and regenerative medicine (Orkin and Hochedlinger, 2011). Adult stem cells on the contrary have a limited differentiation potential but are important in maintaining and repairing tissues. Embryonic development, organ formation, and tissue homeostasis are biological processes that are based on stem cell differentiation. In the process, stem cells are closely regulated to change both their molecular and phenotypic state to be dedicated to a lineage or adopt functional specialization. This migration is controlled by a complicated interaction of inherent genetic programs and external signaling pathways, such as Wnt, Notch, Hedgehog and bone morphogenetic protein (BMP). The issues with these mechanisms of regulation may result in abnormal differentiation, which plays a role in the cause of diseases, including cancer, neurodegenerative disorders, and developmental abnormalities. An example is where defective differentiation will lead to the maintenance of undifferentiated cells that have uncontrolled growth, which is one of the characteristics of tumorigenesis. Discovering the molecular mechanism of stem cell differentiation has grown more significant in the area of regenerative medicine and treatment. Classical models of gene regulation, which are mostly based on transcription factors, do not provide a high level of understanding on the dynamism and context specificity of

stem cell fate choices (Orkin & Hochedlinger, 2011; Lessard and Crabtree, 2010). In the recent studies, the critical importance of epigenomic and transcriptomic regulation in the process of regulating gene expression and cell identity has been brought to light (Atlasi and Stunnenberg, 2017; Allis and Jenuwein, 2016). DNA methylation, histone modifications, chromatin remodeling, and higher-order genome organization of epigenomic regulatory processes affect chromatin accessibility and transcriptional activity, but not DNA sequence (Schubaeler, 2015; Bannister and Kouzarides, 2011; Bonev and Cavalli, 2016; Stadhouders et al., 20 The modifications can be viewed as molecular switches which shut down or open gene expression in differentiation. Besides epigenomic control, another important level of control is the transcriptomic processes. Not only are protein-coding genes regulated by transcriptomics, but non-coding RNAs, including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) also play a crucial role in transcriptomics (Flynn & Chang, 2014). They are important molecules of post-transcriptional regulation that manipulate the stability of mRNA, translation and other gene expression networks. Moreover, alternative splicing and RNA editing are among the mechanisms that add to the diversity of transcriptomes and allow controlling cellular differentiation with a fine precision. Notably, epigenomic and transcriptomic systems are closely related to each other, and they act as integrated regulatory systems (Atlasi and Stunnenberg, 2017; Tanay and Regev, 2017). These layers of regulation can be studied in detail due to the development of new high-throughput sequencing methods, such as RNA sequencing (RNA-seq), chromatin immunoprecipitation sequencing (ChIP-seq), atomic chromatin access sequencing (ATAC-seq), etc. (Buenrostro et al., 2015). Recently, single-cell and multi-omics have given increased insights into the heterogeneity of cells and dynamics of differentiation (Cao et al., 2018; Clark et al., 2018; Argelaguet et al., 2019). Although these have been made, there are still serious challenges such as incorporation of large-scale omics datasets and establishing the causal relationship between regulatory mechanisms. This review also seeks to summarize available information on the molecular mechanisms controlling stem cell differentiation along with epigenomic and transcriptomic changes and to point to the new multi-omics methods and future directions in research.

2. LITERATURE REVIEW: STATE OF RESEARCH

Molecular regulation of stem cell differentiation has been rapidly changing over the last decades such that multi-layered regulatory programs comprising of epigenomic and transcriptomic interactions have replaced simple models of transcription factor regulation (Orkin and Hochedlinger, 2011; Lessard and Crabtree, 2010). Most of the earlier research looked at how important the main transcription factors were, including OCT4, SOX2, and NANOG that were determined as key regulators of pluripotency and lineage commitment. These models, which were based on transcription factors, offered the first basis on the understanding of stem cell fate decision; but they could not offer the dynamism and context dependency of the differentiation processes. Later studies further extended this framework with the addition of epigenomic regulation, the role of which in regulating gene expression is of great significance in DNA methylation and histone modification (Schubeler, 2015; Bannister and Kouzarides, 2011). DNA methylation was identified to be a key part of silence of lineage-inappropriate genes whereas H3K4me3 and H3K27me3 histone modifications were found to activate and repress chromatin, respectively. The idea of bivalent chromatin domains also helped to highlight the state of developmental genes as poised in stem cells and becoming activated by differentiation signals in a very short period of time (Atlasi and Stunnenberg, 2017). Higher-order genome organization and chromatin remodeling have been also found to be necessary tools to regulate access to chromatin and gene regulatory landscapes (Bonev and Cavalli, 2016; Stadhouders et al., 2019). Simultaneously, the development of transcriptomic technology has given better understanding of the dynamics of gene expression in the process of stem cell differentiation. With the advent of RNA sequencing (RNA-seq), it became possible to study the genome-wide alterations in gene expression, which has also identified lineage-specific patterns of gene expression and regulatory interactions (Tsankov et al., 2015). Most recently, the single-cell RNA sequencing (scRNA-seq) has transformed the process by measuring the heterogeneity of cells and discovering intermediate cell types throughout the differentiation process (Semrau et al., 2017). These methods have revealed complicated transcriptional programs and regulatory pyramids that would not be revealed by bulk analysis. Combination of epigenomic and transcriptomic has brought more insight in the regulation of stem cells. The use of multi-omics technology, which involves a combination of ChIP-seq, ATAC-seq, and RNA-seq, has made it possible to build complex gene regulatory networks (Cao et al., 2018; Clark et al., 2018; Ma et al., 2020). Systems biology methods have been used to simulate how chromatin states and transcriptional programs are interacting with each other, and thus offer a comprehensive perspective on differentiation process (Tanay and Regev, 2017; Stuart et al., 2019). It has been demonstrated that epigenomic and transcriptomic processes are closely related and act as coordinating systems of regulation, and not as layers. Although these have been improved, there are some challenges that still exist. One of the greatest constraints of the contemporary research is the absence of a regulatory framework, in which a cohesive system of epigenomic and transcriptomic processes is merged into various biological conditions. Also, the combination of multi-omics data is still technically and computationally difficult, which frequently results in the lack of consistency in data interpretation. It is also still a major challenge to establish a causal role of epigenetic changes on transcriptional outcomes to bring the findings to clinical use. As the background to give a comparative overview of the important works in this field, Table 1 will provide the summary of the representative research works in the field with emphasis on the models and methods used in the experiments, their main findings, and limitations of the articles (Xie et al., 2013; Pijuan-Sala et al., 2019).

Table 1. Summary of Key Studies on Epigenomic and Transcriptomic Regulation of Stem Cell Differentiation

Author (Year)	Model/System	Technique	Key Findings	Limitations
Bernstein et al. (2006)	Human ESCs	ChIP-seq	Identified bivalent chromatin domains regulating developmental genes	Limited resolution of dynamic changes
Meissner et al. (2008)	Mouse ESCs	Bisulfite sequencing	Demonstrated DNA methylation dynamics during differentiation	Bulk analysis lacks cellular heterogeneity
Young (2011)	Human ESCs	Gene regulatory network analysis	Defined core transcriptional circuitry of pluripotency	Limited integration with epigenomic data
Buenrostro et al. (2015)	Mammalian cells	ATAC-seq	Revealed chromatin accessibility landscapes	Does not directly measure transcriptional output
Trapnell et al. (2014)	Various stem cells	RNA-seq / scRNA-seq	Identified transcriptional trajectories of differentiation	Computational complexity
Stuart & Satija (2019)	Single-cell datasets	Multi-omics integration	Advanced integration of scRNA-seq and epigenomic data	Integration bias and batch effects

3. Biological Basis of Stem Cell Differentiation

Stem cell differentiation is a central biological phenomenon by which unspecialized cells obtain the specialized structural and functional features. This is involved in the development of embryos, formation of tissues; and regeneration of tissues in the postnatal stage. The developmental potential of the stem cells has been broadly classified into embryonic stem cell (ESCs), induced pluripotent stem cell (iPSCs) and adult stem cell. ESCs are a product of inner cell mass of the blastocyst and have the pluripotent potential which facilitates their derivatives to form derivatives of all three germ layers: ectoderm, mesoderm, and endoderm. Likewise, reprogrammed somatic cells that have been reinstated to pluripotency by the ectopic expression of particular transcription factors (iPSCs) are a source of an ethical and clinically potential alternative to ESCs. Adult stem cells, on the other hand, are multipotent or tissue-restricted and play the major role in the maintenance and repair of particular tissues in particular organs. Differentiation takes place in a series of developmental events where the initial stage is the maintenance of pluripotency, then lineage priming, lineage commitment, progenitor expansion and termination maturation. Through the lineage commitment, the stem cells slowly lose their self-renewal capacity and develop molecular cues that guide them to a particular cell fate. The initial significant lineage segregation in the pluripotent stem cells generates the three germ layers. The ectoderm lineage produces mostly neural and epidermal cell types, the mesoderm lineage produces muscle, blood, bone, and cartilage, and the endoderm lineage produces internal organ-derived epithelial cells, like hepatocytes, pancreatic cells and lung/intestine epithelial cells. Pluripotent stem cells differentiate into these three germ layers as demonstrated in Figure 1 and which further undergo progressive lineage restriction to form specific cell types. Stem cell identity and differentiation are regulated by a central transcriptional network that is centered on OCT4, SOX2 and NANOG at the molecular level. The transcription factors are also necessary in the process of sustaining pluripotency and inhibiting precature differentiation. OCT4 is a global controller of self-renewal and early developmental capability and SOX2 is involved in this process together with OCT4 to maintain the undifferentiated state. NANOG also maintains pluripotency through the strengthening of transcriptional circuits which suppress the expression of lineage-specific genes. The down-regulation of the activity or expression of these factors is generally linked with quitting pluripotency and embarking on differentiation programs. Lineage commitment in conjunction with the transcriptional control is highly dependent on extracellular signalling pathways that convey cellular microenvironment developmental signals. WNT, Notch and bone morphogenetic protein (BMP) signaling are among the most significant ones. The Wnt signaling has a context-dependent part in the maintenance of pluripotency as well as lineage specification, especially in mesodermal differentiation. Notch signaling plays a vital role in cell fate, maintenance of progenitors and time of differentiation particularly in the neural and hematopoietic systems. The BMP signaling is responsible of controlling early embryonic patterning and directing differentiation to various lineages based on cellular context and signaling strength. Several other pathways such as fibroblast growth factor (FGF), transforming growth factor-beta (TGF-B)/Activin, and Hedgehog also play a role in the molecular control of lineage development. These signaling pathways play a key role in lineage commitment and the production of differentiated cell types as indicated in Figure 1, are large regulatory inputs.

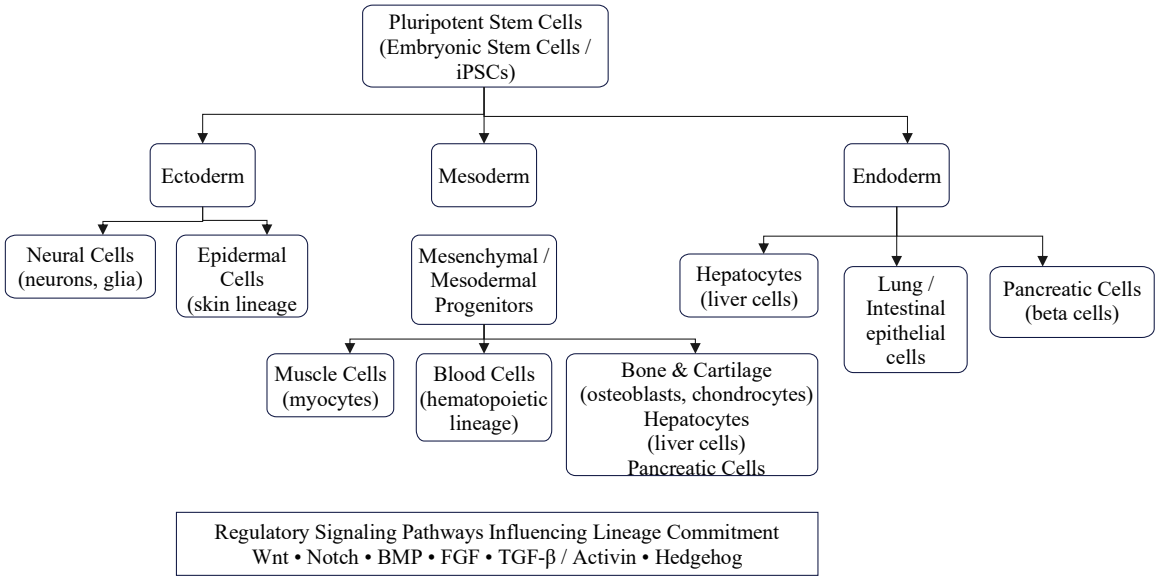

Figure 1. Stem Cell Differentiation Pathways and Lineage Commitment

This diagram shows how pluripotent stem cells differentiate by progressing through the three main germ layers: the ectoderm, mesoderm and endoderm and then differentiate into specialized cell types. It also emphasizes the regulatory role of major signaling pathways, such as Wnt, Notch, BMP, FGF and TGF- beta/Activin in lineage commitment.

4. Molecular Regulation of Stem Cell Differentiation

4.1 Epigenomic Regulation

The regulation of the epigenome is the key to the regulation of stem cell differentiation through the regulation of chromatin structure and gene accessibility without affecting the underlying DNA sequence. Using these mechanisms, the temporal and spatial regulation of gene expression is carried out accurately, and stem cells can be induced to switch between a pluripotent state and a lineage-specified one. DNA methylation which normally takes place at cytosine bases located in CpG dinucleotides is one of the most well-researched epigenetics processes. The DNA methylation is commonly related to the concept of transcriptional repression, and it is also needed to silence the pluripotency genes during differentiation and activate the lineage-specific gene programs. The dynamics of the appearance and disappearance of DNA methylation patterns guarantee the preservation of the lineage commitment and maintenance of the cellular identity. The other important regulatory process is the histone modifications, which can be either methylation or acetylation of histone tails. Certain histone modifications like the H3K4me3 are linked with active gene transcription, and the H3K27me3 is associated with gene repression. Many developmental genes in pluripotent stem cells have bivalent domains, with the activation and repressive marks present in these domains, and holding genes in an active state until developmental cues trigger their activation. Gene expression is also regulated by chromatin remodeling complexes like SWI/SNF family which alters the nucleosome positioning and chromatin accessibility. These ATP-dependent complexes are involved in binding of transcription factors to regulatory regions to allow or inhibit transcription activity in lineage specification. Besides the local chromatin changes, three dimensional (3D) genome organization has come out as a very important aspect in the regulation of epigenomics. The chromosome positioning of the nucleus such as topologically associating domains (TADs) and enhancerpromoter looping interactions are important in the coordination of the gene expression programs. The alteration of chromatin structure in the process of differentiation permits activating lineage-specific genes and suppressing pluripotency-related genes. In order to provide the greatest epigenetic mechanisms and functional relevance in stem cell differentiation, Table 2 provides a comparative view of major epigenetic changes, their enzymes, and their biological role in the lineage commitment processes.

Table 2. Epigenetic Modifications and Functional Roles in Differentiation

Epigenetic Modification	Key Enzymes/Factors	Functional Role	Impact on Differentiation
DNA Methylation	DNMT1, DNMT3A, DNMT3B	Gene silencing through CpG methylation	Represses pluripotency genes; stabilizes lineage commitment
DNA Demethylation	TET enzymes (TET1/2/3)	Removal of methyl groups	Reactivates lineage-specific genes

Histone Acetylation	HATs (e.g., p300/CBP)	Chromatin relaxation and gene activation	Promotes transcription of differentiation genes
Histone Deacetylation	HDACs	Chromatin condensation and gene repression	Maintains transcriptional control during lineage restriction
H3K4me3 (Active Mark)	MLL/SET complexes	Activates gene transcription	Supports lineage-specific gene expression
H3K27me3 (Repressive Mark)	Polycomb Repressive Complex 2 (PRC2)	Gene repression	Silences developmental genes until activation
Chromatin Remodeling	SWI/SNF complexes	Alters nucleosome positioning	Enables transcription factor binding
3D Genome Organization	CTCF, Cohesin	Chromatin looping and TAD formation	Coordinates enhancer–promoter interactions

4.2 Transcriptomic Regulation

Transcriptomic regulation is an essential control level of stem cell differentiation, which determines the expression programs of gene expression that form the basis of lineage specification and cell identity. In contrast to epigenomic pathways controlling accessibility of chromatins, transcriptomic pathways have a direct effect on the production, processing and destabilization of the RNA molecules, which shape the production of functional genes. One of the fundamental aspects of transcriptomic regulation is mRNA expression dynamics that is characterized by close regulation of gene expression through activation and repression in differentiation. The pluripotent stem cells have their own profiles of transcription in which they express genes related to the self-renewal and pluripotency. These genes are silenced as differentiation goes on, and lineage specific genes are activated in a coordinated fashion. These transcriptional transitions have been found to be gradual, being studied by high-throughput RNA sequencing, to correlate with intermediate states between them. Besides protein coded transcripts, non-coding RNAs (ncRNAs) also form a key part of the stem cell differentiation regulation process. One of them is the microRNAs (miRNAs), which are short RNA molecules that participate in post-transcriptional gene regulation, by degrading or repressing mRNA target. Indicatively, there are particular miRNAs that are known to have an inhibitory effect on pluripotency genes and differentiation. Long non-coding RNAs (lncRNAs) are molecular scaffolds which direct chromatin-modifying complexes to target genomic loci and have an effect on transcription. Likewise, circular RNA (circRNA) have also become significant regulators, which are capable of becoming miRNA sponges, ability to regulate the expression networks of genes and play a role in lineage-specific differentiation. Another essential mechanism is the process of alternative splicing whereby once gene produces different splicing variants of mRNA with different functional characteristics. The process immensely increases the diversity of proteomics and allows regulating the processes of cellular functioning on a fine scale during differentiation. Asymmetric cell division is also common in lineages: such events are called alternative splicing events, which play a role in functional differentiation of differentiated cells. To present a systematic review of the major non-coding RNAs that contribute to stem cell differentiation, Table 3 has listed examples of ncRNAs, target, and functional role in the control of lineage choice and gene expression cascades.

Table 3. Non-Coding RNAs in Stem Cell Differentiation

RNA Type	Example	Target/Function	Role in Differentiation
miRNA	miR-145	Targets OCT4, SOX2, KLF4	Promotes differentiation by suppressing pluripotency
miRNA	miR-302 family	Regulates cell cycle genes	Maintains pluripotency and controls differentiation timing
lncRNA	HOTAIR	Chromatin remodeling via PRC2	Regulates gene silencing during differentiation
lncRNA	MALAT1	RNA processing and transcription regulation	Supports lineage-specific gene expression
circRNA	circRNA CDR1as	miRNA sponge (miR-7)	Modulates regulatory networks in differentiation
circRNA	circHIPK3	Interacts with multiple miRNAs	Influences cell proliferation and differentiation

A combined system of transcriptomic and epigenomic regulation mechanisms constitutes the regulation of the stem cell fate. Epigenomic changes like DNA methylation and histone changes and interact with transcriptomic mechanisms, including mRNA expression, non-coding RNA activity and alternative splicing to control gene expression programs as shown in Figure 2. This regulatory network, which is integrated eventually dictates lineage commitment and production of differentiated cell types.

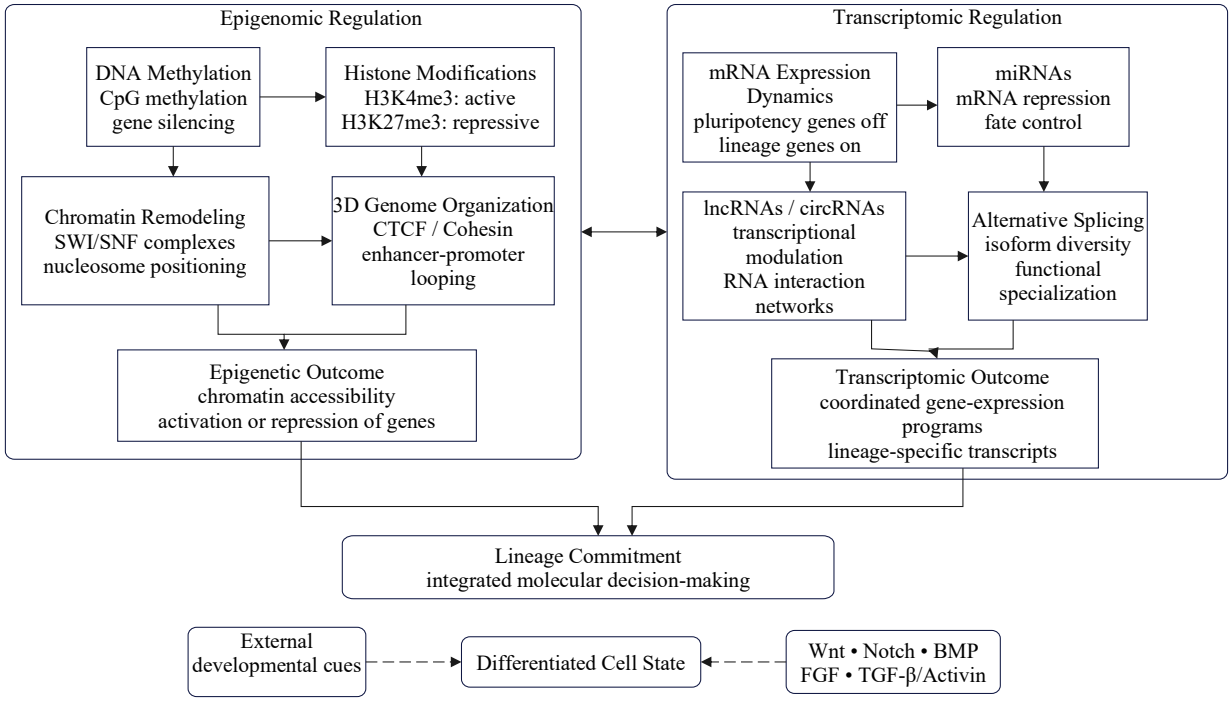

Figure 2. Integrated Epigenomic and Transcriptomic Mechanisms Regulating Stem Cell Differentiation

This illustration represents the interconnection between epigenomic and transcriptomic regulatory pathways and shows how together, chromatin changes and changes associated with RNAs are combined to regulate the expression levels of genes, lineage commitment and differentiation.

5. Integrated Multi-Omics and Regulatory Networks

Proper appreciation of the stem cell differentiation process involves a multifaceted interpretation of the process instead of the isolated analysis of the various molecular processes per se. Over the past few years, it is becoming clear that epigenomic and transcriptomic processes are tightly coupled to each other and thus can be seen to be a highly coordinated activity to form interdependent and interlinked regulatory networks that determine lineage commitment and cellular specialization. The epigenomic processes, e.g. the DNA methylation and histone modification, the chromatin reorganization, and the three-dimensional organization of the genome, determine the availability of the regulatory regions and lay the structural platform on which transcription transpires. Meanwhile, it is transcriptomic processes, such as mRNA expression dynamics, the role of non-coding RNAs in controlling the expression of these epigenetic states, and alternative splicing, that defines how these epigenetic states are converted to functional gene expression products. The epigenome and transcriptome cross-talk is the key to the control of stem cell fate. The epigenetic changes affect the binding of transcription factors, the activity of enhancers and promoter accessibility, which affect transcriptional initiation and elongation. Transcriptomic regulating factors, especially long non-coding RNAs and microRNAs on the other hand can initiate recruitment of chromatin-modifying complexes or change epigenetic landscapes forming two-way feedback of chromatin state and gene expression. This mutual contact permits the dynamic response of the stem cells to developmental stimuli, as well as the high specificity of the stem cell in executing lineage-specific differentiation programs. Figure 3 indicates that through cross-talk of bidirectional epigenomic and transcriptomic controls an integrated regulatory network is generated that eventually leads to lineage commitment. In this context, gene regulatory networks (GRNs) offer systems level details of the interactions of transcription factors, chromatin regulators and RNA molecules that regulate differentiation. These networks are not linear but they are highly interconnected networks that include feedback loops, feed forward networks as well as context dependent interactions that allow strong yet adaptable developmental responses. GRNs in stem cells regulate the inhibition of pluripotency-related genes and activation of the lineage-related transcriptional units. The combination of epigenomic and transcriptomic data sets has greatly enhanced the reconstructions of such networks enabling the researcher to determine the major regulatory nodes and signal-sensitive pathways in the process of differentiation. Systems biology In terms of systems biology, stem cell differentiation is most aptly viewed as an emergent property of multilayered molecular relations as opposed to the action of single genes. Computational modeling, network inference, and large-scale omics profiling are integrated into systems-level approaches to understand the dynamic behavior of regulatory systems over developmental time. The resulting differences have shown that differentiation pathways are orchestrated by synchronized changes in chromatin accessibility, transcriptional state and changes in RNA processing that engage with extracellular signaling pathways including Wnt, Notch, BMP, FGF, and TGF-2/Activin. The integration strategies of multi-omics have also contributed to the increased advancement in this area by increasing availability. The combination of RNA-seq, ChIP-seq, ATAC-seq, Hi-C are used, and the analysis of the chromatin state, transcriptional activity, and genome architecture can be conducted at the same

time. There has been a growing trend in the computational frameworks of integrative character to coordinate these datasets and determine common regulatory signatures and cell-state maps of differentiation. Although these improvements have been made, even now, significant issues exist such as heterogeneity of data, batch, computation complexity, and causality establishing across omics layers. In sum, it may be seen that integrated multi-omics methods allow using a more comprehensive picture of stem cell differentiation, as it allows connecting molecular regulation on several biological levels. This all-inclusive epigenomic-transcriptomic regulatory network is represented in figure 3, which shows the intersection of chromatin-controlled, RNA-mediated receptiveness and developmental indications in lineage-specialization differentiation plans.

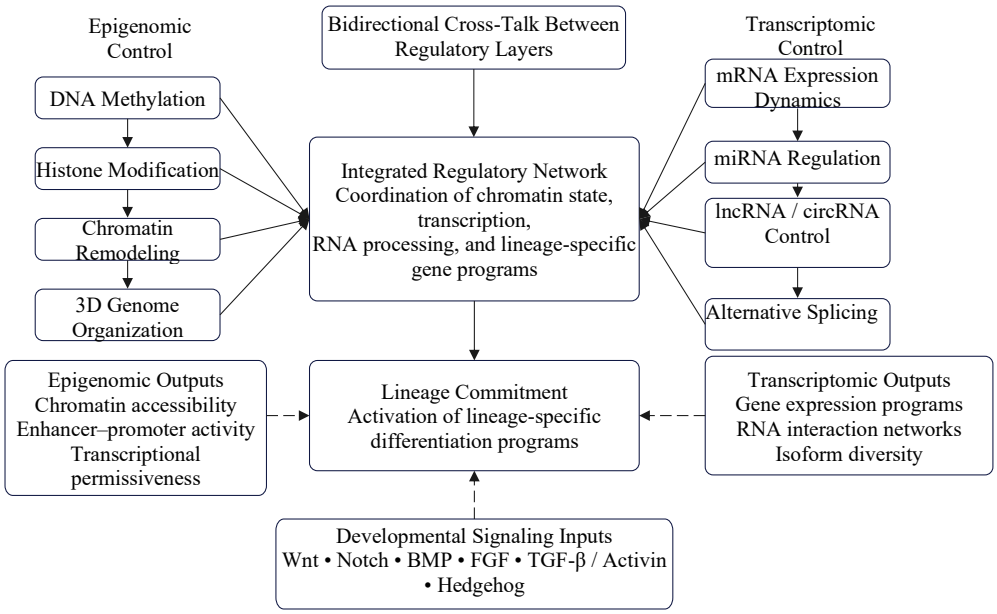

Figure 3. Multi-Omics Integration Framework for Stem Cell Lineage Commitment

This figure shows a system level perspective of the two way trafficking of epigenomic and transcriptomic regulatory layers in differentiation of stem cells. It reveals the interplay between DNA methylation, histone regulation, chromatin organization and mRNA regulation dynamics, non-coding RNA regulation, and alternative splicing conditioning the lineage commitment process in response to developmental signaling pathways.

6. APPLICATIONS IN BIOMEDICAL RESEARCH

The epigenomic and transcriptomic processes of molecular regulation of stem cell differentiation have considerable implications on the diverse fields of biomedical research. The ability to regulate the cell fate decision by these regulatory layers has now been applied to develop improved therapeutic applications and novel biomedical uses. Regenerative medicine is one of the most noticeable uses because stem cells can be employed in the repair or replacement of damaged organs and tissues. With a high level of control over epigenetic and transcriptional condition, scientists can steer the stem cells in due direction, e.g. neuronal, cardiac or hepatic cells. This has breaked new horizons when it comes to curing ailments like spinal cord injuries, myocardial infarction and liver diseases. Combination of multi-omics information also leads to higher potential in the development of functionally mature and stable cell types that can be used in clinical applications. Stem cells have been applied in the area of tissue engineering namely with biomaterials and scaffolding-based systems to produce functional tissue constructs. The epigenomic and transcriptomic profiling can be used to mechanize the differentiation conditions and enhance the structural and functional characteristics of the engineered tissues. This has been useful especially in the development of artificial skin, cartilage and bone tissues and organoid systems that replicate the architecture of tissues in vivo. The other important use is in disease modeling and in this case, the stem cells, especially, induced pluripotent stem cells (iPSCs), are employed in modeling disease-specific cellular environment on a cell culture dish. It is particularly crucial in the study of such complicated illnesses like cancer and neurodegenerative disorders. Transcriptomic and epigenomic studies can be used to identify the disease-linked regulatory changes which can offer the understanding of the pathogenic mechanisms and possible therapeutic targets. The iPSC models of the patient enable the study of genetic and epigenetic differences that lead to the development of the disease. Moreover, the systems based on stem cell have found their way into drug discovery and precision medicine. Screening platforms based on differentiated cells obtained by using the stem cells can be used to determine both the efficacy and toxicity of drugs in a physiological environment. Biomarkers (epigenomic and transcriptomic signatures) can be predicted to determine the responses of drugs given to a particular patient and customize the therapy to that patient. The strategy is advantageous to the creation of bespoke therapies, especially in cancer and rare genetic diseases. On the whole, the presence of epigenomic and transcriptomic controls in the stem cell studies has made major contributions in the field of biomedical application in filling the gap between basic biology and clinical application.

7. CHALLENGES AND FUTURE PERSPECTIVES

In as much as the molecular control of stem cell differentiation has made great progress, there are still numerous issues that restrict the potential of the epigenomic and transcriptomic methods in research and therapeutic practice. It is necessary to overcome these weaknesses in order to convert the multi-omics knowledge into credible and scalable biomedical applications. Integration of data across multi-omics layers is also one of the biggest challenges. Epigenomic, transcriptomic, and other high-throughput datasets are usually obtained on various platforms, resolutions and at various experimental conditions and thus their integration is complicated. The fact that there are variations in data formats, batches, and noise may cause variations in interpreting such errors. It is also acute to develop powerful computational systems that can be used to integrate multi-omics data to accurately represent regulatory networks and differentiation processes. Tightly connected with this is computational constraints especially with respect to processing the volume and the complexity of high dimensional biological data. Multi-omics datasets cannot be analyzed using simple algorithms, machine learning models, and network inference techniques. Nevertheless, these methods may require significant amounts of computing resources and knowledge and thus restrict accessibility and reproducibility. In addition, most existing models are more of a correlation as opposed to causation and this is why it is hard to distinguish clear regulatory processes. The other issue that is significant is reproducibility. The difference in experimental procedures, sequencing technology and pipeline of data analysis can lead to discrepancies of results across research. Experimental designs, data preprocessing procedures and analytical pipelines have to be standardized in order to make results reliable and comparable. Reproducibility and transparency in stem cell research can also be improved by the practice of open data and sharing computational tools. Besides technical issues, ethical considerations are as well important especially on the application of embryonic stem cells and genome-editing technologies. Ethical considerations such as consent, privacy, and other factors that may lead to the misuse of genetic information have to be considered. There is a need to have regulatory frameworks and ethical guidelines in order to have responsible research and clinical translation. In the future, innovative technologies can provide a solution to most of these issues. Multi-omics data is more and more being assisted by the application of artificial intelligence (AI) and machine learning to predict various outcomes of differentiation and uncover the key regulatory factors. Such techniques can reveal the patterns that are not clear and enhance better gene regulatory network models. On the same note, CRISPR-based genome and epigenome editing technologies offer potent methods of authenticating regulatory actions as well as explicitly controlling gene expression. CRISPRi and CRISPRa systems provide a controlled way of modulating the epigenetic state and furthermore, allow for controlled differentiation and therapeutic intervention providing new avenues. It is hoped that future studies will be dedicated to uniting single-cell multi-omics, spatial transcriptomics, and real-time monitoring technologies in order to follow the dynamic aspects of differentiation in high detail. With these innovations and enhanced computational tools and ethical guidelines, the fuller and more accurate idea of the stem cell biology will be achieved, which will eventually lead to the creation of personalized and regeneration treatments.

8. CONCLUSION

The differentiation of stem cells is regulated by a multicomponent interaction between multiple regulatory layers of molecular mechanisms. The structural and functional framework that is used to regulate the accessibility of genes is formed by epigenomic processes, such as DNA methylation, histone modifications, chromatin remodeling, and three-dimensional organization of the genome. Simultaneously, these epigenetic states are converted to functional gene expression programs by transcriptomic resources (i.e. mRNA expression dynamics, non-coding RNA regulation, and alternative splicing). The combination of these interrelated systems provides the accurate control of the lineage determination and formation of the stable cellular identities. The combination of epigenomic and transcriptomic data has been very useful in overcoming the stem cell biology understandings. Multi-omics technologies have made possible the identification of important regulatory networks and molecular signatures that promote differentiation, which offer a more global and systems-level view. This combative perspective is critical to the understanding of the complexity of stem cell fate choices and in the transition between the fundamental research and clinical practice. In the future, further development of multi-omics, single-cell and computational modeling technologies are likely to allow improving our knowledge of differentiation in greater detail. Artificial intelligence and genome-editing applications like CRISPR will be used to make predicting, manipulating and controlling the behavior of stem cells far more precise. These applications promise much with regard to the regenerative medicine, disease modelling, and personalized treatment plans, which will eventually result in better healthcare outcomes.

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