Differentiation Mechanisms of Hematopoietic Stem Cells (HSCs)

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Abstract. Hematopoietic stem cells (HSCs) are a type of stem cell that are mostly present in the bone marrow and oversee producing all types of blood cells, and play a crucial role in maintaining the body's hematopoietic system. This paper provides an in-depth review of recent advances in hematopoietic stem cell research, focusing on differentiation mechanisms and key regulatory factors, with a focus on recent advances in understanding molecular pathways and the application of cutting-edge technologies such as CRISPR-Cas9 gene editing. The study identifies the significance of CD34 and CD38 as early differentiation markers, analyzing their roles throughout the differentiation process. Furthermore, the paper explores the impact of Notch signaling and transcription factors on cell fate decisions, uncovering crucial insights into HSC differentiation. Additionally, it reviews the research progress in the clinical applications of gene therapy, and provides a detailed explanation of the cell fate determination process, which involves transcription factors, signaling pathways, and epigenetic regulation. These findings provide a comprehensive reference for researchers and offer potential directions for clinical applications and stem cell therapies. The paper aims to synthesize complex data to enhance the practical application of novel technologies in HSC research.

Keywords: Hematopoietic Stem Cells (HSCs), Differentiation Mechanisms, TF.

1. Introduction

Early research on Hematopoietic Stem Cells (HSCs) primarily focused on the observation of blood cell morphology, dating back to the era of optical microscopes in the 17th century. However, substantial HSC research truly began in the mid-20th century, with advancements in bone marrow transplantation technology, gradually recognizing HSCs as critical players in the treatment of various hematological diseases. Hematopoietic Stem Cells (HSCs) play an essential role in the hematopoietic system. Their ability to self-renew and their multipotency enable them to create red blood cells, white blood cells, and platelets, among other blood cell types. These properties make HSCs crucial for maintaining the homeostasis of the blood system [1,2]. Additionally, the study of HSC differentiation has offered significant insights into the behavior of a variety of stem cell types. As one of the earliest adult stem cells to be investigated, research into HSC differentiation mechanisms has informed studies on stem cells in other tissues and organs, further advancing the field of regenerative medicine [3]. In recent years, one major focus of HSC research has been exploring their role and application in the aging process. Studies have shown that changes in HSC proliferation and function are closely linked to the onset of several aging-related diseases. Moreover, the potential applications of HSCs in regenerative medicine have garnered increasing attention. Research into hematopoietic stem cell transplantation (HSCT) holds considerable potential. The technology and standards for HSCT have seen significant improvements, with standardized protocols established to enhance the safety and effectiveness of transplantation procedures [4]. With advancements in technology, gene therapy based on studies of common HSC genetic disorders has shown immense potential. The use of the CRISPR/Cas system in HSC gene therapy has achieved groundbreaking progress, such as using CRISPR/Cas9 to correct mutations associated with diseases like β-thalassemia and sickle cell disease [4]. However, the complexity of these technologies and the vast amount of data demand that we consolidate and summarize existing research findings to aid researchers in better applying these technologies. This review aims to provide a detailed account of the HSC differentiation process and offer new perspectives for the application and development of emerging technologies such as gene

editing. This paper will focus on the key mechanisms of HSC differentiation, elaborating on the interplay of transcription factors, signaling pathways, and epigenetic regulation. It will highlight early differentiation markers (such as CD34 and CD38) in terms of structure, function, and development, as well as the regulatory factors that influence cell fate decisions. Additionally, the study will analyze the critical roles of transcriptional regulators (TFs) in establishing and maintaining appropriate gene expression patterns in specific cell types, with particular emphasis on core transcription factors like GATA-1 and SCL/TAL1 in the differentiation process. Current research and application of HSCs still face several key challenges. Despite significant advances in in vitro expansion of HSCs in recent years, many technical difficulties remain. Existing expansion methods, while increasing the number of differentiated HSCs, often fail to fully preserve their functional characteristics, particularly their ability for long-term self-renewal [5]. Furthermore, there are considerable differences in the in vitro expansion performance of HSCs from different sources. However, with continued technological advancements, these issues are expected to be gradually resolved, driving the broader application of HSCs in regenerative medicine and disease treatment. This paper aims to provide references and new perspectives for future clinical applications and stem cell therapies involving HSCs.

2. Hematopoietic Stem Cell Differentiation Mechanism

The mammalian hematologic system encompasses more than ten distinct types of mature cells, all of which derive from a singular type of cell—the hematopoietic stem cell (HSC). Within the hematopoietic system, only hematopoietic stem cells (HSCs) possess both multipotency and self-renewal capability [6]. Self-renewal refers to the ability of hematopoietic stem cells (HSCs) to produce more HSCs of the same type without differentiation. As mature blood cells typically have a short lifespan, HSCs continuously generate more differentiated progenitor cells by finely regulating the balance between self-renewal and differentiation, while maintaining an appropriate HSC pool size throughout their lifespan. Therefore, the study of HSC self-renewal and differentiation mechanisms has always been a key scientific question. Although evidence indicates that the reduction in hematopoietic stem cell content in mice is due to the quiescent (G0) state of the HSC cell cycle. The mechanisms of stem cell differentiation rely on the analysis and validation of experimental results. The process of differentiation for hematopoietic stem cells (HSCs) is complex and is affected by the interplay of transcription factors, signaling pathways, and epigenetic controls. Essential transcription factors, including RUNX1, GATA-1, and SCL/TAL1, guide the differentiation of HSCs into distinct blood cell lineages by modulating the expression of specific genes. In addition, epigenetic processes like DNA methylation and histone alterations control the differentiation of hematopoietic stem cells (HSCs) by influencing both gene accessibility and the levels of gene expression. The interaction of these factors collectively determines the fate and function of HSCs, providing important insights for the research and treatment of related hematological diseases.

3. The early differentiation marker of HSCs

CD34 was first discovered in 1987 and proved to be a cell-surface protein. CD34 is widely expressed on the surface of hematopoietic stem cells and various progenitor cells. All three CD34 family proteins possess an extracellular domain rich in serine, threonine, and proline, which undergoes extensive O-glycosylation and sialylation [7]. This results in an actual molecular weight significantly greater than the experimentally predicted molecular weight (35-60 kDa). Meanwhile, sialylation and glycosylation modifications enhance the stability of CD34 on the cell membrane and reduce immune recognition. Additionally, the C-terminus of CD34 contains a unique binding site, which is a domain capable of interacting with other membrane proteins. L-selectin (CD62L) is commonly selected to coordinate with CD34. Numerous studies on CD34 have demonstrated the necessity of its research. CD34 is considered an important marker for hematopoietic stem cells (HSCs), playing a critical role in the differentiation, migration, and homing processes of HSCs [8].

In the context of hematopoietic stem cell transplantation, migration and homing are two primary processes. Homing describes the process where hematopoietic stem cells and progenitor cells (HPC) that have been infused intravenously move from the peripheral blood (PB) to the bone marrow (BM). The low homing efficiency of HSCs is a significant issue currently. Homing efficiency directly impacts the success rate of transplantation. Studies have shown that the homing of HSCs is influenced by multiple factors, including the expression of cell surface receptors, the conditions of the microenvironment, and the pre-transplantation handling methods [9]. The CD34 protein, along with its related family members such as podocalyxin, has been observed to facilitate the transport and migration of hematopoietic cells. Podocalyxin protease seems to be involved in the process of cell morphogenesis, while proteins from the CD34 family are thought to either promote or inhibit cell adhesion. Additionally, recent studies have shown that these proteins may have broader functions, particularly with podocalyxin playing an important role in chemotaxis and asymmetric cell division [8]. According to published articles, CD34 functions differently across various hematopoietic stem cell subpopulations. HSC, MSC, muscle satellite cells, and keratocytes are among the most studied cell types. As an important marker of hematopoietic stem cells, CD34's clinical applications cover stem cell transplantation, the diagnosis and treatment of hematological diseases, and emerging gene therapy research. In stem cell transplantation, CD34 expression is used for stem cell isolation, purification, and post-transplantation monitoring, improving transplant success rates and assessing patient recovery. In the diagnosis and treatment of hematological diseases, detecting CD34+ cells aid in evaluating and monitoring the treatment of diseases such as acute leukemia and chronic blood disorders. Additionally, CD34's application in gene therapy is gradually expanding. Research has shown that CRISPR-Cas9-targeted editing of the BCL11A enhancer in autologous CD34+ cells can increase fetal hemoglobin in the bone marrow and blood without the need for transfusions, offering a new therapeutic approach for transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) [10]. And monitoring stem cells post-transplantation, enhancing success rates and assessing patient recovery. In hematologic diseases, detecting CD34+ cells aids in disease evaluation and treatment monitoring for conditions like acute leukemia and chronic blood disorders. Additionally, CD34 is gaining traction in gene therapy. Recent research shows that CRISPR-Cas9-targeted editing of the BCL11A enhancer in autologous CD34+ cells can increase fetal hemoglobin in bone marrow and blood without relying on transfusions, providing a novel approach to treating transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) [10].

4. The regulatory mechanism of cell fate determination

4.1. The role of transcription factors

Transcription factors (TFs) are crucial for establishing and maintaining correct gene expression patterns in specific cell types. As key proteins in gene expression regulation, they play a vital role in directing cells toward specific lineages [11]. Various transcription factors control the expression of target genes by selectively binding to the promoter or enhancer regions of these genes. General transcription factors play a crucial role in the formation of the initiation complex. These include proteins from the TATA-binding protein (TBP) family and various factors within the TFIID complex. The classical TFIID complex is composed of the TATA-binding protein (TBP) and 8-14 TBP-associated factors (TAFs). Notably, TAF1/2 interacts with the initiator motif, TBP interacts with the TATA box, and TAF6/9 interacts with the downstream promoter element (DPE), eventually positioning RNA polymerase II at the correct initiation site [12]. Common specific transcription factors can alter the local structure of the promoter region (e.g., Oct4 performs a similar function in embryonic stem cells, regulating the transcription of genes associated with the maintenance of stem cell pluripotency [18]). Genome-wide localization analysis of Oct4 binding sites, along with other embryonic stem cell (ESC) factors, has revealed a set of co-regulated or co-bound targets [13]. This suggests that Oct4 may regulate transcriptional programs primarily through cooperation with other transcription factors or co-regulators, thereby maintaining the pluripotency of embryonic stem cells.

Similarly, Pax6 is thought to be expressed from the early developmental stages of the CNS, regionalizing the neuroectoderm and neural tube [14]. Meanwhile, downstream transcriptomic factors regulate the signaling pathway through Notch signaling, indicating that Pax6's various context-dependent functions interact with each other. For example, Pax6 may be involved in the regulation of Fabp7, Ninein, and Lewis X, which are involved in stem cell self-renewal. Pax6 induces Ngn2, Tbr2, and Dmrta1, thereby promoting neural differentiation. Generally, signal transduction plays a key role in determining cell fate during tissue formation, including in the central nervous system, and is crucial for cell proliferation and differentiation[14].

4.2. Signal path

A signaling pathway is a sequence of molecular interactions used by cells to convey information. When a cell receives external stimuli, such as hormones, growth factors, or environmental changes, these signals are conveyed through specific proteins and molecules within the cell, ultimately influencing cellular behavior. This includes processes such as proliferation, differentiation, or apoptosis [15,16]. The study of signaling pathways is of paramount importance for understanding the onset and progression of various diseases. Moreover, it provides potential targets for drug development. The differentiation mechanisms of hematopoietic stem cells (HSCs) involve various signaling pathways, such as the Notch signaling pathway, the retinoid X receptor (RXR) signaling pathway, and the Wnt/β-catenin signaling pathway. The Notch signaling pathway plays a crucial role in the self-renewal and differentiation of HSCs. Upon binding of the Notch receptor to its ligand, the intracellular domain (ICD) is cleaved by γ-secretase and translocated to the cell nucleus, where it regulates gene expression and influences the fate decisions of HSCs [17]. Studies have shown that Notch signaling is involved not only in the formation of HSCs but also in distinguishing between the fates of HSCs and hematopoietic progenitor cells (HPCs) through different mechanisms [18]. As a member of the nuclear receptor family, the retinoid X receptor (RXR) also regulates the self-renewal and differentiation of HSCs. Treatment of human CD34+CD38−lin− cells with the selective RXR modulator LG1506 can inhibit their differentiation, suggesting that the RXR signaling pathway may play a role in maintaining the undifferentiated state of HSCs [19]. Typically, the signaling pathways involved in HSC differentiation are highly complex and may interact with each other.

4.3. Epigenetic regulation

Epigenetic regulation affects gene expression by modifying the spatial arrangement of chromatin, influencing transcription initiation and elongation, as well as DNA replication and repair, without altering the genetic sequence directly. This allows for reversible and heritable changes in gene expression levels [20]. Wharton’s Jelly Mesenchymal Stem Cells (WJ-MSCs) were first isolated by McElreavey from fibroblast-like cells in the human umbilical cord. [21]. WJ-MSCs possess robust proliferative capacity and strong immunomodulatory properties, making them an excellent model for investigating mechanisms such as transcriptional regulation and epigenetic modifications. The pathways through which epigenetic regulation occurs include DNA methylation, histone modifications, and non-coding RNAs. DNA methylation is catalyzed by DNA methyltransferases (DNMTs), using S-adenosylmethionine (SAM) as a methyl donor to transfer a methyl group to the cytosine-phosphate-guanine (CpG) dinucleotides, primarily at CpG islands. This process leads to the formation of 5-methylcytosine (5mC), completing the methylation of DNA [22]. Ten-Eleven Translocation (TET) enzymes mainly participate in the process of active DNA demethylation, as they oxidize 5mC into unmethylated cytosine intermediates, thereby facilitating the demethylation process [23]. Currently, the expression patterns of TET1 and TET2 differ across various MSC sources, contributing to the ambiguity surrounding the molecular mechanisms of TET-mediated DNA demethylation. This uncertainty could be addressed through CRISPR/Cas9-mediated gene knock-in/knockout of TET genes, enabling researchers to observe how these alterations impact cellular functions and phenotypes, particularly concerning cell differentiation, proliferation, and apoptosis.

5. Conclusion

The study provides a detailed analysis of the differentiation mechanisms of hematopoietic stem cells (HSCs), focusing on key molecular pathways and regulatory factors. It highlights the significance of early differentiation markers, particularly CD34 and CD38, and their roles in the HSC differentiation process. Moreover, the study delves into the impact of Notch signaling and transcription factors on cell fate determination, providing crucial insights into how external signals and intrinsic factors guide the differentiation of HSCs. Recent advancements, such as the application of CRISPR-Cas9 gene editing, have been discussed as transformative tools in the study of HSC genetics and their potential clinical applications. The significance of this research lies in its synthesis of complex molecular data and its contribution to the understanding of HSC behavior. By identifying the key markers and pathways involved in HSC differentiation, the study offers valuable insights that could be translated into practical applications for stem cell therapy and regenerative medicine. Specifically, it underscores the potential for more effective therapies for blood disorders, such as improved bone marrow transplants or targeted genetic therapies using advanced gene-editing techniques like CRISPR-Cas9. However, the study also acknowledges several limitations. HSC differentiation is a complex process, influenced by a wide array of interacting factors, both molecular and environmental. Translating these findings from laboratory research into clinical practice presents challenges, particularly in maintaining the precision and safety of gene-editing techniques. Additionally, while CRISPR-Cas9 shows significant promise, its long-term effects and safety, especially in human applications, remain areas of concern. Further research is necessary to validate these tools in clinical settings and to ensure that their application is both safe and effective. In terms of future directions, the study suggests the need for more precise in vivo models to better understand how HSCs behave in the human body. There is also a call for the exploration of new molecular markers and more refined gene-editing technologies to enhance the control over HSC differentiation. Expanding research in these areas will be crucial for overcoming current limitations and fully realizing the therapeutic potential of HSC-based treatments.

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