Chapter 3

Hematopoietic Stem Cells in Human Medicine

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Abstract

HSCs[1] are multipotent cells responsible for the generation and reconstitution of all blood lineages. HSCs have played a central role in basic research and clinical treatments for hematological and genetic disorders. This review provides an overview of the historical evolution of hematopoietic research and highlights key milestones that led to the identification and functional characterization of HSCs. This review examines the molecular markers, developmental origin, and regulatory mechanisms that define HSC biology, as well as recent advances in their

[1]. Hematopoietic Stem Cells

isolation, ex vivo expansion, and therapeutic use. Special attention is paid to clinical applications such as autologous and allogeneic transplantation, gene editing, and regenerative medicine, along with the ethical and immunological challenges associated with the use of HSCs. Finally, emerging strategies in personalized and regenerative therapies are discussed.

Keywords: Hematopoietic stem cells; Regeneration; Human.

1. Introduction

HSCs are one of the most important types of stem cells that have gained a special place in modern biology and medicine due to their unique ability to self-renew and differentiate into all blood and immune cell lineages. The study of hematopoiesis, or the process of blood formation, has undergone a fundamental transformation over the past two centuries; from the initial observations of blood cell formation to the experimental discovery of the existence of hematopoietic stem cells in the twentieth century. The pioneering research of Thiel and McCulloch in 1961 provided the first experimental evidence of the existence of HSCs and laid the foundation for the modern understanding of stem cell biology [1-3]. In the following decades, significant advances in molecular biology and immunophenotyping techniques led to the identification of key surface markers such as CD34, CD90, and CD49f, which are critical tools for isolating and identifying functional stem cells [4, 5]. In addition to their biological importance, HSCs have revolutionized clinical therapies. HSCT is still the sole proven treatment for a large number of hematological disorders, both malignant and non-malignant. However, advances in gene editing and ex vivo expansion technologies have opened new horizons for reconstructive medicine and personalized gene therapy treatments [6, 7]. Despite these successes, several obstacles remain, including the shortage of matched donors, immune graft rejection, the difficulty in

expanding HSCs on a large scale, and ethical issues related to the use of stem cells and cord blood banks [8-10]. This review's objective is to offer a complete view of the biology, clinical applications, and prospects of hematopoietic stem cells, emphasizing their pivotal role in the advancement of regenerative medicine and personalized therapies.

2. Historical Background

2.1. Discovery of Hematopoietic Stem Cells: A Historical Perspective

The comprehension of hematopoiesis and HSCs[1] has undergone a remarkable transformation over the past two centuries. The formal scientific exploration of hematopoiesis began in the late 1860s when foundational discoveries were made about blood cell formation. Before this period, knowledge regarding blood and its formation was largely superficial, with a focus on the overall blood and circulatory composition rather than a detailed understanding of the processes involved in hematopoiesis [1].

2.2. Foundational Discoveries in Hematopoiesis

The pivotal discoveries by scientists such as Neumann and Bizzozero marked a significant turning point in our understanding of hematopoiesis. In 1868, both researchers independently provided evidence that the BM[2] serves as the primary site for blood cell production. Their findings linked bone marrow to the generation of blood cells, fundamentally changing the perceptions of blood formation during that era [2]. As the field progressed, significant advancements were further contributed to by Paul Ehrlich in the 1880s. Ehrlich introduced innovative staining techniques that enabled the differentiation and classification of various blood cell types. His pioneering work provided crucial insights into the diverse array of white blood cells

[1] Hematopoietic Stem Cells
[2] bone marrow

and their roles within the context of hematopoiesis. These early developments laid the groundwork for a deeper understanding of blood cell formation and heralded a new era in the study of hematopoietic cell biology.

2.3. The Concept of Hematopoietic Stem Cells

The modern concept of HSC[1] emerged from groundbreaking research conducted by Till and McCulloch in 1961. They conducted a series of experiments involving irradiation of mice, which demonstrated the presence of stem cells that could regenerate the full scope of the blood system [3]. Through their innovative approaches, they showed that single cells isolated from the bone marrow could proliferate and differentiate into various blood cell lineages, resulting in the development of spleen cell colonies. This pioneering work established the foundation for our current understanding of HSCs and their essential role in hematopoiesis [3]. The discovery of HSCs revolutionized existing paradigms concerning the origins of blood cell formation, underscoring the significance of a stem cell population capable of self-renewal and multilineage differentiation. HSCs have emerged as powerful tools with immense potential for therapeutic applications in clinical medicine, particularly in the field of regenerative medicine and transplantation [11].

3. Characteristics of HSCs

3.1. Hematopoietic Stem Cell Differentiation

The potential of HSCs has typically been defined through transplantation studies, where donor cells are introduced into recipients that have undergone lethal irradiation and lack a Hematopoietic system in function. This approach has long been considered the standard method for evaluating functional HSCs.

[1] Hematopoietic Stem Cells

The first demonstration of HSCs occurred in 1961, when lethally irradiated mice were successfully rescued through bone marrow transplantation, leading to the detection of hematopoietic colonies in their spleens [3]. As research advanced, scientists sought techniques to isolate HSCs from the BM to better comprehend their functions and the molecular mechanisms regulating them. The isolation of HSCs was made possible using antibodies in conjunction with FACS[1]. In 1988, a study first reported on HSC-enriched cells by employing a combination of multiple surface markers [12]. Since then, numerous research groups have worked diligently to identify additional surface markers that can further enhance HSC purification. Currently, markers such as CD34, Sca-1, c-Kit, and SLAM[2] are frequently utilized for isolating HSCs [13-16]. Similar approaches have also been effective for identifying multipotent and unipotent progenitors, leading to the isolation of various progenitor populations based on their surface markers [17-19]. Two essential traits of HSCs are their capability for self-renewal and their ability to differentiate into multipotent cells, enabling them to produce every kind of blood cell [20]. In contrast, progenitor cells do not have self-renewal capacity and exhibit limited differentiation. To demonstrate the connection between and their progeny, Weissman's team developed a tree-like hierarchical model based on immunophenotyping that outlines the stepwise differentiation process [21, 22]. In this established model, HSCs are categorized into two groups based on the expression of CD34: LT[3] CD34-negative HSCs and ST[4] CD34-positive HSCs. A rare, dormant population in bone marrow, LT-HSCs have the capacity to reconstitute over an extended period of time (>3–4 months), whereas ST-HSCs have a much shorter reconstitution capability (typically <1 month). LT-HSCs give rise to ST-HSCs, which subsequently

[1] Fluorescence activated cell sorting
[2] signaling lymphocyte activation molecule
[3] Long-term
[4] short-term

differentiate into MPPs[1] that lack self-renewal ability [23]. The first differentiation point happens between CMPs[2], which can develop into CLPs[3], myeloid, erythroid, and megakaryocytic lineages, which are limited to lymphoid lineages. The second branching point at CMPs results in MEPs[4] and bipotent GMPs[5]. GMPs mature into granulocytes and monocytes, MEPs produce megakaryocytes and erythrocytes, while CLPs further differentiate into T cells, B cells, NK cells, and dendritic cells. This hierarchical tree model is directed by essential TFs[6] and cytokines that meticulously regulate the differentiation of HSCs[7] into mature blood cells [23-25].

[1] multipotent progenitors
[2] common myeloid progenitors
[3] common lymphoid progenitors
[4] Megakaryocyte-erythrocyte progenitors
[5] granulocyte-macrophage progenitors
[6] transcription factors
[7] Hematopoietic Stem Cells

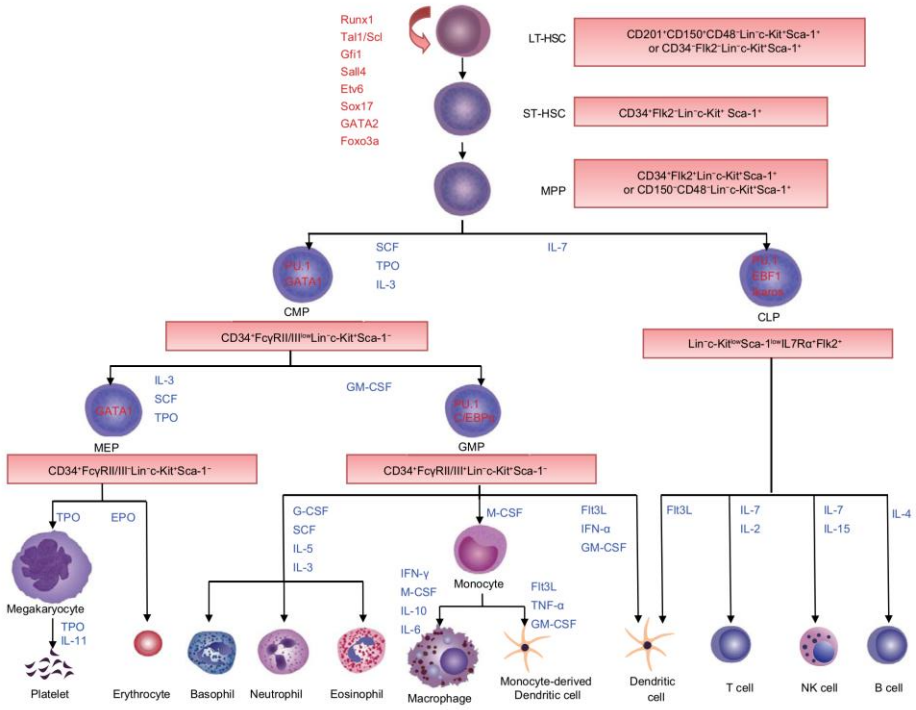

Figure 1. The classical hematopoietic hierarchy posits that long-term hematopoietic stem cells (LT-HSCs) occupy the apex of the cell hierarchy. These LT-HSCs differentiate into short-term HSCs (ST-HSCs), which then give rise to multipotent progenitors (MPPs) with diminished self-renewal capacity. Beyond the MPP stage, a clear bifurcation occurs into myeloid progenitors, known as common myeloid progenitors (CMPs), and lymphoid progenitors, called common lymphoid progenitors (CLPs), marking the initial step of lineage commitment. CMPs can further differentiate into megakaryocyte-erythroid progenitors (MEPs) and granulocyte-monocyte progenitors (GMPs), while CLPs produce lymphocytes and dendritic cells. MEPs mature into megakaryocytes and erythrocytes, whereas GMPs generate granulocytes, macrophages, and dendritic cells. This process of hematopoietic differentiation is tightly regulated by external cytokines and internal transcription factors [12].

3.2. Surface Markers of Hematopoietic Stem Cells: An Overview

The characterization of HSCs[1] has advanced significantly over the years, with extensive research identifying various surface markers associated with these cells. This body of work spans multiple contexts, including homeostasis,

[1] Hematopoietic Stem Cells

ontogeny, immune stress, and disease [13-15]. Despite the wealth of information generated, only a few studies have had a lasting impact on the methodologies used for purifying HSCs, a critical step for their subsequent biological study. Historically, the approaches to isolating human HSCs have undergone numerous revisions in pursuit of achieving the most refined populations for exploration. Advances in flow cytometry techniques have allowed researchers to leverage commonly recognized identification markers to purify these stem cells effectively [16]. Currently, the most prevalent method for HSC identification involves the use of lineage marker-negative (Lin−) cells, in conjunction with the expression of key surface markers such as CD34, CD38, CD45RA, CD90, and CD49f [4]. Recent studies have challenged existing protocols by suggesting the inclusion of additional markers, such as EPCR[1], replacing CD90, to enhance the resolution of HSC[2] identification. Furthermore, the potential use of GPRC5C has been proposed for distinguishing dormant HSCs from their active counterparts [5]. This evolving strategy has led to a revised cell surface marker profile of EPCR+, $Lin^{-}CD34^{+}CD38^{-}CD45RA^{-}CD49f^{+}$, identifying a stem cell frequency of about one in five cells. Below, we delve into specific surface markers associated with HSCs and their biological roles.

CD34

CD34 is a single-pass transmembrane sialomucin protein that is ubiquitously expressed on HSCs and progenitor cells from early fetal growth through to adulthood [30-32]. While a very rare population of CD34-negative HSCs exists, which may be found in umbilical cord blood, CD34-positive cells are fundamental for maintaining adult hematopoiesis [33]. This marker is essential in HSC transplantation procedures, acting as a

[1] Endothelial protein C receptor
[2] Hematopoietic Stem Cells

key criterion for assessing stem cell doses in donor cells prior to therapeutic application. Indeed, CD34 is a strong predictor of long-term hematopoietic reconstitution following transplantation [34]. Despite its critical importance, the specific functional role of CD34 in human HSCs remains largely unknown [29].

CD90 (Thy-1)

CD90, or Thy-1, is a GPI[1]-anchored protein with a V-like immunoglobulin domain. Originally identified in 2007 as a marker that enriches functional HSCs in mouse models, it has since become a common identifier for human HSCs[2] [35]. Though smooth muscle and endothelial cells also express CD90 [36], its functionality in human HSCs has not been well explored. As some studies propose the removal of CD90 from purification protocols, ongoing investigations aim to understand its necessity for the long-term maintenance of HSCs and the impact of its absence on stem cell functionality [37].

CD49f[3]

CD49f, identified in 2011, is an integrin alpha chain subunit that has been instrumental in identifying and further purifying the Lin−CD34+CD38−CD45RA−CD90+ HSC population [4]. This marker is recognized across various stem cell types, including those from epithelial, cardiac, and neuronal tissues [38]. Integrin-like CD49f facilitates interactions with the extracellular matrix, thus influencing stem cell behavior, including differentiation, proliferation, and cell signaling [39, 40].

RET[4]

[1] Glycosylphosphatidylinositol
[2] Hematopoietic Stem Cells
[3] Integrin Subunit Alpha 6
[4] Rearranged During Transfection

RET is a single-pass transmembrane protein that functions as a receptor tyrosine kinase. Its activation occurs through binding with GLFs[1], such as GDNF[2] and its co-receptor, GFRα1 [41]. Previous research has documented the expression of RET in HSCs[3] derived from umbilical cord blood, revealing significant enrichment in the CD49f-positive HSC population [42]. The RET protein has been extensively studied and is known to regulate a variety of downstream signaling pathways, including those mediated by PI3K, RAC1, ERK/AKT, and JAK/STAT, among others [41].

EPCR/CD201[4]

EPCR, classified as a type I transmembrane glycoprotein, can bind protein C and its activated form, known as activated protein C [43]. Initial studies identified EPCR[5] expression in the endothelial cells of blood vessels, the liver, and splenic cells [44]. Subsequent research revealed its expression across a diverse range of cell types, which includes neurons, cardiomyocytes, keratinocytes, neutrophils, and monocytes [45]. Furthermore, EPCR has been shown to mark HSCs[6] that have proliferated when cultivating in vitro [45]. Lately, Anjos-Afonso and colleagues described that EPCR-positive HSCs are positioned at the apex of the hematopoietic hierarchy, with the capability to generate all previously documented immunophenotypic HSC populations following transplantation [46].

GPRC5C[7]

[1] glial family ligands
[2] glial-derived neurotrophic factor
[3] Hematopoietic Stem Cells
[4] Endothelial protein C receptor
[5] Endothelial protein C receptor
[6] Hematopoietic Stem Cells
[7] G Protein-Coupled Receptor Class C Group 5 Member C

Current investigations led by the Cabezas-Walscheid team have recognized GPRC5C, an orphan receptor, as a marker for dormant human HSCs [47]. Through sophisticated in vivo studies, they demonstrated that GPRC5C plays a crucial role in the long-term functionality of HSCs, particularly in maintaining their quiescence and stem cell characteristics [29].

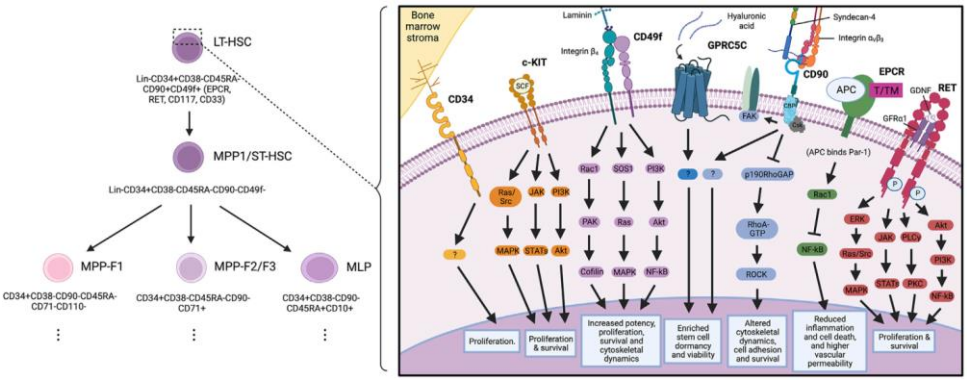

Figure 2. The primary indicators of hematopoietic stem cells and the proposed signaling pathways that control their biology [17].

4. Location and Niches

4.1. Development of Hematopoietic Stem Cells: A Detailed Examination

The ontogeny of HSCs[1] is characterized by multiple, distinct waves of de novo blood cell production during embryonic development, each contributing to the formation of a functional hematopoietic system [48]. These waves are temporally and anatomically segregated, reflecting different cellular origins and developmental mechanisms, and are crucial for establishing the lifelong self-renewable HSC pool in the adult organism [49].

[1] Hematopoietic Stem Cells

The initial wave, known as primitive hematopoiesis, occurs in the yolk sac and is responsible for generating transient, short-lived erythroid cells that are vital for oxygen delivery to the rapidly growing embryo. These primitive erythrocytes originate from mesodermal precursors called hemangioblasts, which are aggregates within the blood islands of the yolk sac. This initial hematopoietic activity also includes primitive macrophages and megakaryocytes, forming a rapid but temporary blood supply [49].

Subsequently, the second wave, termed definitive hematopoiesis, involves the emergence of long-term, self-renewing HSCs primarily from hemogenic endothelium within intraembryonic sites such as the AGM[1] region, fetal liver, and the placenta [50]. The AGM region, in particular, is recognized as the critical site where endothelial cells undergo a trans-differentiation process, called EHT[2], resulting in the formation of HSCs with multilineage potential and self-renewal capacity [50, 51]. These HSCs can be traced back to hemogenic endothelial progenitors that acquire hematopoietic identity through a complex interplay of transcription factors such as RUNX1, GATA2, and SCL/TAL1, regulated by external signaling cues, including Notch and Wnt pathways [50].

The final wave involves the maturation and expansion of HSCs[3] within the fetal liver and eventually their colonization of the bone marrow, where they persist throughout adult life [49]. This transition is governed by both intrinsic transcriptional programs and extrinsic environmental factors, including cytokines and morphogens, which regulate HSC proliferation, quiescence, and self-renewal [52]. Importantly, intrinsic master regulators like RUNX1 and GATA2 are essential for initial HSC emergence but are

[1] Aorta-gonad-mesonephros
[2] Endothelial-to-hematopoietic transition
[3] Hematopoietic Stem Cells

less critical for their maintenance in adult marrow, indicating distinct regulatory networks across developmental stages [53].

Understanding these developmental waves and their underlying molecular biochemistry offers vital insights into optimizing the in vitro generation of HSCs for therapeutic applications. The stepwise nature of HSC ontogeny, driven by coordinated signaling pathways and transcriptional hierarchies, underpins innovative strategies aiming to produce transplantable, long-lived HSCs, ultimately improving treatment options for blood disorders [49].

5. Isolation of HSCs

5.1. Mobilization and collection of HSCs

HSCs are primarily placed in specialized niches within BM[1] [54], where their activity is tightly regulated. However, under certain conditions, HSCs can egress into peripheral circulation, a process referred to as "mobilization." While the precise mechanisms of mobilization are not fully understood, this process is often associated with various hematologic disorders and can be utilized therapeutically. Key agents that promote HSC mobilization include G-CSF[2], SCF[3], SDF-1[4], IL-8[5], and small molecules like AMD3100 (plerixafor) [55, 56].

G-CSF[6] is the most commonly used mobilization agent, although its application can be limited by potential toxic effects and variable patient responses. Recent advances, including the introduction of BIO5192, a VCAM-1/VLA-4 [57] antagonist, have shown promise in enhancing HSC

[1] bone marrow
[2] granulocyte-colony stimulating factor
[3] stem cell factor
[4] stromal-derived factor-1
[5] interleukin-8
[6] Granulocyte-colony stimulating factor

mobilization, especially when paired with G-CSF and plerixafor. In addition, chemotherapy, when combined with G-CSF, is considered the preferred method for mobilizing HSCs[1], particularly for patients needing to decrease tumor burden or those requiring high cell collections. Commonly used chemotherapeutic agents, such as cyclophosphamide (2–4 g/m²), are integrated with G-CSF to optimize mobilization outcomes [58]. The approval for G-CSF doses following myelosuppressive therapy includes filgrastim (5 µg/kg/day SC) and lenograstim (150 µg/m²/day SC). While the combination of chemotherapy with cytokines can improve collection yields, it poses risks such as treatment-related toxicity and the necessity for in-hospital care. Thus, understanding the dynamics of HSC mobilization remains crucial for optimizing therapeutic strategies in patients requiring stem cell transplantation. The isolation of HSCs poses significant challenges due to their low prevalence in hematopoietic tissues and the absence of unique identifying markers. The phenotypic similarities between HSCs, differentiating progenitor cells, and mature blood cells complicate their effective recovery. Consequently, efficient HSC isolation often necessitates multiple separation steps, each differing in selectivity and capacity. Non-antibody-based methods [59], such as density gradient centrifugation, are frequently utilized to enrich HSCs by removing denser, mature cell types. However, this technique may result in a 10-30% loss of HSCs due to overlapping cell densities. In vivo treatment with cytotoxic drugs can enhance HSC frequency by selectively eliminating rapidly dividing mature cells. Antibody-mediated isolation [60] offers the advantage of achieving higher purity through both positive and negative selection strategies. Positive selection often involves a single antibody and can yield 25- to 100-fold enrichment of HSCs, particularly through CD34

[1] Hematopoietic Stem Cells

selection. Recent advancements in magnetic separation techniques, such as column-free systems using small submicron magnetic particles (EasySep™), have mitigated some challenges associated with traditional methods. Negative selection, by contrast, relies on antibody cocktails to deplete unwanted mature cells while preserving HSCs[1], allowing for tailored enrichment strategies. Combining these techniques optimally enhances HSC yield and purity, facilitating their application in clinical and research settings.

5.2. Source (bone marrow and cord blood) advantage or disadvantage

HSCs possess unique self-renewal and differentiation capabilities, making them a vital resource for treating hematological disorders, immunodeficiencies, and inherited metabolic disorders. HSCs can be harvested from several sources: mobilized PB[2], BM[3] [61], and CB[4]. Recently, CB has gained popularity as a source of HSCs due to its accessibility, better tolerance of HLA mismatches, and a lower risk of GVHD[5] compared to other sources [62-65]. Although CB has a lower overall cell yield, it contains a higher frequency of progenitor cells [66] and has been shown to provide CD34+ cells that proliferate more rapidly than those from BM [67]. Moreover, CB-derived HSCs exhibit enhanced engraftment potential compared to their PB or BM counterparts [68]. Recent investigations have further identified CB as a source of progenitor or non-hematopoietic stem cells, including endothelial and mesenchymal precursors [69, 70]. However, challenges remain; achieving high purity and recovery rates of HSCs from CB is difficult due to its high content of

[1] Hematopoietic Stem Cells
[2] Peripheral blood
[3] Bone marrow
[4] Umbilical cord blood
[5] Graft-versus-host disease

thrombocytes and nucleated erythroid precursors, which complicate mononuclear cell isolation. Current methodologies for PB sample processing do not always translate effectively to CB. For instance, studies indicate variable purity rates of CD34+ cells from CB, with reports of mean purities around 41% after initial selection and improving to 85% following a second pass through the separation column [71]. Overall, while CB offers significant advantages, the complexities of cell isolation and purity must be addressed for optimal application in clinical settings. Other research indicates that both committed and primitive progenitor cells derived from cord blood exhibit faster proliferation rates in response to cytokine stimulation in vitro compared to their bone marrow counterparts [72]. Notably, a significantly larger proportion of cord blood $CD34^{+}CD38^{-}$ cells proliferate in vitro, and each cord blood cell, regardless of whether it is $CD34^{+}CD38^{+}$ or $CD34^{+}CD38^{-}$ is capable of producing nearly an order of magnitude more progeny than similar cells from bone marrow. [72]. The higher percentage of cord blood HSCs cycling in vivo contributes to the faster initiation of proliferation in vitro, although quiescent cord blood cells' increased ability to respond to stimulation also plays a role.

6. Clinical Relevance

6.1. Bone marrow and stem cell transplants Autologous or allogenic

Bone marrow or HSC[1] transplantation presents significant challenges, primarily due to the scarcity of suitable donors. The optimal donors are typically healthy, histocompatible family members, but they are only available for approximately one-third of patients in need. Despite the establishment of international registries for unrelated donors and umbilical cord blood HSC repositories, nearly 50% of patients still lack access to

[1] Hematopoietic stem cell

potentially life-saving transplants due to insufficient compatible donor availability. Patients with hereditary blood disorders often require repeat transfusions of RBCs[1] or platelets, a process that hinges on the availability of compatible donors. While allogeneic HSC transplantation serves as the only definitive cure for many genetic blood disorders, its effectiveness is restricted by the necessity of HLA[2]-matched donors. Furthermore, this form of transplantation carries considerable risks, including graft failure, GVHD[3], delayed immune reconstitution, and even the possibility of disease recurrence [73]. To address these limitations, extensive efforts are being directed toward developing safe and effective ex vivo gene therapy approaches [74]. One promising avenue involves autologous transplantation strategies utilizing genetically corrected HSCs, which could potentially eliminate the dependence on donor availability while reducing associated risks. Through these innovative therapies, researchers aim to improve treatment outcomes for patients suffering from inherited blood disorders, ultimately enhancing their quality of life and survival prospects.

7. HSCs[4] in Research and Therapy

7.1. Gene editing and HSCs

Gene editing has profoundly advanced the field of HSC therapy, harnessing the accessibility of HSCs for precise genetic manipulation and their ability to reconstitute blood and immune systems. Technological progress from early methods relying on homologous recombination facilitated by donor plasmids was initially hampered by low efficiency. The recognition that DSBs[5] could enhance DNA repair shifted the paradigm,

[1] Red blood cells
[2] Human leukocyte antigen
[3] Graft-versus-host disease
[4] Hematopoietic stem cell
[5] Double-strand breaks

leading to the creation of programmable nucleases, including ZFNs[1], TALENs[2], and, most notably, CRISPR/Cas systems [75]. These tools enable targeted modifications—gene correction, disruption, or insertion—at specific genomic loci with greater precision, providing a safer alternative to traditional viral vector-based gene addition, which carries risks of insertional mutagenesis.

Initially, autologous HSC-based therapies primarily involved inserting functional copies of disease-causing genes via viral or non-viral delivery systems [76]. Contemporary strategies utilize genome editing for the permanent correction of pathogenic mutations directly within patient-derived HSCs. This approach holds promise for treating hereditary blood disorders such as hemoglobinopathies, primary immunodeficiencies, and congenital cytopenias—conditions originating from genetic defects affecting blood cell development. Correcting or replacing defective HSCs could restore a healthy hematolymphoid system, offering a potential cure.

Advances in genome editing include refined techniques like base editing and prime editing, which allow precise nucleotide changes without DSBs[3], and epigenome editing, enabling gene expression regulation without altering DNA sequences. These innovations, alongside iPSC[4] technology, open new avenues for targeting monogenic blood diseases [77-81]. However, challenges remain, such as managing mutation loads in long-cultured iPSCs and minimizing off-target effects [82, 83]. Overall, ongoing efforts aim to improve editing efficiency, safety, tolerability, and broad accessibility, positioning personalized gene editing as a transformative approach for treating genetic hematologic disorders.

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[1] zinc finger nucleases
[2] Transcription activator-like effector nucleases
[3] Double-strand breaks
[4] Induced pluripotent stem cell

7.2. HSC expansion and ex vivo manipulation

HSCs[1] are a rare subset of cells located in the bone marrow, crucial for lifelong blood formation and for restoring the hematopoietic system following hematopoietic stem cell transplantation. To enhance the safety and effectiveness of HSCT[2], researchers are exploring various strategies. One promising approach involves transplanting larger quantities of HSCs derived from ex vivo expansion of the cells. However, cultivating and maintaining functional HSCs outside the body has long posed a challenge, hindering the widespread application of this technique [84, 85]. The ex vivo expansion and manipulation of HSCs help improve clinical HSC transplantation therapies. Despite over six decades of clinical use, HSCT remains a high-risk procedure, and access to it is limited for many patients. Those undergoing allogeneic HSCT need to find a compatible human HLA-matched donor to minimize the risk of GVHD[3]. Unfortunately, about 70% of patients lack a related, matched donor A significant advancement in the field occurred in 2023 when the U.S. FDA[4] approved the first HSC product derived from ex vivo expanded cells. Omisirge (omidubicel-onlv, also known as NiCord) was authorized for use in adult patients with hematologic malignancies following myeloablative conditioning. This product utilizes cord blood HSCs[5] expanded with nicotinamide riboside, a form of vitamin B3. Nicotinamide was first identified as an HSC enhancer in 2012, demonstrating its ability to promote HSC proliferation by inhibiting the deacetylase Sirtuin 1 [86]. Another promising candidate is UM171, which has shown encouraging results in phase I/II clinical trials [21, 22].

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[1] Hematopoietic stem cells
[2] Hematopoietic stem cells transplantation
[3] Graft-versus-host disease
[4] Food and Drug Administration
[5] Hematopoietic stem cells

UM171 has been found to significantly expand long-term engraftable HSCs[1]by approximately 30-fold over a 10-day culture period. Although its precise mechanism was initially unclear, recent studies reveal that UM171 induces the degradation of the histone demethylase LSD1 and the associated repressive complex CoREST, thus modulating epigenetic pathways [89]. Furthermore, recent research suggests that replacing serum albumin with synthetic polymers such as PVA[2] can substantially enhance HSC expansion, as demonstrated in mouse models [90]. These developments highlight ongoing progress in improving HSC expansion techniques, which are crucial for increasing the safety and efficacy of stem cell transplantation therapies. Ex vivo expansion of HSCs has seen significant advancements with the development of optimized culture conditions. One such study created a xeno-free, serum-free culture medium (StemPro™ HSC Expansion Medium) supplemented with FLT3L, KITL, TPO, IL3, and IL6, which led to substantial increases in viable nucleated cells from human $CD34^+$ cells derived from mobilized peripheral blood, cord blood, and bone marrow. The average fold increases in cell numbers were 96-, 178-, and 80-fold, respectively, compared to day 0 [91]. Another review underscores the critical role of the bone marrow niche, detailing how its regulatory factors influence HSC functions. The review highlights various strategies for ex vivo expansion, with an emphasis on the importance of niche-associated factors that support HSC self-renewal and multipotency [92]. Additionally, the continuous presence of TPO in long-term bone marrow cultures has been shown to promote both long- and short-term HSC repopulation, suggesting that HSCs can self-replicate ex vivo under specific conditions [93, 94]. Research also identifies

[1] Hematopoietic stem cells
[2] polyvinyl alcohol

dermatopontin (Dpt) as a crucial factor for HSC maintenance, as its overexpression under non-supportive conditions restored HSC survival and clonogenicity, thus enhancing ex vivo cultures [95]. Furthermore, the exploration of insulin-like growth factor 2 and angiopoietin-like proteins has revealed their potential to support HSC expansion, contributing to the improvement of cultured HSCs [96]. Finally, Fbxw7α's role in maintaining HSC quiescence and stemness has been confirmed, with its overexpression leading to dormancy and sustained reconstitution capacities, offering a promising approach for HSC maintenance [94, 97].

8. Future prospects: regenerative medicine and personalized therapies

Regenerative medicine and personalized therapies are rapidly advancing through the integration of cutting-edge technologies and individualized approaches to treatment. Stem cells—especially iPSCs[1]are at the forefront of this transformation, enabling targeted therapies, regenerative treatments, and personalized drug testing, although ethical and accessibility issues remain [98]. Innovations in regenerative medicine now include organ transplantation, tissue engineering, artificial intelligence, and nanorobotics, all of which offer promising therapeutic applications [99]. Recent research also highlights a shift from traditional cell-based therapies to acellular strategies using EVs[2], with demonstrated potential in the repair of organs such as the heart, eye, and skin [34]. Additionally, stem cell therapy is being combined with precision medicine, gene editing, and bioengineering to treat complex diseases like cancer and neurological disorders more effectively [32]. Personalized regenerative strategies, tailored to each patient's genetic and clinical profile, are

[1] induced pluripotent stem cells
[2] Extracellular vesicles

enhancing treatment efficacy, particularly in oncology and cardiovascular care [101].

9. Challenges and Ethical Considerations

9.1. Difficulties in HSC isolation and culture

HSCs[1] are vital for treating various diseases and hold significant research potential. However, their expansion in vitro remains a challenge, as current culture conditions often lead to rapid differentiation and a loss of stemness. Factors such as oxidative stress, normoxia, and DNA damage contribute to these difficulties, which hinder effective HSC [2]expansion protocols [102]. The application of HSCs in clinical settings is also limited by the low in vitro concentrations routinely achieved in bioreactors. Despite advancements, bioreactor systems struggle to maintain high cell densities and uniform culture conditions, which are essential for optimizing HSC production. Key factors like pH, dissolved oxygen, and nutrient uptake require careful monitoring to improve yields, but these systems often fall short of producing sufficient HSCs for large-scale applications [103]. Additionally, stress-induced differentiation during collection and culture poses another challenge. Exposure to suboptimal conditions triggers stress responses such as ER[3] and replicative stress, compromising HSC self-renewal, differentiation, and engraftment potential. To maintain HSC functionality, strategies to mitigate culture-induced stress are necessary [102]. Medium composition also plays a critical role in HSC expansion. While cytokine supplementation has shown promise, the addition of non-cytokine supplements yields variable results, necessitating further research to identify optimal medium compositions

[1] Hematopoietic stem cells
[2] Hematopoietic stem cells
[3] endoplasmic reticulum

[104]. Furthermore, the transition of HSCs from laboratory to clinical applications involves challenges such as graft rejection risk due to animal-derived materials. To address this, the use of serum-free or xeno-free media is recommended, though these options are costly and may still contain non-human proteins [105]. Recently, current immunomagnetic separation techniques for isolating $CD117^{+}$ HSCs are inefficient, yielding low numbers and presenting limitations for experimental applications [106]. Further research comparing traditional 2D and 3D culture systems is needed to determine the best approach for HSC maintenance and expansion [107].

9.2. Immunological barriers in transplantation

HSC[1] transplantation is a vital therapeutic strategy for various hematological disorders, but its success is often limited by immunological barriers that affect both engraftment and function. Allogeneic HSCs are recognized as foreign by the recipient's immune system, leading to graft rejection. Research has shown that both T and B lymphocytes play significant roles in this process, with B cells identified as a primary barrier to hematopoietic engraftment in allosensitized recipients. This highlights the importance of humoral immunity in graft rejection. Additionally, GVHD[2] occurs when donor-derived immune cells attack the recipient's tissues, resulting in significant morbidity and mortality. The severity of GVHD is influenced by factors such as the degree of HLA[3] mismatch and the presence of specific immune cells that initiate the attack. To address these immunological challenges, strategies like T lymphocyte depletion from the graft have been explored to reduce the risk of GVHD, although this may

[1] Hematopoietic stem cell
[2] Graft-versus-host disease
[3] Human leukocyte antigen

also reduce the graft-versus-tumor effect. Another approach involves the use of NK[1] cells from HLA-disparate donors, which may enhance graft-versus-leukemia activity while minimizing GVHD [108, 109]. In conclusion, understanding and overcoming immunological barriers in HSC transplantation is essential to improving patient outcomes. Ongoing research into immune modulation and graft engineering holds promise for enhancing the success of HSC-based therapies.

9.3. Ethical issues in stem cell use (including cord blood banking)

The ethical issues surrounding UCB[2] collection, storage, and use are significant, focusing on principles such as beneficence, autonomy, and justice. The ethical debate also involves the distinction between autologous and allogeneic UCB use, the validity of informed consent, and the moral implications of private versus public UCB banking [110-112]. Additionally, a comprehensive review identifies various ethical concerns related to UCB, such as ownership, informed consent, and medical indications. The review further delves into the ongoing debate between public and private banking models, while also considering the commercialization, patenting, and data protection issues inherent in the practice. Furthermore, the ethical considerations in UCB banking include the vital role of informed consent and the disparities between private and public banking models. Concerns regarding the commercialization of stem cell resources are also addressed, with a call for equitable access to these valuable biological materials [110, 113]. Another article outlines policies, practices, and ethical issues related to UCB banking, particularly focusing on ownership, informed consent, and ethical considerations in both private

[1] Natural killer
[2] Umbilical cord blood

and public banking models [114]. Moreover, a comparative analysis of national and international UCB banking guidelines reveals the ethical challenges associated with informed consent, the differences between public and private banking, and the use of UCB in research and drug production. This study stresses the necessity for uniform ethical standards across various jurisdictions to ensure consistency in practice [115].

9.4. HSCs in the treatment of leukemia, lymphoma, and other blood disorders

HSCT[1] has become an essential component in the management of hematopoietic cancers, including lymphoma and leukemia, and other blood disorders. G-CSF[2] is the most commonly used agent for stem cell mobilization; however, combining it with plerixafor or cyclophosphamide significantly enhances mobilization efficiency, especially in poor mobilizers [116, 117]. The integration of precision medicine and immunotherapies—including CAR-T cells and immune checkpoint inhibitors has further improved HSCT outcomes by enhancing malignant cell targeting and reducing relapse rates [118]. In AML[3], LSCs[4] are a major cause of relapse due to their resistance to standard chemotherapy. Developments in the biology of LSCs have resulted in the creation of targeted treatments, which, when combined with HSCT[5], can improve disease control and support long-term remission [116, 119]. MSCs[6] also play an important adjunctive role in HSCT by reducing GVHD[7] and promoting graft acceptance, particularly in high-risk patients, due to their strong

[1] Hematopoietic stem cell transplantation
[2] Granulocyte-colony stimulating factor
[3] Acute myeloid leukemia
[4] leukemic stem cells
[5] Hematopoietic stem cell transplantation
[6] Mesenchymal stem cells
[7] graft-versus-host disease

immunomodulatory properties [120]. A large cohort study involving over 40,000 patients with diffuse DLBCL[1] demonstrated that HSCT leads to high remission and survival rates in relapsed or refractory cases. However, outcomes are influenced by factors such as disease stage, patient age, and comorbidities. The study supports incorporating modern therapies alongside HSCT for optimal results [122]. In pPCL[2], while HSCT offers survival benefits, overall prognosis remains poor. Early identification of suitable candidates and combining HSCT with chemotherapy and novel targeted therapies are essential to reduce relapse rates [56]. Finally, a deeper understanding of the molecular and cellular mechanisms involved in HSCT—including immune interactions between donor and recipient—along with innovations like stem cell engineering and gene editing, could significantly enhance the effectiveness of HSCT, especially in treatment-resistant blood cancers [123].

10. Conclusion

HSCs[3] are the basis of contemporary hematology and regenerative medicine due to their capacity for self-renewal and specialization in multiple lineages. Recently, great progress has been made in their characterization, identification of their molecular markers, and development of efficient methods for their isolation and expansion. In addition, HSC transplantation is a promising treatment for many hematological and genetic diseases. Gene editing, ex vivo expansion, and customized transplantation are also emerging therapies. However, there are still obstacles such as immunological barriers, donor shortages, and ethical dilemmas surrounding the use of stem cells and cord blood.

[1] large B-cell lymphoma
[2] primary plasma cell leukemia
[3] hematopoietic stem cells

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