

Advances in Human Embryonic Stem Cells-Based Therapy for Leukemia

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Abstract. Leukemia refers to a malignant clonal disease triggered by hematopoietic stem cells (HSCs). The leukemia cells are at a standstill at different phases of cell development because of the self-renewal enhancement and differentiation disorder. In the bone marrow and other hematopoietic tissues, leukemia cells proliferate and accumulate rapidly, inhibiting normal hematopoiesis and infiltrating other organs and tissues. Among current clinical treatments, though hematopoietic stem cell transplantation (HSCT) serves as the optimal therapeutic choice for leukemia currently, it faces challenges such as difficulties in donor matching and high risks of complications. Human embryonic stem cells (hESCs), derived from the inner cell mass of embryos, have unlimited self-renewal and multi-directional differentiation potential. These cells exhibit notable value in basic research and disease modeling. hESC-derived HSCs can proliferate at a rapid rate and exhibit strong self-renewal ability. These unique biological traits offer a promising approach for leukemia therapy. However, clinical translation of hESCs still faces severe challenges such as tumorigenicity and low differentiation efficiency. This review summarizes the research progress of hESCs in leukemia treatment, providing a reference for future studies.

Keywords: Human embryonic stem cells, Leukemia, Stem cells therapy.

1. Introduction

Leukemia is a malignant hematological disease which has the uncontrolled proliferation of abnormal leukocytes and impairment of normal hematopoiesis characteristics. Its complex pathogenesis is related to genetic, environmental, and hematological factors. The proliferation of leukemia cells in the bone marrow and other hematopoietic tissues suppress normal hematopoiesis. These cells can also infiltrate other organs. Leukemia can be divided into four broad categories: acute myeloid leukemia (AML), acute lymphocytic leukemia/lymphoma (ALL), chronic myeloid leukemia (CML), and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) [1]. Unlike solid tumors, leukemia cells uniquely possess innate migratory and invasive abilities. Malignant leukocytes retain the motility and survival capacity of normal leukocytes in the circulation, while acquiring the ability for uncontrolled proliferation [1]. Clinically, disease severity is different and presents various symptoms. For instance, acute leukemia often brings high fever or severe bleeding, while chronic leukemia's symptoms are relatively mild, such as anemia, enlarged lymph nodes and spleen, and abnormal proliferation of lymphocytes. These symptoms are often caused by the infections of viruses, immune dysfunction, long-term exposure in physical and chemical environments, etc. Up to now, the treatment options for leukemia include molecular targeted therapy, chemotherapy, radiotherapy and HSCT. Among these, HSCT is the only curative option for many patients, but some factors like the scarcity of matching donors, the graft-versus-host disease (GVHD), and the insufficient quality and quantity of hematopoietic stem cells in traditional sources such as bone marrow and umbilical cord blood (UCB) affected seriously on this treatment, resulting in a high risk of complications. In contrast, human embryonic stem cells may partially solve these problems as they provide an alternative and renewable source of stem cells [2].

hESCs, first isolated from blastocytes, have emerged as a promising candidate to address these unmet needs in leukemia therapy [3]. Distinguished by their unlimited self-renewal capacity and pluripotency, hESCs can differentiate into various cell types in the human body, including functional HSCs and immune effector cells [3]. Compared with HSCs, hESCs have broader differentiation

potential and avoid the limitations of finite in vitro proliferation and aging [4]. Such advantages make hESCs a renewable and standardized resource for generating transplantable cells, establishing disease models, and developing targeted therapies for leukemia.

In recent years, research on hESCs in leukemia therapy has progressed rapidly, expanding from basic studies on differentiation mechanisms to preclinical exploration of innovative applications. These include the generation of high-quality HSCs to overcome donor shortages in HSCT, and the production of natural killer (NK) cells for tumor-targeting immunotherapy. However, the translation of hESC-based therapies to the clinic is hindered by critical technical and ethical challenges: genetic instability during in vitro culture, the teratoma formation hazard of residual undifferentiated cancer cells, low efficiency in generating functional, etc.

This review focuses on the biological characteristics and unique advantages of hESCs, systematically summarizes their therapeutic applications in leukemia, and critically discusses current technical bottlenecks. By integrating current progress with future directions, this review aims to provide insights into the potential of hESCs as a revolutionary tool in leukemia therapies and highlight directions for future research to accelerate their clinical translation.

2. Biological Characteristics and Advantages of hESCs

hESCs are extracted from embryos produced through in vitro fertilization (IVF), and are collected from the inner layer of the blastocyst. Although these embryonic cells are undifferentiated, they possess self-regulation and self-renewal capacity [3]. James Thomson is known as the pioneer of hESC research and predictor of future discoveries in the field [3]. He describes hESCs as having ‘developmental plasticity’, which allows them to integrate into developing tissues at various stages while maintaining their ability to develop normally [3]. hESCs are stable in their pluripotency, overall expression of gene, karyotype, the capacity of repairing DNA, telomerase maintenance, and growth characteristics. These properties offer a renewable source of various cell types for future cell-based therapies in leukemia treatments [5].

In contrast to other types of stem cells, hESCs have significant advantages. Adult stem cells typically only differentiate into the cell types of their original tissue or closely related types, while hESCs have a broader differentiation potential. For instance, HSCs can only form blood cells. hESCs, in theory, can differentiate into a wide range of types of body cells. Furthermore, the proliferative capacity of adult stem cells in vitro is limited. After a certain period of cultivation, they will enter an aging state, and their growth rate will slow down or even stop, making it impossible to produce enough cells required for research [4]. And hESCs in theory, possess the ability for unlimited self-renewal and can be continuously expanded, providing an abundant source of cells for treatment and research. Furthermore, compared with induced pluripotent stem cells (iPSCs), hESCs exhibit stability in terms of differentiation potential and epigenetic state. Although the research on iPSCs has been sufficiently mature, the technological advancements to decipher the impact of genetic and epigenetic alterations as well as variability in cell clones is still limited. In addition, hESCs, due to their strong proliferative ability and relative ease of in vitro cultivation, are more suitable for gene editing (e.g., CRISPR/Cas9) and transgenic modification than most adult stem cells [6]. hESCs are used for investigating gene functions, establishing disease models, and conducting potential gene therapies.

With technological advances, interest in research and expansion of hESCs has become even more intense, which has triggered an ethical controversy. Initially, government regulations permitted research only on existing hESC lines and prohibited the creation of new ones. Over time, these regulations have gradually relaxed. Embryos used to establish hESC lines typically come from the leftover products of IVF procedures or are donated by couples specifically for scientific research purposes [3].

3. Therapeutic Application of hESCs in Leukemia

To achieve more effective treatment of leukemia, scientists are constantly exploring the potential roles that hESCs can play in the treatment of leukemia. Since hESCs have the ability of unlimited self-renewal and multi-directional differentiation capacity, they are regarded as ideal candidates in regenerative medicine. In leukemia therapy, the most crucial application value of hESCs lies in their ability to differentiate into functional HSCs, providing a new alternative source for HSCs, providing an alternative source for stem cell transplantation. Studies have shown that the HSCs from early human embryos possess exceptionally strong proliferative capability. A 2020 study published by the University of Edinburgh reported that HSCs from the AGM region of hESCs proliferate 200 to 500 times faster than those from UCB [2]. These HSCs can efficiently rebuild the blood system after transplantation, providing an ideal model for in vitro expansion techniques [2]. Compared with adult-derived HSCs, those derived from embryos exhibit stronger self-renewal ability, fewer epigenetic modification, and greater metabolic adaptability [2]. These characteristics make them an excellent cell source for transplantation therapy in leukemia patients. However, clinical translation still faces challenges. In animal models, the implantation rate of hESC-derived HSCs is generally below 10%, which is far lower than the threshold required for clinical hESC-derived HSCs. Moreover, residual undifferentiated hESCs may form teratomas, which necessitates the development of more precise sorting techniques.

Application of hESCs in leukemia treatment has extended beyond simple hematopoietic reconstitution and has developed a variety of innovative therapeutic strategies, including immunotherapy and transplantation support therapy, etc. NK cells are the key effector cells of the innate immune system. Several studies have shown that donor-derived NK cells play a crucial role in the elimination of cancer cells in patients with acute leukemia [7]. hESCs, due to their unique property of unlimited proliferation, has become an ideal source for standardized NK cell products. Research has shown that NK cells derived from H9 line hESC line can effectively clear human tumor cells in vivo, as demonstrated by bioluminescent imaging [8]. In addition to enhanced in vitro killing of various tumor targets, the in vivo tumor clearance by H9 hESC-derived NK cells was more effective compared with NK cells derived from UCB [8]. These findings show that hESCs have served as an alternative cell source for clinical therapy. Furthermore, although hESCs have not yet been directly used in clinical transplantation, their derivatives already have transplantation support. The use of embryonic stem cells has introduced new insights into treatment. They possess regenerative potential and can repair damaged tissues and cells. They are an important source for stem cell transplantation therapy. However, they may also lead to uncontrolled cell proliferation, potentially triggering the formation of cancer. Recently, Wang et al. successfully generated HSCs from both hESCs and human iPSCs [9]. Their study confirmed the differentiation of hESCs into hematopoietic cells. This approach could provide sufficient hematopoietic cells for the treatment of leukemia [9].

hESCs research in leukemia has gradually progressed from basic research toward clinical translation. With advances in differentiation technologies and gene editing, personalized hESC-derived cell therapies are expected to become an important component of curative leukemia treatment in the next 5 to 10 years, bringing new hope to patients with hematological malignancies.

4. Technical Bottlenecks and Solutions in hESC-based Leukemia Therapy

During the development process of hESCs, there are still some technical bottlenecks. Increasing evidence indicates that human pluripotent stem cells (hPSCs), including hESCs, are prone to genetic instability in vitro culture [10]. Recurrent genetic alterations confer selective advantages in culture, leading to the overgrowth of abnormal, culture-adapted cells [10]. These changes suggest a potential malignant transformation of abnormal cells, which may appear as the characteristics of decreased differentiation and increased proliferation. Undifferentiated hESCs that remain after the differentiation process may form teratomas, thereby increasing the risk of tumor-forming in patients. Furthermore, although hESCs can differentiate into hematopoietic stem cells, providing a potential

source for leukemia treatment, the low differentiation efficiency into transplantable functional HSCs and the lack of sustaining long-term hematopoiesis reconstitution may increase other risks in patients [11]. In addition, because of the hESCs derived from human embryos, their use appeals to ethical controversies and is subject to strict regulation in many countries.

To address the issues discussed above, scientists are continuously conducting further research to minimize treatment risks and to provide a reliable scientific foundation for advancing hESCs applications in leukemia treatment. In the previous section, we noted that hESCs have a risk of tumorigenesis. Scientists have developed a monoclonal antibody (mAbs) against hESCs, named it as an anti-stage-specific embryonic antigen (SSEA)-5, which can highly and specifically bind a previously unidentified antigen called the H type-1 glycan [12]. To completely remove the teratoma-forming cells, scientists identified additional pluripotency surface markers (PSMs), which show a wide range of dynamic expression during the possession of differentiation (e.g. CD9, CD30, CD50, etc.) [12]. This strategy can eliminate the teratoma-forming potential of incompletely differentiated hESCs cultures. Moreover, researchers applied transcription factors in hPSCs making them directly differentiate into haemogenic endothelium cells (HEC), and then filtered 26 candidate haematopoietic specific stem cells transcription factors. They successfully identified seven transcription factors that were sufficient to convert haemogenic endothelium into haematopoietic stem and progenitor cells which could be applied in the treatment of leukemic diseases [13]. However, the direct conversion method still requires full validation in preclinical models and adapted to gene-free trace technologies, along with easy application in readily available and expandable somatic cell types. This will require deeper understanding of the gene regulatory networks and epigenetic mechanisms that control HSC identity [14]. Meanwhile, another landmark study created the widely recognized E8 medium, a chemical defined culture medium free of serum and feeder cells [15]. Although originally crafted for iPSCs, E8 medium quickly emerged as the gold standard for cultivating pluripotent stem cells, including hESCs, as it enhances cell quality, reduces batch differences, and simplifies the culture process. It has since found broad use in directed differentiation protocols [15]. This medium stands as a critical technology to realize standardized production.

The current core challenge of hESCs in leukemia treatment lies in ensuring both safety and the generation of functional hematopoietic stem cells. With the advancements in fields such as cell purification and differentiation, hESC-derived cells are expected to become a revolutionary treatment for leukemia. The emergence of iPSCs has also enabled personalized solutions; however, challenges related to tumorigenicity and differentiation efficiency still required ongoing improvements.

5. Conclusion

This paper systematically addresses the progress in hESCs and their potential uses in the therapy of leukemia. It points out that hESCs hold significant potential for leukemia treatment, providing a possible alternative to existing treatments such as HSCT. Their unique characteristics of unlimited self-renewal and multi-directional differentiation enable them to serve as valuable sources for generating functional HSCs and immune effector cells. However, clinical applications of hESCs face obstacles from technical challenges, such as tumorigenic risks stemming from genetic instability and poor efficiency of differentiation into functional HSCs.

The review also emphasizes recent advancements that have laid a solid basis for overcoming these challenges and pointing the way to future clinical applications. Besides technical hurdles, proper use of hESCs is still a pressing concern, and ethical debates related to their use continue to spark wide-ranging discussions around the world.

As cell proliferation, differentiation techniques, and gene editing keep advancing, hESC-derived therapies are expected to become a powerful method to treat leukemia over the next 5 to 10 years, bringing new hope to patients with hematological malignancies. The author holds that future research should focus on enhancing safety, improving functional outcomes, and accelerating clinical translation to realize the full therapeutic potential of hESCs.

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