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Hematopoietic Defects and iPSC Disease Modeling: Lessons Learned

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Summary

Hematopoiesis is a paradigm for stem cell biology in that it centers on differentiation of a self-renewing pluripotent precursor into multiple committed cell types with specific functions. The use of induced pluripotent stem cells (iPSCs) as a disease modeling tool has revealed numerous insights into the underlying pathophysiology of hematological diseases – those disorders arising from defective hematopoiesis. Likewise, studying hematopoiesis and the defects that can arise offer clues to understanding general stem cell survival and differentiation.

Introduction

Hematopoiesis is the process of forming the cellular components of blood. These components (erythrocytes, lymphocytes, granulocytes, platelets, monocyte/macrophages, dendritic cells, mast cells) differentiate from a common hematopoietic stem cell. Transcription factors, growth factors, and the extracellular environment direct specific genetic programs to drive development towards a particular cell type. Hematological diseases generally reflect inappropriate regulation or alterations of the genetic program controlling these complex processes during hematopoiesis. These diseases range from disruption of blood cell production leading to absence of one or more cellular components (bone marrow failure syndromes) to faulty maturation processes such as disordered globin chain switching (hemoglobinopathies) to excessive hematopoietic cell production (leukemias).

In vitro cellular differentiation is the process of forming a target tissue of interest from a common progenitor cell in a laboratory setting. Experimentally, induced pluripotent stem cells (iPSCs) have provided numerous culture based models of human disease. iPSCs originate from adult somatic cells that have been reprogrammed by enforced expression of transcription factors that drive pluripotency, and represent functional equivalents of embryonic stem cells which can differentiate into tissues of any germ layer (Takahashi &

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Yamanaka, 2006; Takahashi et al., 2007). IPSCs offer a platform for providing patient specific, scalable biologic material of various tissue types useful in investigating the pathophysiology of a disease, in assessing the effectiveness/toxicity of pharmaceuticals in tissues of interest, and in generating transplantable tissues for clinical applications (Lee et al., 2012; Onder & Daley, 2012; Rowland et al., 2012). The ability to generate tissues from iPSCs for experimentation is particularly useful in rare conditions or when primary tissues, such as bone marrow, are difficult or invasive to obtain or have been destroyed by the disease process (Z. Ye, Chou, & Cheng, 2012). Obtaining hematopoietic tissues for experimentation is challenging and can limit study design. The low prevalence of some hematological conditions hinders recruiting a statistically sufficient number of study patients and related biological materials. Furthermore, procurement of primary hematopoietic tissues requires an invasive procedure (bone marrow aspiration/biopsy) that can yield inadequate samples, particularly in the pediatric population where hematological diseases commonly present. Therefore, developing methods to model hematological diseases is beneficial to studying these conditions.

In a parallel manner to hematopoiesis, growth factors, cytokines, and media composition drive changes toward a specific genetic program during differentiation of iPSCs. Therefore, while iPSCs have the potential to model hematological diseases, hematological diseases also have the potential to serve as a *in vivo* natural model for iPSC biology. We may learn about the underlying mechanisms of pluripotent cells by examining the pathogenic mechanisms of inappropriate hematopoiesis.

In this short review, we will describe advances related to disease modeling of hematopoietic conditions with iPSCs. We will provide examples of new insights uncovered using this method and discuss how studying these diseases may inform improved development of pluripotent cells for laboratory experimentation.

Bone marrow failure syndromes

Bone marrow failure (BMF) syndromes are rare, usually inherited disorders marked by a developmental deficiency within one or many cell lines of the hematopoietic compartment. The cause of BMF syndromes is often associated with a genetic mutation or polymorphism, which makes iPSC-based disease modeling particularly amenable in these conditions due to the ability to generate gene-corrected controls. The study of BMF can also inform a better understanding of iPSC biology as bone marrow contains naturally developing stem cells that serve as ideal examples of multipotency. There are recent examples of novel insights into the biology of BMF syndromes and iPSCs gained from each other.

Fanconi anemia (FA), the most common BMF syndrome, results from mutations in genes encoding DNA repair mechanisms. Patients with FA often develop acute myelogenous leukemia and bone marrow failure along with short stature, endocrine defects, and abnormalities of the eyes, ears, skin, and kidneys. Reprogramming somatic cells into iPSCs requires a functional version of the DNA repair pathway involved in FA, which emphasizes these genes importance in maintaining multipotency for both natural bone marrow and laboratory-manipulated iPSCs. Genetic complementation or reprogramming under hypoxic conditions can overcome this requirement in the production of FA-patient specific iPSCs to produce cells with a normal karyotype and full hematopoietic potential (Müller et al., 2012; Raya et al., 2009). In a protocol using conditions without genetic complementation and under normal oxygenation, reprogramming of FA affected somatic cells to iPSCs demonstrated reduced efficiency, higher frequencies of chromosomal abnormalities, and failure to produce teratomas (Yung et al., 2013). Such defects would be expected as reprogramming activates the FA pathway leading to an increased number of double stranded

DNA breaks and cellular senescence (Müller et al., 2012). Studying reprogramming defects and iPSC generation using cells from patients with FA may provide insights to improve reprogramming efficiency in general as well as learn about this specific disease process. For example, lentiviral gene correction in FA-patient specific iPSCs leads to phenotypically normal erythroid and myeloid hematopoietic progenitor cells (Jacome et al., 2009; Raya et al., 2009; Río et al., 2008), providing a proof of concept for the potential benefit of gene therapies.

Shwachman-Diamond syndrome (SDS) results from inappropriate 60S ribosomal subunit maturation and presents with exocrine pancreatic insufficiency and neutropenia (Finch et al., 2011). Recent disease modeling of iPSCs has shown increased protease levels as a common mechanism between the pancreatic and hematopoietic manifestations of SDS (Tulpule et al., 2013). These data suggest that higher proteases can lead to autodigestion of pancreatic acinar and myeloid cells; therefore, anti-proteolytic compounds such as aprotinin are a potential therapeutic option. This work provides an example of a potential impact for iPSC disease modeling in uncovering pharmacologically targetable disease mechanisms.

Dyskeratosis congenita (DC) results in bone marrow failure (anemia and thrombocytopenia), predisposition to cancer, and integumentary system dysfunction appearing similarly to premature aging of the skin, hair, and nails. Studies in iPSCs reprogrammed from DC patients demonstrate the importance of the DC-related pathway in pluripotency. Mutations in dyskerin (*DKC1*) result in blocked telomerase assembly; mutations in telomerase reverse transcriptase (*TERT*) reduce telomerase elongation; and mutations in WRAP53 (also known as *TCAB1*) cause inappropriate telomerase localization (Batista et al., 2011). These defects and the subsequent telomerase shortening in bone marrow lead to eventual loss of pluripotency and were shown to prevent derivation and survival of iPSCs (Batista et al., 2011). However, not only do genes of this pathway cause BMF when disrupted, the upregulation of telomerase RNA component (TERC) has been shown essential in a pluripotent state, which is expected since pluripotency-related transcription factors act on telomerase components (Agarwal et al., 2010). Therefore, while iPSCs can effectively model the defects observed in DC, studying DC may provide insight into improving efficiency of iPSC survival.

Hemoglobinopathy

Diseases affecting hemoglobin are another category of hematological disorders recently modeled using iPSCs. Hemoglobinopathies are inherited conditions marked by abnormal structures of globin chains in the hemoglobin molecule. Thalassemias are also inherited conditions characterized by underproduction of structurally normal hemoglobin molecules, which results in weakening and more rapid destruction of red blood cells.

Patients with sickle cell disease, a hemoglobinopathy, display rigid, abnormally sickle-shaped red blood cells due to a polymorphism in the beta globin chain. Using gene targeted plasmids and zinc finger nucleases, researchers introduced a corrected hemoglobin A allele (HbS/HbA) into iPSCs derived from sickle cell patients (HbS/HbS); these corrected cells expressed 25–40% of the HbA transcript (Zou, Mali, Huang, Dowey, & Cheng, 2011), which is consistent with transcript levels in typically asymptomatic sickle cell heterozygous carriers. Such targeted gene modification using zing finger nuclease enhanced homologous recombination may offer a useful methodology for future gene therapy protocols and for generating patient-specific gene-corrected tissues for use in transplantation or laboratory screening tests (Sebastiano et al., 2011). Thus, disease modeling of SCD, a hematological condition, is a model itself for improving cellular based technologies.

Beta thalassemia is caused by splice site mutation that influences mRNA transcription of the hemoglobin beta globin chain. Fully functioning $\alpha_2\beta_2$ hemoglobin chains may (or may not) be produced in sufficient quantities depending on the mutation, thereby leading to anemia. Researchers reprogrammed and gene-corrected beta thalassemia patient-specific cells into iPSCs; they propose that differentiation of these iPSCs into hematopoietic cells could provide an option for transfusion support in these patients (L. Ye et al., 2009). iPSCs from patients with beta thalassemia have also been a model for a proof of concept study in genetic modification of potential cellular therapy. These scientists developed protocols for optimizing lentiviral integration sites of the beta globin transgene to genomic sites with little oncogenic potential and demonstrated high beta globin gene expression without influencing nearby genes (Papapetrou et al., 2011). Their methods provide another example of how modeling hematologic diseases offers insight to learning about iPSC biology and manipulation in general, as beta thalassemia continues to be a model for understanding the underlying mechanisms of lentiviral integration in iPSCs (Tubsuwan et al., 2013).

Leukemia

Leukemias represent excessive or uncontrolled growth of hematopoietic cells, usually resulting in a large number of immature hematopoietic cells. Given their potential for self-renewal, studying leukemic cells offers parallels for studying iPSCs (Kumano, Arai, & Kurokawa, 2013). Chronic myelogenous leukemia (CML) is a leukemia commonly treated with tyrosine kinase inhibitors such as imatinib, which target the BCR-ABL fusion product. iPSCs derived from imatinib-sensitive CML patient samples were able to overcome tyrosine kinase inhibition; the phosphorylation of kinases such as MAPK3, JNK, and AKT, which are essential to both iPSC and BCR-ABL positive cell survival, were not affected by imatinib treatment (Kumano et al., 2012), suggesting that mechanisms important to *in vitro* stem cell survival may reflect pathogenic mechanisms in resistant hematological cancers.

Conclusions

There are many challenges for cells to maintain a pluripotent state – in both the natural hematopoietic compartment and in a laboratory tissue culture dish. Environmental signals and specific genetic programs determine the cell type survival and fate in a complex manner. iPSC disease modeling of hematological conditions promises to uncover new therapeutic avenues for gene therapy or tissue transplantation / transfusion in patients with hematopoietic defects, such as those reported in hemoglobin-based disorders. Likewise, studying hematopoietic defects promises to show opportunities for optimizing cellular mechanisms underlying multipotency, as observed in studies of the DNA repair defects of Fanconi anemia. These examples demonstrate the benefit of studying hematopoiesis in iPSCs since hematological diseases and stem cell technologies offer models to learn from each other.

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