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Deriving blood stem cells from pluripotent stem cells for research and therapy

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Embryonic stem cells and induced pluripotent stem cells offer promise for research and treatment of hematologic diseases. While broad clinical application in humans is still a distant prospect, there are promising near-term applications in transfusion of platelets and red blood cells.

Introduction

A paradigm shift has taken place in recent years, enabling us to manipulate cell identities. In just a few weeks' time, skin fibroblasts can be converted into pluripotent stem cells, which in turn can be converted into a diversity of tissue lineages. This technology has proven valuable for fundamental studies in hematopoietic stem cell biology, and may prove essential to our understanding of how to treat hematologic malignancy.

The hematopoietic stem cell (HSC) is the best understood of all stem cells and the most effective stem cell exploited for therapy through bone marrow transplant. My research into HSCs began with my interest in chronic myeloid leukemia (CML), which is the classic malignancy of the HSC and an outstanding model for the cancer stem cell hypothesis. The Philadelphia chromosome translocation, which produces the *BCR/ABL* oncogene, can be found in both lymphoid and myeloid lineages, indicating

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the stem cell nature of this cancer. As a graduate student, I demonstrated the direct involvement of *BCR/ABL* as the founding driver mutation in CML. While the development of the targeted therapy imatinib has been transformative, the dominant treatment for Philadelphia-chromosome-positive CML prior to imatinib was bone marrow transplantation, and transplant remains the best hope for cure for many patients with intractable blood disease, both malignant and genetic. However, there are limitations to bone marrow transplant, including a lack of matched donors and morbidity and mortality related to graft-versus-host disease. These limitations have compelled me to explore alternative sources of HSCs.

Alternative sources of hematopoietic stem cells

My search for alternative sources of HSCs started with the realization that pluripotent embryonic stem (ES) cells are the precursors of all tissue lineages. By deriving ES cells using somatic cell nuclear transfer from mice carrying genetic disease, then repairing the gene defects and coaxing ES cells to become HSCs, my lab pursued combined gene repair and cell therapy to correct a genetic blood disease [1]. For this model, we started with a biopsy of the tail tip from a mouse with *rag2* gene deficiency, and cultured dermal fibroblasts. Then, using micromanipulation, we transferred the nucleus of the dermal fibroblast into an egg from which we had already removed the DNA. This process of nuclear transfer created a reconstructed pseudozygote, mimicking fertilization. A blastocyst formed, from which embryonic cells could be isolated. We then used homologous recombination to repair the disease locus, and employing the *hoxb4* gene, amplified hematopoietic elements that we used to transplant the *rag2*–deficient mouse, thereby restoring immune function in a proof-of-principle experiment for combining gene and cell therapy [1].

Nuclear transfer proved much more difficult to replicate in humans because of poor access to quality donor oocytes. We attempted to perform human somatic cell nuclear transfer into donor eggs that were harvested in the context of fertility treatment, but which were of poor quality: they had failed to fertilize during in vitro fertilization and would have been discarded had they not been donated for research. While we failed using these poor quality oocytes, over the last year three different groups were successful in performing somatic cell nuclear transfer on human cells using healthy oocytes specifically donated for research use [2–4]. While nuclear transfer technology is not efficient enough to be widely applied, there are important lessons to be learned about the mechanism by which eggs reprogram somatic nuclei. It is also essential to understand whether or not embryonic stem cells created using nuclear transfer have any advantages or limitations relative to other stem cells.

Highly practical alternatives to nuclear transfer now exist. By introducing four genes normally active in embryonic stem cells into fibroblasts or blood cells, somatic cells can be reprogrammed to pluripotency, generating the functional equivalent of embryonic stem cells. These induced pluripotent stem cells (IPS cells) have practical advantages relative to stem cells created by nuclear transfer, as transcription factor reprogramming is enormously efficient and produces a very high quality embryonic stem cell that can be used in all types of manipulations. iPS cells can be converted into a multitude of cells like dopaminergic neurons, motor neurons, hepatocytes, and blood cells. Indeed, reprogramming and cell fate conversion is among the most promising new avenues of stem cell biology and has ushered in a new paradigm, indicating that the differentiated identity of somatic cells is no longer sacrosanct. We can manipulate cellular identity for biomedical research, and potentially one day for therapeutic applications.

Barriers to HSC generation

While we have created pluripotent stem cells, we are still challenged to identify protocols that faithfully replicate embryonic development in a Petri dish to develop the cells of interest. Embryonic and IPS cells, being embryonic tissues, reproduce a primitive, embryonic or sometimes fetal program of hematopoietic development. Cells of the erythroid lineage express embryonic and fetal globins, and one of the major challenges left to solve is understanding how to developmentally mature these cells. It is clear from attempts to direct lineage differentiation along various myeloid or lymphoid pathways that the understanding of blood development in vitro is incomplete. It may be hubris to imagine that