Hope for hematological diseases

Tweaking growth media helps cultured hematopoietic stem cells thrive

By Adam C. Wilkinson

The development of methodologies for growing cells ex vivo has been essential for advancing the field of regenerative medicine. The in vitro stabilization of embryonic (pluripotent) stem cells, for example, has afforded unprecedented biological insights, breakthrough technologies, and new therapeutic paradigms (1). Determining the equivalent culture conditions necessary for growing adult tissue stem cells holds a similar scientific and clinical potential, but this goal remains largely unmet.

During my scientific career studying hematopoiesis (blood formation), my research efforts have often been frustrated by the lack of suitable culture conditions for the stable growth of hematopoietic stem cells (HSCs). Because HSCs are a rare bone marrow cell type, this has been a major barrier to understanding the mechanism of blood formation and to developing new therapies for hematological diseases.

Stable, ex vivo HSC expansion has the potential to revolutionize current clinical therapies. With the capacity for both self-renewal and multipotent differentiation, HSCs can reconstitute the entire blood system after transplantation and engraftment within a recipient (2). This remarkable activity provides the scientific basis for clinical HSC transplantation (HSCT), the pioneering cell therapy that for more than half a century has offered a cure to so many patients suffering from hematological diseases (3).

Although HSCT is potentially curative, it remains a risky therapy. Two of the major roadblocks to its wider and safer use are the availability of immune-compatible allogeneic donor HSCs—necessary to avoid severe graft-versus-host disease—and the need for patients to undergo genotoxic chemotherapy and/or radiation bone marrow pre-conditioning, which can cause substantial morbidity and mortality and limit patient access.

The ideal HSCT therapeutic paradigm (at least for nonmalignant hematological diseases) would be the transplantation of healthy autologous patient-derived HSCs without preconditioning. CRISPR/Cas9 genome editing technologies have opened the door for efficient autologous HSC gene correction for numerous hematological diseases (4). Unfortunately, however, the clinical success of these new HSCT strategies (as well as existing lentivirus- or retrovirus-based HSCT gene therapies) remains challenging because of the limited window for ex vivo perturbation (usually <72 hours) and the often inadequate numbers of HSCs collectable from patients. Additionally, genotoxic preconditioning is still required to improve the chances of engraftment.

Nongenotoxic preconditioning, such as metabolic and antibody-based strategies (5–7), has been suggested for HSCT, but achieving HSC engraftment without any preconditioning would be ideal. Such a therapeutic strategy would be possible if much larger numbers of HSCs were available for HSCT because HSCs do engraft in the nonconditioned setting, but only at low efficiencies (8).

The existence of substantial scientific and clinical potential of ex vivo HSC expansion has stimulated an extensive search for supportive culture conditions (9). However, current ex vivo conditions only sustain human HSCs short term (1 to 2 weeks), during which expansion of functional HSCs is limited. Rather than displaying potent self-renewal ex vivo, HSCs rapidly differentiate and lose their “stemness.” This has led to the assumption that we are still missing essential self-renewal–promoting factors. Alternatively, we proposed that HSC media contained additional contaminants that skewed HSCs to differentiation (10).
To test this hypothesis, I took a reductionist approach to optimize media conditions that we had previously shown could maintain mouse HSCs for 7 days and supported approximately twofold functional expansion (11). The results were dramatic. Using our new “cleaned-up” culture condition, we were able to grow HSCs for more than 2 months ex vivo and achieve ~900-fold expansion (10).

The biggest factor in establishing optimal ex vivo HSC expansion conditions was the removal of serum albumin. As a major blood component, serum albumin has long been considered an essential supplement for hematopoietic cell cultures. However, the use of serum or serum albumin introduces numerous biologically active impurities (11). In our HSC cultures, even recombinant serum albumin contained contaminants that HSCs were highly sensitive to and that inhibited HSC self-renewal.

I discovered that serum albumin can be entirely replaced with the synthetic polymer polyvinyl alcohol within our HSC media. The use of this biologically inert albumin replacement to remove these HSC differentiation-inducing contaminants dramatically improved HSC expansion (10).

This substitution—combined with the right levels of stem cell factor, thrombopoietin, and fibronectin—enabled us to grow bulk populations of HSCs in long-term cultures and to expand single HSCs clonally. Using clone-splitting assays, we were even able to definitively prove ex vivo HSC self-renewal (10).

This new HSC culture system also provided an important clinical insight. When large numbers of ex vivo–expanded HSCs (generated from just 50 freshly isolated HSCs) were transplanted into nonconditioned recipient mice, durable engraftment was achieved. We are now working to demonstrate the efficacy of this intervention in combination with CRISPR/Cas9–based autologous HSC gene correction.

THE NEXT STEPS
Our mouse HSC expansion system offers a valuable technology with which to uncover new biological mechanisms and to develop therapeutic approaches for regenerative medicine and cell therapy. Additionally, our recent progress in expanding mouse HSCs has also provided an important roadmap with which to achieve stable ex vivo human HSC expansion, a route that will, I hope, ultimately lead to safer clinical HSCT therapies.

REFERENCES AND NOTES

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