Serial Transfer of Human Hematopoietic and Hepatic Stem/progenitor Cells

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[Abstract] A range of assays have been developed to determine the stemness or stem cell activity of human stem cells. The key assays of stem cells are functional: they must show self-renewal and the ability to generate the appropriate tissue. The best assays available to study this property in putative human stem cells involve xeno-transplantation into immune-deficient mice. Demonstration of both long-term (2-3 months) multi-lineage reconstitution of human blood or liver in a murine host and the ability of the putative stem cells to mediate reconstitution of a secondary host upon re-isolation from the primary mouse are generally accepted as the gold standard for demonstrating the presence of human hematopoietic and hepatic stem cells. Here, we describe a method of reconstituting NOD-scid IL-2Rγ$^{-/-}$(NSG) mice with CD34^{+} stem cells from human fetal liver and repurification of CD34^{+} cells for serial transplantation.

Materials and Reagents

1. NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (The Jackson Laboratory, stock number: 005557)
2. CD34^{+} fetal liver cells
3. StemSpan™ SFEM (STEMCELL Technologies, catalog number: 09650)
4. ACK Lysing Buffer (Life Technologies, catalog number: A1049201)
5. Liver perfusion medium (Life Technologies, catalog number: 17701-038)
6. Liver digestion medium (Life Technologies, catalog number: 17703-034)
7. RoboSep™ Buffer (STEMCELL Technologies, catalog number: 20104)
8. Trypan blue solution (Sigma-Aldrich, catalog number: T8154)
9. EasySep™ Human Cord Blood CD34 Positive Selection Kit (STEMCELL Technologies, catalog number: 18096)
10. DMEM

Equipment

1. Biosafety cabinet

2. Petri dishes (100-mm^2)
3. 137 Cs gamma irradiator
4. Insulin syringe (29 G 1 cc) (BD Biosciences, catalog number: 320310)
5. Syringe (5 ml) (BD Biosciences, catalog number: 309646)
6. Heating pad or warming lamp
7. Butterfly needle (BD, catalog number: 368659)
8. Cell strainer (100 µm) (BD Biosciences, catalog number: 352360)
9. Falcon tube(15 ml)

Procedure

A Engraftment of primary recipient mouse

1. CD34$^+$ fetal liver cells are purified based on the protocol "Isolation of CD34$^+$ Cells from Human Fetal Liver and Cord Blood" (Chen and Chen, 2013).
2. Monitor breeder pairs for the birth of new litters.
   Note: Engraftment procedures should be performed on newborn pups 24 to 48 h post-natal.
3. Prepare CD34$^+$ fetal liver cells suspend in StemSpan at 2.5 x 10^5 cells/50 µl/pup.
   Note: Freshly prepared or previously frozen preparations may be used.
4. Place 24- to 48-h post-natal pups from a single litter into a 100 mm^2 petri dish along with a small amount of bedding material from the breeder cage.
5. The petri dish is put into a 137 Cs gamma irradiator. Irradiate pups with 1 Gy whole body irradiation.
6. The petri dish is then brought back into biosafety cabinet. A second sterile petri dish is prepared with cotton nestlet from parent cage.
7. One pup is taken at a time from the irradiated dish and held firmly, yet with great care, by thumb and index finger of one hand. Tilt the pup back so that abdomen is exposed. The liver will be visible on the right flank of the pup (Figure 1). Disinfect area with alcohol pad.
8. The other hand holds a 29 G 1 cc insulin syringe loaded with 50 µl of fetal liver cells.
9. The needle (perpendicular to body) will be inserted straight in, with bevel facing upwards, approximately 3 mm into the pup.
10. The cells are then carefully and slowly injected into the pup's liver.
11. Once injected, the needle is removed and gentle pressure is applied to the area. The injected pup is then placed in the second petri-dish.
12. Steps A7-11 are repeated until all the pups have been injected.
13. The pups are carefully placed back into their parents' cage and covered with the cotton nestlet so they will smell familiar to parents.

14. The pups will be monitor everyday for seven days. Any pups exbihiting severe weight loss, dehyration, dyspnea should be euthanized immediately. From experience, the pups are mostly unaffected by the injection.

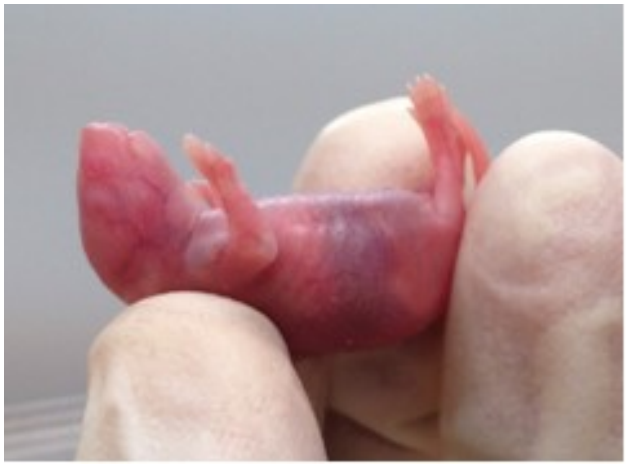

Figure 1. Liver: site of injection

B Cell repurification and reconstitution of secondary recipient mouse

15. 8 to 10 weeks later, the primary mice are used for repurification of human hematopoietic stem cells and hepatic progenitor cells respectively.

For repurification of hematopoietic stem cells from femurs

16. Femurs are harvested and Remove as much muscle as possible around the femur bone.
17. Attach a 27 G needle to a 5 ml syringe filled with PBS.
18. Place the needle into the bone marrow (red middle of the bone), and flush out cells onto a 100 μm cell strainer in a 5 cm petri dish.
19. Mesh cells and transfer the pass-through to a 15 ml Falcon tube.
20. Pellet cells at 400 *x g* for 5 min.
21. Lyze red blood cells with ACK lysis buffer.
22. Re-purify $CD34^+$ cells by magnetic selection with EasySep™ Human Cord Blood CD34 Positive Selection Kit.

For repurification of hepatic progenitor cells from livers

23. Cannulate portal vein (Figure 2) with a 27 G buffer fly needle.
24. Make incision in inferior vena cava (Figure 2).

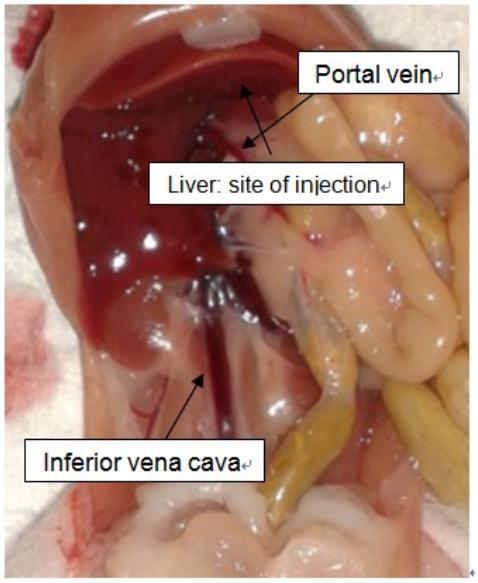

Figure 2. Portal vein and inferior vena cava

25. Mouse livers were first perfused with pre-warmed liver perfusion medium at 0.7 ml/min for 10 min, then with pre-warmed liver digestion medium for 10 min.
26. Carefully transfer the digested liver to a petri dish and dis-associate liver cells into single cell suspensions with curved forceps.
27. The cell suspensions were washed with ice-cold DMEM at 50 *x g* for 5 min.
28. Assay cell viability by Trypan Blue dye.
29. $CD34^+$ cells were re-purified from the cell suspensions with EasySep^{TM} Human Cord Blood CD34 Positive Selection Kit.

C Reconstitution of secondary recipients

30. $CD34^+$ cells of desired numbers were injected into sublethally irradiated newborn NSG pups.
31. After 8 to 10 weeks, samples *e.g.* blood and livers are harvested for analysis.

Acknowledgments

This protocol was developed and adapted from the previous publication Chen *et al.* (2013a) and (2013b).

References

1. Chen, Q., Khoury, M., Limmon, G., Choolani, M., Chan, J. K. and Chen, J. (2013a). Human fetal hepatic progenitor cells are distinct from, but closely related to, hematopoietic stem/progenitor cells. *Stem Cells* 31(6): 1160-1169.
2. Chen, Q., and Chen, J. (2013b). Isolation of CD34+ cells from human fetal liver and cord blood. *Bio-protocol* 31(23): e991.