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Isolation and expansion of the hepatic progenitor cell (HPC) population

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Abstract

HPCs were isolated from genetically normal mice, using cell surface markers, were highly expandable and phenotypically stable in vitro. Genetically normal HPCs were transplanted into adult mice livers where hepatocyte Mdm2 could be repeatedly deleted, creating a non-competitive repopulation assay. Transplanted HPCs contributed significantly to restoration of liver parenchyma, regenerating hepatocytes and biliary epithelia, highlighting their in vivo lineage potency. HPCs are therefore a potential future alternative to hepatocyte or liver transplantation for liver disease.

Subject terms: Cell biology Cell culture Isolation, Purification and Separation

Keywords: Liver progenitor cells

Introduction

Isolation a defined population of hepatic progenitor cells from mouse liver by FACS and expansion of hepatic progenitor cells in vitro.

Reagents

Liver Digest Medium (Gibco)
Williams'E Medium with 10% FCS (Gibco)
HPCs Expansion Medium
Diluted Trypsin
FACS antibody

Equipment

Miltenyi GentleMACS Dissociator

Procedure

Preparation of mouse liver non-parenchymal cell fraction

1. Sacrifice mouse
2. In vivo perfusion with 5-10 ml of PBS from the IVC follows by 5-10ml of Liver Digest Medium (Gibco)
3. Harvest the liver without including the gall bladder and transfer to a tube with (Williams'E medium + 10% FCS).
4. Cut the liver into fine pieces and incubate in Liver Digest Medium at 37C for 20 mins
5. Mesh the liver more either with mechanical chopping with a scalpel or Miltenyi GentleMACS.
6. Pass the mesh through 100um cell strainer and then top up with 25ml of Williams'E medium + 10% FCS
7. Spin tubes at 50G for 5 mins with acceleration =4 deceleration=4
8. Transfer supernatant to a new 50 ml falcon while passing through a 40um strainer, discard the pellet.
9. Spin tubes at 50G for 5 mins with acceleration =4 deceleration=4
10. Transfer supernatant to a new 50 ml falcon while passing through a 40um strainer, discard the pellet.
11. Spin tubes at 300G for 5 mins with max acceleration max deceleration
12. Keep pellet, discard supernatant
13. Incubate with red cell lysis buffer for 2 mins on ice.
14. Wash with 10 ml of PBS at 300G
15. Spin tubes at 300G for 5 mins with max acceleration max deceleration
16. The resulting pellet will be the non-parenchymal fraction of the liver. Can either plate or continue with FACS.

FACS of mouse liver non-parenchymal cell fraction

1. Block with either Fc Block or neat FCS for 10 mins at room temperature
2. Spin at 300G for 5 mins, discard supernatant
3. Incubate with antibody mix for 30mins – 1 hour on ice. ~50-100 ul per sample

Antibody Mix

Manufacturer Antibody Clone Dilution
eBiosciences CD45 PE 30-F11 1/100
eBiosciences CD31 PE 390 1/100
eBiosciences Ter119 PE TER119 1/100
eBiosciences EpCAM APC G8.8 1/200
eBiosciences CD133 FITC 13A4 1/50
Biolegend CD24 PeCy7 M1/69 1/200

4. Wash with FACS Buffer (PBS +2%FCS)

5. Spin at 300G for 5 mins, discard supernatant.
6. Resuspend samples in 300ul of FACS buffer and transfer to FACS tube with 40um cell strainer cap and sort for CD45-/CD31-/Ter119-/EpCAM+/CD24+/CD133+
7. Before sorting, add life dead marker to exclude dead cells, DAPI/7AAD

Culturing mouse liver NPCs / HPCs

1. Coat plates with 1 mg/ml Rat Tail Collagen 1 (Sigma)
2. Leave in incubator for approximately 4 hours to dry
3. Plate cells with liver expansion medium

HPCs Expansion Medium Recipe (Williams' E + 10% FCS)
Chemicals required Stock concentration Dilution Preparation
17.6 mM NaHCO3
20mM HEPES pH 7.5
10 mM Nicotinamide
1mM Sodium Pyruvate
1X ITS
100nM Dexamethasone
0.2 mM Ascorbic Acid
14mM glucose 1.4 M
10ng/mL IL-6 10 ug/mL
10ng/mL HGF 20ug/mL
10ng/mL EGF 100ug/mL

4. Wait for colony to form
5. After colony formation, change medium every 2-3 days
6. Try and prepare medium relatively fresh, in 5ml aliquots (or smaller)

Passaging primary mouse HPCs

1. Incubate with diluted trypsin until cells start to detach (might take a while)
2. Flush colony with Williams'E + 10%FCS
3. Collect cells and spin at 300G for 5 mins.
4. Replate cells on collagen coated plates

Diluted Trypsin (Tsuchiya et al, Gastroenterology 2005):
0.25% Trypsin no EDTA
20% Knockout Serum Replacement
1mM CaCl2

Associated Publications

This protocol is related to the following articles:

- Hepatic progenitor cells of biliary origin with liver repopulation capacity
  Wei-Yu Lu, Thomas G. Bird, Luke Boulter, Atsunori Tsuchiya, Alicia M. Cole, Trevor Hay, Rachel V. Guest, Davina Wojtacha, Tak Yung Man, Alison Mackinnon, Rachel A. Ridgway, Timothy Kendall, Michael J. Williams, Thomas Jamieson, Alex Raven, David C. Hay, John P. Iredale, Alan R. Clarke, Owen J. Sansom, and Stuart J. Forbes

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Competing financial interests

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Readers' Comments

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