Liver Stem Cell

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Abstract: Primary liver cells (hepatocytes) are the liver's chief functional cells that perform most of the liver's complex metabolic tasks. It is possible to grow hepatocytes that could provide significant biomedical advantages, including cell therapy, and the liver stem cells are good candidate. For the patients suffering from liver cancer, the liver stem cells could be safe and credible source of liver cells as an alternative to liver transplant. Growing liver cells form liver stem cells could also be useful in detecting safe drug screening methods. Stem cell therapy is already a popular intervention for treating blood and immune system conditions, as well as numerous skin diseases. It involves a careful medical procedure of introducing adult cells that are grown from early cells or stem cells, into damaged tissues. Liver stem cells are the precursors for a subset of liver cancer, hepatocellular carcinoma.
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The liver in an animal adult healthy body maintains a balance between cell renew and cell loss. The cell damage can stimulate the new cell growth and in liver it is hepatocyte self-replication. The transplanted hepatocyte can undergo clonal expansion which shows that the hepatocytes themselves can play the function as stem cells in the liver. Severe liver injury can activate a potential stem cell compartment located within the intrahepatic biliary tree, giving rise to cords of bipotential (oval cells) within the lobules that can differentiate into hepatocytes and biliary epithelial cells. A third population of stem cells with hepatic potential resides in the bone marrow; these haematopoietic stem cells can contribute to regeneration and restore normal liver function (Forbes, et al, 2012).

Primary liver cells (hepatocytes) are the liver's chief functional cells that perform most of the liver's complex metabolic tasks. It is possible to grow hepatocytes that could provide significant biomedical advantages, including cell therapy, and the liver stem cells are good candidate. For the patients suffering from liver cancer, the liver stem cells could be safe and credible source of liver cells as an alternative to liver transplant. Growing liver cells form liver stem cells could also be useful in detecting safe drug screening methods.

Stem cell therapy is already a popular intervention for treating blood and immune system conditions, as well as numerous skin diseases. It involves a careful medical procedure of introducing adult cells that are grown from early cells or stem cells, into damaged tissues. Liver stem cells are the precursors for a subset of liver cancer, hepatocellular carcinoma. The possibility of liver stem cell therapy, especially for those with chronic liver diseases, could definitely ease the worry of hundreds of people anxiously waiting for liver transplants. Selecting CD45 negative cells is used in the isolation of liver stem cells. Magnetic antibody metod is applied in the isolation liver stem cells.

Hepatocellular carcinoma (HCC) and intrahepatic cholangiocellular carcinoma (ICC) are common liver tumors. Originating from one pluripotent liver stem cell both tumor entities can occur in a cirrhotic liver. According to Wengert et al report, several risk factors have been identified as causative for both carcinomas. Surgical resection, interventional procedures and transplantation are available as curative treatment options when diagnosed in time. The common characteristic features and morphology in cross-sectional imaging by ultrasound (US), multidetector computed tomography (CT) and magnetic resonance imaging (MRI) were used. It showed a better understanding of the carcinogenesis model of both liver tumors originating from one pluripotent liver stem cell (Wengert, et al, 2014).

The precursor to oval cells is considered to be a facultative liver stem cell (LSC). Recent lineage tracing experiments indicated that the LSC is SRY-related HMG box transcription factor 9 positive (Sox9(+)) and can replace the bulk of hepatocyte mass in several settings. Quantitative analysis showed that Sox9(+) cells contributed only minimally (<1%) to the

hepatocyte pool, even in classic oval cell injury models (Tarlow, et al, 2014).

Glycogen storage disease type I (GSDI), an inborn error of carbohydrate metabolism, is caused by defects in the glucose-6-transporter/glucose-6-phosphatase complex, which is essential in glucose homeostasis. With the feasibility of novel cell-based therapies, including hepatocyte transplantations and liver stem cell transplantations, it is essential to consider long term outcomes of liver replacement therapy. (Boers, et al, 2014).

A method for the feeder-independent culture of PICM-19 pig liver stem cell line was recently devised, but the cell line's growth was finite and the cells essentially ceased dividing after approximately 20 passages over a 1 year culture period. Talbot and Caperna reported the isolation, continuous culture, and initial characterization of a spontaneously arising feeder-independent PICM-19 subpopulation, PICM-19FF, that maintained replication rate and hepatocyte functions over an extended culture period. Accordign to Talbot and Caperna's report, PICM-19FF cells grew to 90-98 % confluency after each passage at 2 week intervals, and the cells maintained a high cell density after 2 years and 48 passages in culture (average of 2.6 × 10(6) cells/T25 flask or 1 × 10(5) cells/cm(2)). Morphologically, the PICM-FF cells closely resembled the finite feeder-independent PICM-19 cultures previously reported, and, as before, no spontaneous formation of 3D multicellular ductules occurred in the cells' monolayer. Their bipotent stem cell nature was therefore not evident (Talbot and Caperna, 2014).

Saito, et al studied a 43-year-old man patient with chronic hepatitis B without history of hepatocellular carcinoma (HCC) and first diagnosed with thrombosis in right portal vein trunk and portal vein branches and ruptured esophageal varices in October 2011, and underwent endoscopic variceal ligation, but ruptured repeatedly. Computed tomography (CT) scan showed that portal vein thrombosis had low density from early to late phase. This is the first case of HCC only in portal vein without liver parenchyma tumor nodules, with difficult differential diagnosis from a non-malignant portal vein thrombosis (Saito, et al, 2013).

Various stem cell populations have been described in distinct models of liver regeneration (Dahlke, et al, 2004).

One example of the protocols for the liver stem cells analysis (Rountree, et al, 2015):

1. Parenchymal and non-parenchymal separation from whole liver

1) Prepare the enzyme solution for digestion using a 15 cc tube with screw top in 10 ml sterile PBS containing 5 mg collagenase, 5 mg pronase, and 1 mg DNAse.
2) Euthanize mouse using institution approved (Institutional Animal Care and Use Committee) method such as CO_2 asphyxiation. Wipe abdominal area of euthanized animals with 70% ethanol solution. Using sterile instruments, open abdominal cavity and explant liver en-block. Remove gall-bladder from explanted liver. Transfer whole liver to laminar flow hood in closed sterile dish.
3) This procedure is completed in a laminar flow hood. Using a sterile razor blade, mince liver with combination of multiple horizontal and vertical cuts for 1 minute in sterile dish. Place ¼ of minced liver pulp in 15 cc tube with 10 cc PBS with collagenase, pronase, and DNAse from step 1.1 above. Repeat with each ¼ minced liver.
4) Place tubes with ¼ minced liver pulp and enzymes into water bath at 37°C for 45 minutes, with shaker at 1-2 cycles/second. Wipe tubes with 70% ethanol after removal from water bath prior to transfer to laminar flow hood.
5) This procedure is completed in a laminar flow hood. Strain digested liver pulp through 70 micron mesh filter to collect into a sterile dish. Using 2 ml aliquots of sterile DMEM:F12 media with 10% heat inactivated fetal bovine serum, rinse the filter and use the rubber end of a syringe plunger to mash the digested pulp through the filter. Repeat 5 times to make total volume of filtrate approximately 20 mL. Divide the filtrate into 2 equal 15 mL tubes.
6) All transfers should be completed in laminar flow hood, and use refrigerated centrifuge at 4°C if available. Centrifuge at 50 x g for 1 minute. Save supernatant #1 and discard parenchymal pellet Centrifuge supernatant #1 at 50 x g for 1 minute. Save supernatant #2 and discard pellet. Centrifuge supernatant #2 at 50 x g for 1 minute. Save supernatant #3 and discard pellet. Centrifuge final supernatant #3 for 180 x g for 8 minute to obtain non-parenchymal fraction.

2. Red cell lysis

Work in laminar flow hood, keep cells cold, and use solutions cooled to 4°C.

1) The night before the procedure, prepare red cell lysis buffer by diluting 10X concentration stock BD Pharm Lyse buffer with a 1:10 dilution with sterile distilled water. 1X solutionshould be stored for 30 days at 4°C.
2) The night before the procedure, prepare Miltenyi buffer using sterile PBS, 0.5% bovine serum *Albumin*, and 2mM EDTA. Filter solution using vacuum filter unit with 0.45 micron filter. Cover top of tilter unit with original plastic lid and secure with plastic wrap. Storeentire filter unit at 4°C for 12 hours to degas EDTA. Filter unit can be replaced with standard sterile cap after 12 hours.
3) This procedure is completed in a laminar flow hood. Using a 5 ml sterile tube, re-suspend non-parenchymal pellet from step 1.6 above into 1 mL of 1X diluted red blood cell lysis buffer from 2.1 above. Cap the tube for transfer out of the laminar flow hood.
4) Gently vortex for 5seconds and incubate for 15 minutes at 4°C protected from light.
5) Centrifuge at 200 x g for 5 minutes.
6) This procedure is completed in a laminar flow hood. Discard lysed RBCs in supernatant, and re-suspend pellet in 1 ml ice-cold and sterile Miltenyi buffer.
7) Centrifuge at 200 x g for 5 minutes.
8) This procedure is completed in a laminar flow hood. Discard supernatant and re-suspend pellet in 1 ml ice-cold and sterile Miltenyi buffer.
9) Remove 10 µl of PBS cell suspension and add 10 µl tryan blue. Count remainingnon-parenchymal cells using hemocytometer.

3. CD45 hematopoietic cell depletion from non-parenchymal fraction

Work in laminar flow hood, keep cells cold, and use solutions cooled to 4°C.

1) Suspend cells in 100 µL of Miltenyi buffer per 107 cells up to 108 total cells.
2) Apply 20 µL Miltenyi CD45 microbead antibody for each 107 cells and incubate at 4°C for 15 minutes.
3) Add additional 2 ml Miltenyi buffer and centrifuge at 200 x g for 5 minutes. Remove supernatant. Re-suspend cell pellet (up to 108 total cells) in 1 ml Miltenyi buffer.
4) In laminar flow hood, filter cells using Miltenyi LD magnetic column. Start by placing column in magnetic holder (Miltenyi MidiMACS or QuadroMACS). Place sterile 5 ml tube below filter to catch filtrate. Prepare column by loading 2 ml Miltenyi buffer.
5) Once pre-filter wash is complete, load cells onto LD column. Once the cell suspension is within the column, add 1 ml Miltenyi buffer and repeat 1 ml Miltenyi buffer wash 2 additional times. Do NOT use plunger provided with column to increase speed of filtration. ONLY collect filtrate when the filter in placed in the magnetic holder.
6) Centrifuge the collected filtrate of approximately 5 ml (the column holds the remaining 1 ml) of CD45-depleted non-parenchymal cells at 200 x g for 5 minutes. Discard the column with the retained CD45 positive cells.

4. Flow cytometry isolation of CD133 positive cells

1) Prepare oval cell media. Use 1:1 DMEM:F12 medium with 10 % heat inactivated fetal calf serum as base, and add insulin (1 µg/ml), HEPES (5 mol/L), and Penicillin/Streptomycin (1% volume/volume). Filter solution using vacuum filter unit with 0.45 micron filter.
2) Re-suspend cells in Miltenyi buffer at 100 µL per 10^7 cells. Add 2 µL of CD133-PE conjugated antibody. Using a second group of cells, incubate with IgG-PE conjugated antibody as a control. Retain a third group of cells without staining as an unstained control for FACS.
3) Incubate at 4°C for 15 minutes in the dark. Re-suspend in 2 ml staining buffer. Centrifuge at 200 x g for 5 minutes. Discard supernatant and re-suspend pellet in 1 ml Miltenyi buffer.
4) This step is conducted using standard flow cytometry cell sorting procedures, which may be institution specific. Using unstained cells and IgG PE stained cells, adjust sorting parameters for optimized gating of CD133+ cell population. PE (R-Phytoerythrin) can generally be used with any flow cytometer that has a laser that emits at 488 nm. The peak emission for PE is 575 nm and is detected in the FL-2 channel. Note: Using a BD FACS Calibur or BD FACS Vantage machines, with the Cell Quest program for data collection, we use Forward Scatter and Side Scatter view in the log scale to identify cell populations, with side scatter set to 250. FL1 and FL2 are both in log scale and set to 550. These parameters provide an initial starting place to view liver non-parenchymal

cells, and are adjusted as needed based on staining intensity of positive and negative populations.

5) Isolate the CD133+ cell population using CD133+ gate and collect the cells in sterile filtered cell media.

5. Cell culture methods

1) Centrifuge FACS collected cells at 200 x g for 5 minutes. Re-suspend cell pellet in oval cell media with approximately 5000 cells per ml. May start with higher concentrations, up to 50,000 cells/ml for initial experiments, and reduce as technique improves and overall cell viability and yield improves.
2) Plate cells onto BD Biocoat Laminin coated 96 well plates using 1000 cells/cm^2. Place in humidified cell culture incubator at 37°C, with 5% CO_2. After 24 hours, add Hepatocyte Growth Factor (50 ng/nl) and Epidermal Growth Factor (20 ng/ml).
3) For single cells, isolate cells directly into 50 µl of oval cell media in each well of a 96 well Laminin coated plate. Use single cell FACS settings for strict selection of one positive cell only. After 24 hours, add 50 µl of oval cell media with HGF and EGF as above in step 5.2. Change media fully after 5-7 days.
4) Once the expanding colonies are greater than 50% confluent, which typically occurs after 2 weeks, depending on total number of cells plated and cell viability, the cells may be split 1:3 as below.
5) Split cells using Trypsin 0.05%-EDTA. Apply just enough to cover well bottom, 50-100 µL/well on 96 well plate Place in incubator at 37°C for 3-5 minutes.
6) Add 100 µL of media to each well and transfer all liquid to 5 ml tube. Add 1 mL media to each tube and centrifuge at 200 x g for 5 minutes.
7) Re-suspend cells in media plate in laminin coated dish, using cells from 1 well to plate into 3 new wells (1:3 ratio).

6. Confirmation of bi-potential status using RT-PCR

This procedure is detailed in the RNeasy protocol handbook, which is supplied with the RNeasy Kit.

1) Use RNeasy micro columns for 96 well plate colonies. Aspirate culture media from each well. Add 75 µl Buffer RLT (from RNeasy Kit) directly to each well. Scrape plate bottom with sterile rubber policeman. Pipettelysate into micro-centrifuge tube and vortex mixture for 1 minute. Add 70% ethanol to lysates and mix by pipetting.
2) Transfer the solution to RNeasy column placed in a 2 ml collection tube (as supplied in RNeasy Kit) and centrifuge in micro-centrifuge for 15 seconds at 10,000 rpm. Discard eluted filtrate.
3) Re-use the collection tube. Add 350 µL Buffer RW1 (RNeasy Kit) to the RNeasy column and centrifuge for 15 seconds at 10,000 rpm to wash the column membrane. Discard eluted wash.
4) Add 10 µL DNase I stock solution to 70 µL Buffer RDD (both supplied in RNeasy Kit).Add the 80 µL DNase I Buffer RDD incubation mix to the RNeasy column membrane and incubate for 15 minutes at room temperature.
5) Add 350 µL Buffer RW1 (RNeasy kit) to the RNeasy column and centrifuge for 15 seconds at 10,000 rpm to wash the membrane. Discard eluted wash and collection tube.
6) Place the RNeasy column in a new 2 ml collection tube (supplied in RNeasy kit). Add 500 µL Buffer RPE(RNeasy Kit) to the RNeasy column and centrifuge for 15 seconds at 10,000 rpm to wash the membrane. Discard eluted wash.
7) Prepare 80% ethanol using RNase-free water (RNeasy Kit). Add 500 µL of 80% ethanol to the RNeasy columnand centrifuge for 2 minutes at 10,000 rpm. Discard eluted wash.
8) Place the RNeasy column in a new 2 ml collection tube (RNeasy Kit) and centrifuge at full speed for 5 minutes. Discard any eluted wash and collection tube.
9) Place the RNeasy column in a new 1.5 ml collection tube (RNeasy kit). Add 14 µL RNase-free water (RNeasy Kit) to the center of the column membrane and centrifuge for 1 minute at full speed. Collect filtrate with purified RNA and transfer to ice if creating cDNA immediately or store at minus 80°C for future use.
10) Reverse transcription using Omniscript. Dilute RNase inhibitor to a final concentration of 10 units/ µL in ice-cold 1x Buffer RT Vortex for 5 seconds, and pulse centrifuge for 5 seconds. Prepare a fresh master mix on ice according to page 13 of Omniscript protocol. Vortex for 5 seconds, and pulse centrifuge for 5 seconds. Recommend to prepare a volume of mastermix 10% greater than that required for the total required for all reactions.

11) Add template RNA to the individual tubes containing master mix. Vortex for 5 seconds, and pulse centrifuge for 5 seconds. Incubate for 60 min at 37°C.
12) PCR amplification of hepatocyte (*Albumin*) and cholangiocyte specific genes using HotStarTaq DNA polymerase Prepare reaction mix per page 15 of HotStarTaq protocol book. Recommend diluting stock primers to concentration of 20 pm/µl, and with 1 µL/reaction tube used for forward and reverse primers. Depending on number of cells initially used for RNA extraction, we recommend dividing final cDNA among 3 reaction tubes (β-actin for loading control, *Albumin*, and KRT19) to start.
13) PCR primer design is listed in Table 1.
14) Place reaction tubes into thermocycler. Recommend following program for initial experiments: 95°Cfor 15 minutes x 1 cycle followed by 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds x 35 cycles, followed by 72°C for 10 minutes.
15) PCR products are analyzed using ethidium bromide impregnated agarose gel.

7. Confirmation of tumor potential of CD133+ stem cells

1) This procedure can be done with freshly isolated cells from step 4 or using cells that have been cultured from step 5. We recommend performing initial experiments with cultured cells, as freshly isolated cells will have reduced viability and reduced yield.
2) Trypsinize cells using Trypsin 0.05%-EDTA from step 5.5 above. After 3-5 minute incubation at 37°C, add 100 µL of media to each well and transfer all liquid from each well to individual 5 ml tubes (1 well = 1 tube). Add 1 ml media to each tube and centrifuge at 200 x g for 5 minutes.
3) Re-suspend cells in 1 ml ice cold PBS. Remove 10 µl of PBS cell suspension and add 10 µl trypan blue Using trypan blue exclusion, determine numbers of live cells. If using FACS isolated cells, cell number will be determined by FACS isolation count.
4) Centrifuge cells at 200 x g for 5 minutes and re-suspended in PBS at a concentration of 1 x 10^6 live cells/100 µl. Add 100 µl Matrigel. Six-week-old immune-deficient Nude mice were injected subcutaneously using 28 gague needle. Inject 1 x 10^6 cells in 200 µl per site.
5) Mice are monitored for tumor growth daily. Once tumors form, typically after 3-4 weeks incubation, tumor volume is measured using calipers (height x length x width).

8. Representative Results:

From normal, healthy murine liver, the expected cellular yield of CD133+liver stem cells is 1,000 to 5,000 per liver. These cells are relatively rare in quiescent liver and will not expand well in culture. We do not recommend using single cell analysis on normal liver and the yield of viable cells that will expand is extremely low.

For livers with significant chronic injury, such as the DDC 0.1% diet for 6 weeks[1] or genetic modification resulting in chronic injury, such as the MAT1a$^{-/-}$ or liver specific Pten$^{-/-}$ mice,[2-4]the expected number of cells isolated increases, with up to 100,000 cells isolated/liver (Figure 2). If using genetic models, knowledge of spontaneous tumor rate is critical, as these procedures for liver stem cell isolation should be conducted in tumor free animals. For example, if the MAT1a$^{-/-}$ model forms spontaneous tumors at 18 months, we recommend using animals no later than 15-16 months, prior to any reported spontaneous tumors.

Isolation of single cells from chronic injury models will yield several (3-9 colonies/96 well plate) colonies that expand from single cells once the procedure is mastered, and cell viability is ensured. Figure 3 present representative phase contrast images of colonies derived from single CD133+ cells expanded after 7 days.

Confirmation of bi-potential status is conducted using *Albumin* and Krt19 RT-PCR. Colonies from expanded single cells will demonstrate both expression for markers of hepatocytes (*Albumin*) and cholangiocytes (*Krt19*). Figure 4 demonstrates bi-potential expression from three isolated colonies, with approximately 25 cells/colony at 7 days.

CD133+ stem cells from normal liver and chemically induced liver injury (e.g. DDC 0.1% diet for 6 weeks) will not form tumors in nude mice. CD133+ stem cells from specific genetic models (MAT1a$^{-/-}$ or liver specific Pten$^{-/-}$ mice) will form tumors in nude mice if isolated late in late pre-tumor chronic injury phase. This tumor forming phenotype is currently identified as a cancer stem cell.[2-4]For example, the MAT1a$^{-/-}$ mice form spontaneous liver tumors at 18 weeks of age. CD133+ liver stem cells isolated at 15-16 weeks, during a late chronic injury phase of liver disease, will form tumors in nude mice. Figure 5 demonstrates representative tumors from bilateral

injection of 1 x 10^6 cells expanded in vitro from single CD133+ liver stem cells.

The following are recent reports in the liver stem cell studies:

Atsafack, S. S., J. R. Kuiate, et al. "Toxicological studies of stem bark extract from Schefflera barteri Harms (Araliaceae)." BMC Complement Altern Med **15**(1): 581.

The use of herbal medicines as complements or alternatives to orthodox medicines has been on the increase. There has been the erroneous belief that these medicines are free from adverse effects. Schefflera barteri is popularly used in the West region of Cameroon for the treatment of various diseases such as diarrhea, spasm, pneumonia and animals bite. Considering the ethnopharmacological relevance of this plant, this study was designed to investigate the possible toxic effects of the stem bark extract of S. barteri. Phytochemical analysis of stem bark extract of S. barteri revealed the presence of anthocyanins, anthraquinons and saponins. Acute toxicity results showed that the LD50 was greater than 16000 mg/kg. Sub-acute treatment significantly ($P < 0.05$) increased the level of serum transaminase, proteins and HDL cholesterol. On the other hand, the extract significantly ($P < 0.05$) reduced the level of leucocytes as well as neutrophils, basophils and monocytes in female. No significant variation of serum creatinine, LDL cholesterol, serum triglycerides as well as liver, spleen, testicles and ovaries proteins was noted. Histopathological analysis of organs showed vascular congestion, inflammation of peri-portal and vacuolization of hepatocytes at the level of the liver. Leucocytes infiltration of peri-portal veins were noticed on lungs and liver cells as well as inflammatory peri-bronchial and basal membranes seminar tube merely joined on lungs and testis respectively. The results suggest that acute administration of the stem bark extract of S. barteri is associated with signs of toxicity, administration over a long duration provokes hepatotoxicity, testes and lungs toxicities.

Bandow, K., J. Kusuyama, et al. "AMP-activated protein kinase (AMPK) activity negatively regulates chondrogenic differentiation." Bone **74**: 125-33.

Chondrocytes are derived from mesenchymal stem cells, and play an important role in cartilage formation. Sex determining region Y box (Sox) family transcription factors are essential for chondrogenic differentiation, whereas the intracellular signal pathways of Sox activation have not been clearly elucidated. AMP-activated protein kinase (AMPK) is a serine-threonine kinase generally regarded as a key regulator of cellular energy homeostasis. It is known that the catalytic alpha subunit of AMPK is activated by upstream AMPK kinases (AMPKKs) including liver kinase B1 (LKB1). We have previously reported that AMPK is a negative regulator of osteoblastic differentiation. Here, we have explored the role of AMPK in chondrogenic differentiation using in vitro culture models. The phosphorylation level of the catalytic AMPK alpha subunit significantly decreased during chondrogenic differentiation of primary chondrocyte precursors as well as ATDC-5, a well-characterized chondrogenic cell line. Treatment with metformin, an activator of AMPK, significantly reduced cartilage matrix formation and inhibited gene expression of sox6, sox9, col2a1 and aggrecan core protein (acp). Thus, chondrocyte differentiation is functionally associated with decreased AMPK activity.

Berger, D. R., B. R. Ware, et al. "Enhancing the functional maturity of induced pluripotent stem cell-derived human hepatocytes by controlled presentation of cell-cell interactions in vitro." Hepatology **61**(4): 1370-81.

Induced pluripotent stem cell-derived human hepatocyte-like cells (iHeps) could provide a powerful tool for studying the mechanisms underlying human liver development and disease, testing the efficacy and safety of pharmaceuticals across different patients (i.e., personalized medicine), and enabling cell-based therapies in the clinic. However, current in vitro protocols that rely upon growth factors and extracellular matrices (ECMs) alone yield iHeps with low levels of liver functions relative to adult primary human hepatocytes (PHHs). Moreover, these low hepatic functions in iHeps are difficult to maintain for prolonged times (weeks to months) in culture. Here, we engineered a micropatterned coculture (iMPCC) platform in a multiwell format that, in contrast to conventional confluent cultures, significantly enhanced the functional maturation and longevity of iHeps in culture for at least 4 weeks in vitro when benchmarked against multiple donors of PHHs. In particular, iHeps were micropatterned onto collagen-coated domains of empirically optimized dimensions, surrounded by 3T3-J2 murine embryonic fibroblasts, and then sandwiched with a thin layer of ECM gel (Matrigel). We assessed iHep

maturity by global gene expression profiles, hepatic polarity, secretion of albumin and urea, basal cytochrome P450 (CYP450) activities, phase II conjugation, drug-mediated CYP450 induction, and drug-induced hepatotoxicity. Controlling both homotypic interactions between iHeps and heterotypic interactions with stromal fibroblasts significantly matures iHep functions and maintains them for several weeks in culture. In the future, iMPCCs could prove useful for drug screening, studying molecular mechanisms underlying iHep differentiation, modeling liver diseases, and integration into human-on-a-chip systems being designed to assess multiorgan responses to compounds.

Cantz, T., A. D. Sharma, et al. "Concise review: cell therapies for hereditary metabolic liver diseases-concepts, clinical results, and future developments." Stem Cells **33**(4): 1055-62.

The concept of cell-based therapies for inherited metabolic liver diseases has been introduced for now more than 40 years in animal experiments, but controlled clinical data in humans are still not available. In the era of dynamic developments in stem cell science, the "right" cell for transplantation is considered as an important key for successful treatment.

Chung, M. M., Y. L. Chen, et al. "The neuroprotective role of metformin in advanced glycation end product treated human neural stem cells is AMPK-dependent." Biochim Biophys Acta **1852**(5): 720-31.

Diabetic neuronal damage results from hyperglycemia followed by increased formation of advanced glycosylation end products (AGEs), which leads to neurodegeneration, although the molecular mechanisms are still not well understood. Metformin, one of the most widely used anti-diabetic drugs, exerts its effects in part by activation of AMP-activated protein kinase (AMPK). AMPK is a critical evolutionarily conserved enzyme expressed in the liver, skeletal muscle and brain, and promotes cellular energy homeostasis and biogenesis by regulating several metabolic processes.

Hamidieh, A. A., F. Moeininia, et al. "Efficacy of hepatic T2* MRI values and serum ferritin concentration in predicting thalassemia major classification for hematopoietic stem cell transplantation." Pediatr Transplant **19**(3): 301-6.

Liver biopsy has been performed for many decades for classifying the patients with TM. Meanwhile, using non-invasive methods such as T2* MRI technique has been recently much more considered to determine the hepatic iron overload. Ninety-three pediatric HSCT candidates with TM who underwent liver biopsy were included in this study. Hepatic T2* MRI values and serum ferritin concentrations were assessed to investigate and determine the useful method in detection of patients with TM class III whom received different conditioning regimens, in comparison with class I and II. Twenty (21.5%) patients were categorized as class III. Hepatic T2* MRI could detect TM class III patients with 60% sensitivity and 87.67% specificity (LR+: 4.867, accuracy: 81.72%), while predictive feature of ferritin values for distinguishing patients with TM class III was not statistically significant (p-value >0.01). Combination of T2*MRI with age (T2*-age) could detect TM class III with 85% sensitivity and 72.6% specificity (LR+: 3.1, accuracy: 75.27%).T2*-age may be considered as an alternative and non-invasive method to liver biopsy for differentiation and classification of patients with TM before transplantation.

Hayakawa, Y., G. Jin, et al. "CCK2R identifies and regulates gastric antral stem cell states and carcinogenesis." Gut **64**(4): 544-53.

Progastrin is the incompletely cleaved precursor of gastrin that is secreted by G-cells in the gastric antrum. Both gastrin and progastrin bind to the CCK2 receptor (Cckbr or CCK2R) expressed on a subset of gastric epithelial cells. Little is known about how gastrin peptides and CCK2R regulate gastric stem cells and carcinogenesis. Interconversion among progenitors in the intestine is documented, but the mechanisms by which this occurs are poorly defined. DESIGN: We generated CCK2R-CreERT mice and performed inducible lineage tracing experiments. CCK2R+ antral cells and Lgr5+ antral stem cells were cultured in a three-dimensional in vitro system.

Li, J., Y. Yu, et al. "Establishment of a novel system for the culture and expansion of hepatic stem-like cancer cells." Cancer Lett **360**(2): 177-86.

Hepatocellular carcinoma (HCC) is a major primary liver malignancy in adults. Despite the progress made, the outcome of the treatment to this disease is less than satisfactory as the post therapy tumor recurrence is almost inevitable. Accumulating pieces of evidence have suggested that the recurrence is due to the existence of a subpopulation of the HCC cells that possess the

properties of stem cells and are resistant to radiation and chemotherapy.

Liu, F., X. Kong, et al. "TGF-beta1 acts through miR-155 to down-regulate TP53INP1 in promoting epithelial-mesenchymal transition and cancer stem cell phenotypes." Cancer Lett **359**(2): 288-98.

It has been shown that acquisition of epithelial-mesenchymal transition (EMT) and induction of cancer stem cell (CSC)-like properties contribute to metastasis of cancers in many studies; however, the molecular mechanisms underlying EMT and CSC phenotypes in liver cancer cells remain to be elucidated. MiR-155 is an important microRNA associated with tumour progression. Here, we report that miR-155 regulates not only the epithelial-mesenchymal transition but also the stem-like transition in liver cancer cells. Utilizing quantitative RT-PCR, we found that the expression of miR-155 is positively related to the levels of CD90, CD133 and Oct4 in enriched spheres.

Ludtke, A., L. Oestereich, et al. "Ebola virus disease in mice with transplanted human hematopoietic stem cells." J Virol **89**(8): 4700-4.

The development of treatments for Ebola virus disease (EVD) has been hampered by the lack of small-animal models that mimick human disease. Here we show that mice with transplanted human hematopoetic stem cells reproduce features typical of EVD. Infection with Ebola virus was associated with viremia, cell damage, liver steatosis, signs of hemorrhage, and high lethality. Our study provides a small-animal model with human components for the development of EVD therapies.

Makela, T., R. Takalo, et al. "Safety and biodistribution study of bone marrow-derived mesenchymal stromal cells and mononuclear cells and the impact of the administration route in an intact porcine model." Cytotherapy **17**(4): 392-402.

Bone marrow mononuclear cells (BM-MNCs) and bone marrow-derived mesenchymal stem stromal cells (BM-MSCs) could have therapeutic potential for numerous conditions, including ischemia-related injury. Cells transplanted intravascularly may become entrapped in the lungs, which potentially decreases their therapeutic effect and increases the risk for embolism. METHODS: Twelve pigs were divided into groups of 3 and received (99m)Tc- hydroxymethyl-propylene-amine-oxime-labeled autologous BM-MNCs or allogeneic BM-MSCs by either intravenous (IV) or intra-arterial (IA) transplantation. A whole body scan and single photon emission computed tomography/computed tomography (SPECT/CT) were performed 8 h later, and tissue biopsies were collected for gamma counting.

Ohshima, M., A. Taguchi, et al. "Intraperitoneal and intravenous deliveries are not comparable in terms of drug efficacy and cell distribution in neonatal mice with hypoxia-ischemia." Brain Dev **37**(4): 376-86.

Most therapeutic agents are administered intravenously (IV) in clinical settings and intraperitoneally (IP) in preclinical studies with neonatal rodents; however, it remains unclear whether intraperitoneal (IP) injection is truly an acceptable alternative for intravenous (IV) injection in preclinical studies. The objective of our study is to clarify the differences in the therapeutic effects of drugs and in the distribution of infused cells after an IP or IV injection in animals with brain injury. IP and IV administration of dexamethasone attenuated the brain injury to a similar degree. IP administration of MK-801 attenuated brain injury, whereas IV administration of MK-801 did not. The IV group showed a significantly greater number of infused cells in the lungs and brains in the MSC cohort and in the spleen, liver, and lung in the MNC cohort compared to the IP group. In the macaque, MNCs were detected in the spleen and liver in large amounts, but not in the brain and lungs. This study demonstrated that the administration route influences the effects of drugs and cell distribution. Therefore, a preclinical study may need to be performed using the optimal administration route used in a clinical setting.

Sabetkish, S., A. M. Kajbafzadeh, et al. "Whole-organ tissue engineering: Decellularization and recellularization of three-dimensional matrix liver scaffolds." J Biomed Mater Res A **103**(4): 1498-508.

To report the results of whole liver decellularization by two different methods. To present the results of grafting rat and sheep decellularized liver matrix (DLM) into the normal rat liver and compare natural cell seeding process in homo/xenograft of DLM. To compare the results of in vitro whole liver recellularization with rats' neonatal green fluorescent protein (GFP)-positive hepatic cells with outcomes of in vivo recellularization process. Whole liver of 8

rats and 4 sheep were resected and cannulated via the hepatic vein and perfused with sodium dodecyl sulfate (SDS) or Triton + SDS. Several examinations were performed to compare the efficacy of these two decellularization procedures. In vivo recellularization of sheep and rat DLMs was performed following transplantation of multiple pieces of both scaffolds in the subhepatic area of four rats.

Schmidt, F., N. Hilger, et al. "Flow cytometric analysis of the graft-versus-Leukemia-Effect After Hematopoietic Stem Cell Transplantation in Mice." Cytometry A **87**(4): 334-45.

Acute Graft-versus-Host-Disease (aGvHD) is one of the major complications following allogeneic hematopoietic stem cell transplantation (HSCT). Although rather helpful, the use of conventional immunosuppressive drugs leads to general immunosuppression and is toxic. The effects of CD4(+) T-cells, in respect to the development of aGvHD, can be altered by administration of antihuman CD4 monoclonal antibodies, here MAX.16H5 IgG1 . This approach must be tested for possible interference with the Graft-versus-Leukemia-Effect (GvL). Thus, in vitro experiments were conducted, exposing P815 leukemic cells to bone marrow and splenocytes from cd4(-/-) -C57Bl/6 mice transgenic for human CD4 and HLA-DR3 (triple transgenic mice, [TTG]) as well as previously irradiated splenocytes from Balb/c(wt) mice. Using flow cytometry, the vitality of the various malignant and graft cells was analyzed over the course of 4 days. The survival rate of P815 cells did not change significantly when exposed to MAX.16H5 IgG1 , neither did the viability of the graft cells.

Soga, M., Y. Ishitsuka, et al. "HPGCD Outperforms HPBCD as a Potential Treatment for Niemann-Pick Disease Type C During Disease Modeling with iPS Cells." Stem Cells **33**(4): 1075-88.

Niemann-Pick disease type C (NPC) is a lysosomal storage disease characterized by abnormal accumulation of free cholesterol and glycolipids. Here, we established induced pluripotent stem cell (iPSC) lines from NPC patients. Hepatocyte-like cells (HLCs) and neural progenitors derived from the iPSC lines accumulated cholesterol and displayed impaired autophagy and ATP production. A molecular signature related to lipid metabolism was also impaired in the NPC-iPSC-derived HLCs. These findings indicate that iPSC-derived cells can phenocopy human NPC. We also newly found that 2-hydroxypropyl-gamma-cyclodextrin (HPGCD) could reduce the cholesterol accumulation and restore the functional and molecular abnormalities in the NPC patient-derived cells, and do so more effectively than 2-hydroxypropyl-beta-cyclodextrin treatment. In addition, NPC model mice showed an improved liver status and prolonged survival with HPGCDs. Thus, iPSC lines derived from patient cells are powerful tools to study cellular models of NPC, and HPGCD is a potential new drug candidate for future treatment of this disease.

Song, Y. M., C. H. Lian, et al. "Effects of bone marrow-derived mesenchymal stem cells transplanted via the portal vein or tail vein on liver injury in rats with liver cirrhosis." Exp Ther Med **9**(4): 1292-1298.

The aim of the present study was to compare the effects of bone marrow-derived mesenchymal stem cells (BMSCs) transplanted via the portal vein or tail vein on liver injury in rats with liver cirrhosis. BMSCs were isolated from rat bone marrow and labeled with green fluorescent protein (GFP). Then, the labeled BMSCs were injected into rats with liver injury via the portal vein or tail vein.

Ye, J. S., X. S. Su, et al. "Signalling pathways involved in the process of mesenchymal stem cells differentiating into hepatocytes." Cell Prolif **48**(2): 157-65.

End-stage liver disease can be the termination of acute or chronic liver diseases, with manifestations of liver failure; transplantation is currently an effective treatment for these. However, transplantation is severely limited due to the serious lack of donors, expense, graft rejection and requirement of long-term immunosuppression. Mesenchymal stem cells (MSCs) have attracted considerable attention as therapeutic tools as they can be obtained with relative ease and expanded in culture, along with features of self-renewal and multidirectional differentiation. Many scientific groups have sought to use MSCs differentiating into functional hepatocytes to be used in cell transplantation with liver tissue engineering to repair diseased organs. In most of the literature, hepatocyte differentiation refers to use of various additional growth factors and cytokines, such as hepatocyte growth factor (HGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), oncostatin M (OSM) and more, and most are involved in signalling pathway regulation and

cell-cell/cell-matrix interactions. Signalling pathways have been shown to play critical roles in embryonic development, tumourigenesis, tumour progression, apoptosis and cell-fate determination. However, mechanisms of MSCs differentiating into hepatocytes, particularly signalling pathways involved, have not as yet been completely illustrated. In this review, we have focused on progress of signalling pathways associated with mesenchymal stem cells differentiating into hepatocytes along with the stepwise differentiation procedure.

Zhao, Q. W., Y. W. Zhou, et al. "Aktmediated phosphorylation of Oct4 is associated with the proliferation of stemlike cancer cells." Oncol Rep **33**(4): 1621-9.

Oct4 protein encoded by POU5F1 plays a pivotal role in maintaining the selfrenewal of pluripotent stem cells; however, its presence in cancer cells remains controversial. In the present study, we provided evidence that the transcripts of authentic OCT4 gene (OCT4A) and its multiple pseudogenes were detected in a variety of cancer cell lines. A few major bands were also detected by western blotting using an antiOct4A monoclonal antibody. Moreover, an antiOct4pT235 antibody was used to identify a band in the majority of the tested cancer cell lines that coincided with one of the antiOct4A bands which was decreasable by a specific shRNA. The Oct4pT235 signals were also detected in human glioblastoma and liver cancer specimens by immunofluorescence microscopy and immunohistochemistry. U87 glioblastoma cells were cultured in a neural stem cell medium to induce the formation of neurospheres rich in stemlike cancer cells. The levels of Oct4pT235 in the sphere cells were markedly increased compared to their monolayer parental cells, a result that was accompanied by upregulation of the PI3KAkt pathway. Akti1/2, a specific inhibitor of Akt, effectively reduced the level of Oct4pT235 and attenuated the proliferation of U87 sphere cells. ITE, an agonist of the aryl hydrocarbon receptor, also significantly attenuated the Aktmediated phosphorylation of Oct4 in glioblastoma and liver cancer cells, and reduced their tumorigenic potential in a xenograft tumor model. Taken together, we concluded that the Aktmediated phosphorylation of Oct4A or its homolog protein was associated with the proliferation of stemlike cancer cells that may serve as a novel biomarker and drug target for certain types of cancer.

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