We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 7

Hepatocyte Selection Medium

Minoru Tomizawa, Fuminobu Shinozaki,
Yasufumi Motoyoshi, Takao Sugiyama,
Shigenori Yamamoto and Makoto Sueishi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/58394

1. Introduction

Embryonic stem (ES) cells have the potential to differentiate to hepatocytes [1]. However, the use of ES cells may pose ethical problems because they are derived from human embryos. The use of human induced pluripotent stem (hiPS) cells that have been generated from adult somatic cells [2], on the other hand, does not create ethical controversies. HiPS cells are useful tools in drug discovery and regenerative medicine because they can differentiate into functional somatic cells [3]. If hiPS cells could be differentiated into hepatocytes, they would be useful for transplantation into patients suffering from hepatic failure [4]. Complications such as graft-versus-host disease as well as ethical issues could be avoided because patient-specific somatic cells could be generated from hiPS cells isolated from the patient.

The ES and hiPS cells that survive among the differentiated hepatocytes and are transplanted to patients may be tumorigenic [5]. Therefore, methods need to be developed to eliminate ES and iPS cells from the population of differentiated cells used for transplantation. To overcome these problems, a new medium, called "hepatocyte selection medium" (HSM), has been developed and will be discussed in this chapter [6].

First, pluripotency and tumorigenicity of ES and iPS cells will be discussed [7]. Next, current methods of eliminating pluripotent cells will be outlined [8, 9]. All the cells, including human iPS cells, require glucose and arginine to live [10, 11]. They will die without glucose or arginine. Hepatocytes have enzymes to produce glucose from galactose and arginine from ornithine. The unique features of hepatocytes compared with other cells will next be discussed. It was expected that hepatocytes would survive in a medium without glucose or arginine, and supplemented with galactose and ornithine [12] [13]. After this introduction, the formulation

of HSM will be described [14]. Finally, the application of HSM for the selection of cells differentiated from mouse ES and human iPS cells will be presented.

2. Pluripotency and tumorigenicity

The link between pluripotency and tumorigenicity was reported in 1960 based on a study of teratocarcinoma [15]. ES and iPS cells are pluripotent and are capable of self-renewal as well as differentiation into a variety of cell types. Pluripotent cells can, however, be tumorigenic because they proliferate rapidly and exhibit telomerase activity [7]. Therefore, one of the problems faced while using ES and iPS cell-derived cells for transplantation into patients is the risk of tumorigenicity [5]. For example, transplantation of mouse hepatocytes differentiated from ES cells into liver resulted in the formation of teratoma [16]. Tumorigenicity was initially attributed to genomic integration of the viral vectors used for the induction of pluripotency [17]. The Sendai virus was also used to generate iPS cells because it posed no risk of altering the host genome [18]. To reduce this risk, plasmid vectors have been used to introduce reprogramming factors such as Oct3/4, Sox2, Klf4, and c-Myc [19]. In addition, the ES cell-specific microRNA, miR-302, has been used to reduce the iPS cells tumorigenicity by suppressing cyclin E-CDK2 and cyclin D-CDK4/6 [20]. Furthermore, Yakubov et al. introduced RNA synthesized from the cDNA of the four reprogramming transcription factors [21]. Several combinations of reprogramming factors have also been investigated. Nakagawa et al. omitted c-Myc to generate iPS cells, thereby reducing the tumorigenicity because c-Myc is a well-known oncogene [22]. Despite these efforts, the risk of tumorigenicity has not yet been eliminated. The process of pluripotency and tumorigenicity involve self-renewal, proliferation, and active telomerase mechanisms [7]. It is, therefore, necessary to develop methods for the efficient eradication of iPS cells that survive among differentiated somatic cells.

3. Methods of eliminating iPS cells

Flow cytometry, which is commonly used to isolate target cells, was used by Yamamoto et al. to isolate hepatocytes differentiated from the mouse ES cells [8]. These workers generated ES cells expressing green fluorescent protein driven by an albumin promoter/enhancer. However, since albumin is expressed in endodermal cells as well [23], this approach led to isolation of cells other than hepatocytes, such as endodermal cells. Therefore, a different strategy was required to improve hepatocyte isolation. Flow cytometry has also been used to analyze surface antigens specific for hepatocytes. For example, delta-like 1 homolog (DLK1) has been used for isolation of hepatoblasts [9]. The issue with DLK1, however, is that this surface antigen is not expressed in the human adult liver [24]. Therefore, it may not be possible to isolate mature hepatocytes differentiated from hiPS cells using DLK-1 as a marker. In our research we focused on other methods to eliminate iPS or ES cells from heptocytes. Sub-lethal heat shock was shown to induce apoptosis in human ES cells [25], but it might also damage differentiated cells intended for transplantation. Cheng et al. reported the same strategy using suicide genes [26]. They introduced a thymidine kinase gene driven by the Nanog promoter into hiPS cells, which

http://dx.doi.org/10.5772/58394

were subsequently ablated by ganciclovir treatment. This method may be ideal for differentiated hepatocytes, which do not express Nanog, but the toxicity of ganciclovir may be a potential issue. Conesa et al. screened a library of 1120 small chemicals to identify molecules that caused mouse ES cells to undergo apoptosis [27], and found that benzethonium chloride and methylbenzethonium induced apoptosis in hiPS and mouse ES cells but not in human fibroblasts or mouse embryonic fibroblasts. Both reagents are quaternary ammonium salts used as antimicrobial agents; they are also used in cancer therapy and may have damaging effects on hepatocytes. *N*-oleoyl serinol (S18), which is a ceramide analogue, eliminated residual pluripotent cells in embryoid bodies [28]. Interestingly, S18 also promoted neural differentiation of embryoid body-derived cells. This strategy is promising because the reagent not only eradicates undifferentiated cells but also promotes their differentiation into the target cell types.

4. Arginine and urea cycle

Among all the amino acids, the deficiency in arginine is the least tolerated by the cells cultured *in vitro* [29]. Arginine is produced through the urea cycle, which is exclusive to hepatocytes. Indeed, an arginine-deficient medium was the first one used for the hepatocyte selection [10]. Tyrosine also is produced by hepatocytes, and H4 II E, a hepatoma cell line adapted to growth in serum-, arginine-, and tyrosine-free medium, has been established [30]. This cell line expresses ornithine transcarbamylase (OTC) involved in the urea cycle, and phenylalanine hydroxylase (PAH), which catalyzes the synthesis of tyrosine in the liver and kidney [31].

The major role of urea synthesis is the excretion of ammonium ions generated in the process of protein degradation. Urea synthesis is a cyclic process as shown in Figure 1. Ornithine plays the key role in urea synthesis, and OTC mediates the formation of L-citrulline from L-ornithine and carbamoylphosphate. Importantly, this process occurs in liver mitochondria (area bounded with green line in Figure 1). The OTC deficiency, linked to X-chromosome is the cause of hyperammonemia type 2 [32]. The elevated ammonium levels lead to infantile death or mental retardation later in life.

Consequently, it could be expected that hepatocytes can be selected from ES cells in a medium deficient in arginine and tyrosine.

5. Glucose and gluconeogenesis

Glucose is an important source of energy for a majority of cells. Glucose deprivation aids in the hepatocyte selection process because hepatocytes are capable of synthesizing glucose [10]. Pyruvate is the final product of glycolysis, which then enters the tricarboxylic acid cycle. It was shown that pyruvate and glucose deficiency led to neural cell death [11]. Galactose enters glycolysis as a substrate for galactokinase, which is expressed in the liver and kidney [33, 34]. Therefore, it is expected that hepatocytes can survive in a medium deprived of glucose or pyruvate but supplemented with galactose [12] [13].

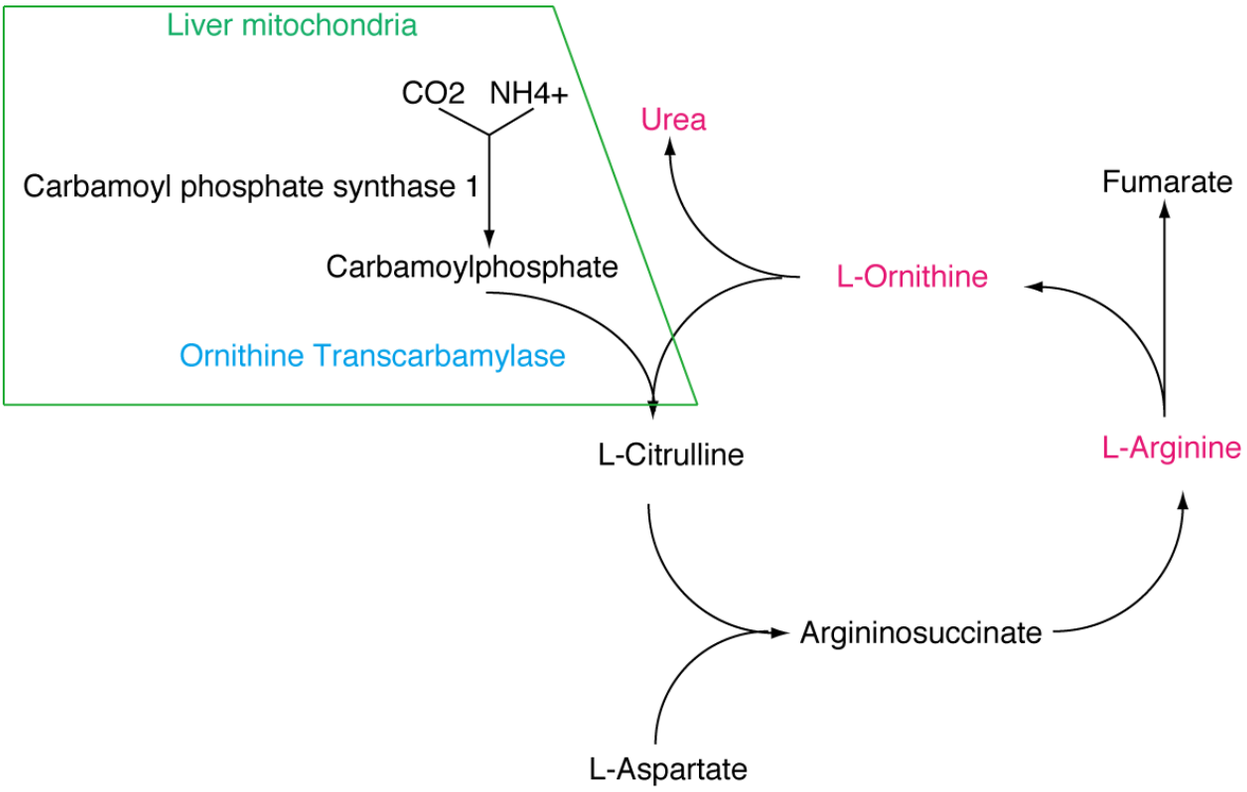

Figure 1. Urea cycle.

Galactose is produced from lactose by hydrolysis in the gastrointestinal tract and is converted to glucose in the liver (Figure 2). Galactokinase catalyzes ATP-dependent phosphorylation of galactose to galactose 1-phosphate which then reacts with uridine diphosphate(UDP)-glucose to produce UDP-galactose converted to UDP-glucose by uridine diposphogalactose 4-epimerase. UDP-glucose is used by glycogen synthase to synthesize glycogen, which is stored in the liver and used as a source of glucose.

Deficiency in the enzymes such as galactokinase, galactose-1-phosphate uridyltransferase, or uridine diphosphogalactose 4-epimerase causes galactosemia. Galactose is then reduced to galactitol, which accumulates in the eye lenses causing cataracts. Deficiency in galactose-1-phosphate uridyltransferase results in accumulation of galactose-1-phosphate and depletion of inorganic phosphate in the liver causing liver failure. This is the reason why children suffering from galactosemia are kept on a galactose-free diet.

6. Hepatocyte selection medium

The hepatocyte selection medium (HSM) was made from powdered amino acids following the formulation of Leibovits-15 medium (Life Technologies, Grand Island, NY). HSM did not contain arginine, tyrosine, glucose, and sodium pyruvate, but was supplemented with galactose (900 mg/L), ornithine (1 mM), glycerol (5 mM), and proline (260 mM) (all from Wako

http://dx.doi.org/10.5772/58394

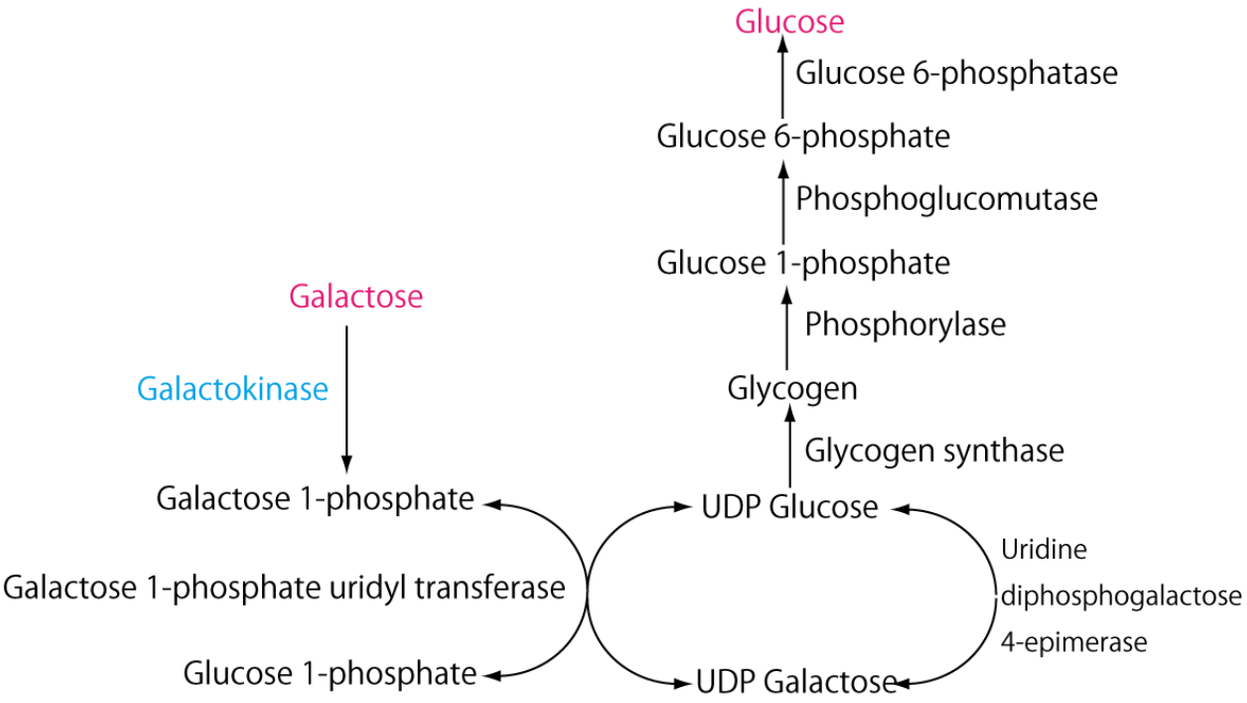

Figure 2. Galactose metabolism.

Pure Chemicals, Osaka, Japan); proline (30 mg/L) was added as a component necessary for DNA synthesis [35]. Aspartic acid as a nonessential amino acid was not included because it can be synthesized from ornithine and arginine. Fetal calf serum (FCS, Life Technologies) at a final concentration of 10% was used to culture mouse ES cells. For human iPS cells, 10% knockout serum replacement (KSR) (Life Technologies) was used instead of FCS to establish xeno-free conditions. Depending on the experiment, FCS and KSR were dialyzed against phosphate buffered saline (PBS) to remove amino acids and glucose.

7. Embryoid bodies in HSM

EB5, a mouse ES cell line provided by Dr. H. Niwa (Center for Developmental Biology, Riken, Kobe, Japan) was maintained in the undifferentiated state in gelatin-coated dishes without feeder cells, in Glasgow minimum essential medium (GMEM) (Sigma Aldrich Japan K.K., Tokyo, Japan) supplemented with 10% FCS (Roche Diagnostics K.K., Tokyo, Japan), 1× nonessential amino acids (NEAA), sodium pyruvate (1 mM), leukemia inhibitory factor (LIF) (1000 U/ml) (Invitrogen Japan, Tokyo, Japan), and 2-mercaptoethanol (0.1 mM) (Wako) [36]. Dissociated ES cells were cultured in hanging drops at a density of 1×10^3 cells per 30 μμl of media without LIF (ESM) to form embryoid bodies. After four days in hanging drop culture, the resulting embryoid bodies were plated onto plastic dishes (Iwaki-Asahi Techno Glass, Tokyo, Japan) precoated with gelatin (Sigma Aldrich). Seven days after their formation, the embryoid bodies transferred to HSM appeared slightly smaller than those in ESM. The cells

comprising the embryoid bodies in ESM differentiated to various cell types 28 days after the formation of embryoid bodies.

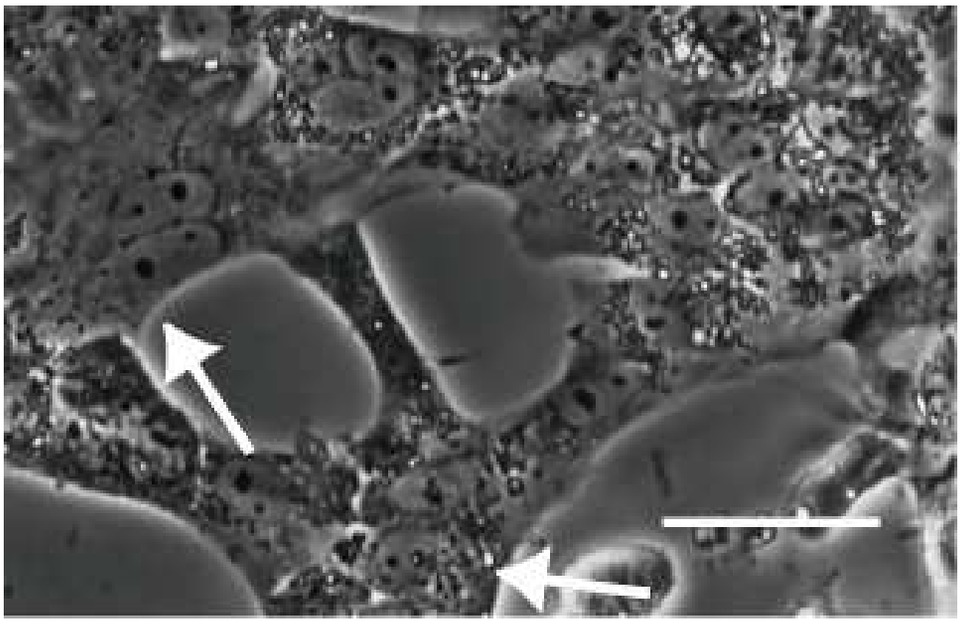

Figure 3. Mouse embryonic stem cells in HSM. Scale bar, 250 μm.

28 days after the formation of embryoid bodies, sizes of colonies in HSM reduced, and the surviving cells appeared cuboidal (Figure 3). Some of these cells were binuclear, which is characteristic of hepatocytes; it was also previously shown that HSM was selective for hepatoblast-like cells [14]. These results suggest that HSM eliminated undifferentiated cells and enriched the population of hepatoblast-like cells.

8. Expression levels of GALK1, GALK2, and OTC

Human fetal and adult hepatocytes express galactokinase and OTC, and would survive in HSM containing galactose and ornithine. If hiPS cells express similar levels of these enzymes, HSM could not be applied for selection of differentiated hepatocytes. Therefore, we compared the expression levels of galactokinase and OTC in hiPS cells with those in human fetal and adult livers. The hiPS cell line 201B7 (RIKEN Cell Bank, Tsukuba, Japan) was cultured feeder-free in ReproFF medium (Reprocell, Yokohama, Japan) in dishes coated with a thin layer of Matrigel (Becton Dickinson, Franklin Lakes, NJ). Two galactokinase isoforms, GALK1 (GenBank: NM_000154) and GALK2 (BC107153), have been identified in humans. The expression levels of GALK1, GALK2, and OTC in the 201B7 cells and fetal and adult livers were compared [6]. The expression levels of these enzymes in the 201B7 cells constituted 22.2% ± 5.0%, 14.2% ± 1.1%, and 1.2% ± 0.2% (mean ± standard deviation) of those in the adult liver, respectively, and the OTC expression was also significantly lower in the 201B7 cells than in the fetal liver. We then cultured 201B7 cells in HSM to assess their survival rates.

http://dx.doi.org/10.5772/58394

9. Human iPS cells in HSM

The 201B7 cells were cultured in 6-well plates coated with Matrigel in the ReproFF medium, which was then changed to HSM (Figure 4). The 201B7 cells started to die and were completely eliminated in three days. Nuclear condensation and fragmentation was observed after staining with hematoxylin and eosin [6]. These nuclei also tested positive by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL). Some of the 201B7 cells that survived in HSM one day after medium change to HSM were immunostained with antibodies against Nanog, SSEA4, and TRA-1-60. The results suggested that the death of undifferentiated 201B7 cells in HSM was caused by apoptosis.

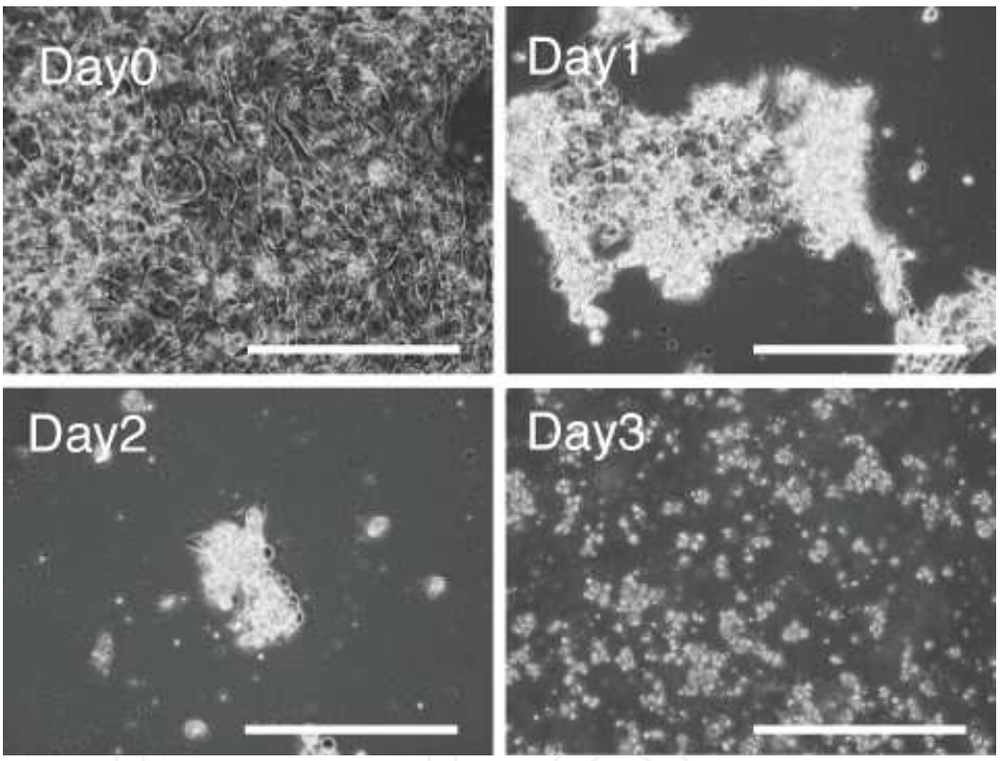

Figure 4. Human iPS cells cultured in HSM. Scale bar, 50 μm. Medium was changed to HSM for human iPS cells in feeder-free culture. All the human iPS cells died on day 3.

10. Primary human hepatocytes

Several protocols for the differentiation of iPS cells to hepatocytes have been reported [3, 37], which describe the differentiation of iPS cells into hepatocyte-like cells which are different from primary human hepatocytes. Recently, a method to generate three-dimensional vascularized liver from iPS cells has been reported [38]. The authors induced hepatic differentiation of human iPS cells by following the protocol described by Si-Tayeb et al [37]. They mixed the iPS cells with vascular endothelial and mesenchymal stem cells, and transplanted them into a

mouse brain. This method is sophisticated and promising, but xenograft rejection may be a problem when the generated liver is transplanted to patients with liver failure. Practical methods for the differentiation of human iPS cell to functional hepatocytes are not available. It is therefore necessary to use primary human hepatoctyes as a model of hepatocytes fully differentiated from iPS cells. Hepatocytes were isolated from a fragment of resected donor liver by using 2-step collagenase perfusion [39].

11. Co-culture of human iPS cells and primary human hepatocytes

Methods have not been established regarding hepatocye differentiation from human iPS cells. It is impossible to select hepatocytes differentiated from human iPS cells from the mixture of human iPS cells. Primary human hepatocytes were used as a model of hepatocytes differentiated from human iPS cells. It was expected that human iPS cells and hepatocytes differentiated from them were mixed. Therefore, co-culure of primary human hepatocytes and human iPS cells was used as a model of the mixtures. Primary human hepatocytes were purchased from Lonza (Walkersville, MD) and cultured as per the manufacturer's instructions. Briefly, hepatocytes were thawed and spread at a density of 1.5×10^5 cells/cm^2 onto CellBIND 24-well plates coated with type I collagen from the bovine dermis (Koken Co., Ltd., Tokyo, Japan) and cultured in the hepatocyte culture medium (HCM, Lonza).

The 201B7 cells and human primary hepatocytes were co-cultured as follows: human primary hepatoctyes were cultured in HCM for 24 h as described above. The 201B7 cells were added to the wells at a density of 3×10^4 cells/well. After 24 h of culture in the ReproFF medium, it was changed to HSM. Human primary hepatocytes survived in HSM, while the human 201B7 cells did not (Figure 5).

12. Potential application of HSM

The HSM that we developed can be safely used for the elimination of hiPS cells because it does not contain hazardous reagents or introduce genetic material. Our results show that hiPS cells die after three days of culture in HSM. Prior to performing the experiments, we compared the hiPS cell viability in media containing crude or dialyzed KSR or combination of insulin (10 μM), dexamethasone (10 μM) and aprotitin (5000 U/ml) (IDA) Unexpectedly, the KSR dialysis and IDA had no effect on hiPS cell survival. As expected, primary human hepatocytes survived in HSM as well as in HCM, which is the recommended medium for their culture.

HSM can be used in clinical practices in situations when hepatocytes differentiated from human iPS cells are transplanted to patients suffering from liver failure.

http://dx.doi.org/10.5772/58394

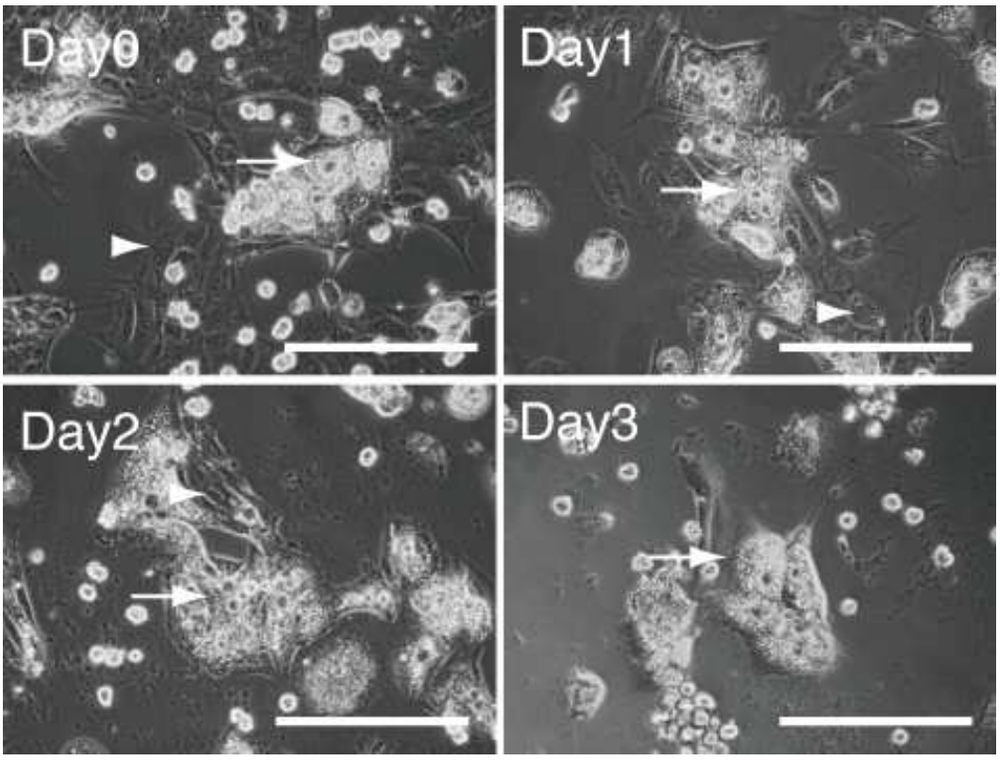

Figure 5. Human iPS cells co-cultured with primary human hepatocytes in HSM. Scale bar, 50 μm; arrow, hepatocytes; arrowhead, 201B7 cells.

13. Conclusion

HSM can be successfully used for the selection of hepatoblast-like cells derived from mouse ES cells. HSM is an ideal medium for the elimination of hiPS cells and the isolation of differentiated hepatocytes without causing any damage. In the future, methods will be established to produce hepatocytes from human iPS cells. Residual human iPS cells are a potential hazard when the hepatocytes will be transplanted for patients with liver insufficiency because the undifferentiated cell harbor tumorigenicity. At that stage, HSM will be an indispensable medium to select hepatocytes differentiated from residual human iPS cells.

Acknowledgements

This work was supported in part by a Research Grant-in-Aid for Scientific Research (C) (Grant No. 23591002) from the Japan Society for the Promotion of Science (JSPS).

Author details

Minoru Tomizawa[1*], Fuminobu Shinozaki[2], Yasufumi Motoyoshi[3], Takao Sugiyama[4], Shigenori Yamamoto[5] and Makoto Sueishi[4]

*Address all correspondence to: nihminor-cib@umin.ac.jp

1 Department of Gastroenterology, National Hospital Organization Shimoshizu Hospital, Yotsukaido City, Japan

2 Department of Radiology, National Hospital Organization Shimoshizu Hospital, Yotsukaido City, Japan

3 Department of Neurology, National Hospital Organization Shimoshizu Hospital, Yotsukaido City, Japan

4 Department of Rheumatology, National Hospital Organization Shimoshizu Hospital, Yotsukaido City, Japan

5 Department of Pediatrics, National Hospital Organization Shimoshizu Hospital, Yotsukaido City, Japan

References

[1] Greenhough S, Bradburn H, Gardner J, Hay DC. Development of an embryoid body-based screening strategy for assessing the hepatocyte differentiation potential of human embryonic stem cells following single-cell dissociation. Cell Reprogram 2013;15(1):9-14.

[2] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131(5):861-72.

[3] Takayama K, Inamura M, Kawabata K, Sugawara M, Kikuchi K, Higuchi M, Nagamoto Y, Watanabe H, Tashiro K, Sakurai F, Hayakawa T, Furue MK, Mizuguchi H. Generation of metabolically functioning hepatocytes from human pluripotent stem cells by FOXA2 and HNF1alpha transduction. J Hepatol 2012.

[4] am Esch JS, 2nd, Knoefel WT, Klein M, Ghodsizad A, Fuerst G, Poll LW, Piechaczek C, Burchardt ER, Feifel N, Stoldt V, Stockschlader M, Stoecklein N, Tustas RY, Eisenberger CF, Peiper M, Haussinger D, Hosch SB. Portal application of autologous CD133+bone marrow cells to the liver: a novel concept to support hepatic regeneration. Stem Cells 2005;23(4):463-70.

[5] Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007;448(7151):313-7.

[6] Tomizawa M, Shinozaki F, Sugiyama T, Yamamoto S, Sueishi M, Yoshida T. Survival of primary human hepatocytes and death of induced pluripotent stem cells in media lacking glucose and arginine. PLoS One 2013;8(8):e71897.

[7] Kooreman NG, Wu JC. Tumorigenicity of pluripotent stem cells: biological insights from molecular imaging. J R Soc Interface 2010;7 Suppl 6(S753-63.

[8] Yamamoto H, Quinn G, Asari A, Yamanokuchi H, Teratani T, Terada M, Ochiya T. Differentiation of embryonic stem cells into hepatocytes: biological functions and therapeutic application. Hepatology 2003;37(5):983-93.

[9] Tanaka M, Okabe M, Suzuki K, Kamiya Y, Tsukahara Y, Saito S, Miyajima A. Mouse hepatoblasts at distinct developmental stages are characterized by expression of EpCAM and DLK1: drastic change of EpCAM expression during liver development. Mech Dev 2009;126(8-9):665-76.

[10] Leffert HL, Paul D. Studies on primary cultures of differentiated fetal liver cells. J Cell Biol 1972;52(3):559-68.

[11] Matsumoto K, Yamada K, Kohmura E, Kinoshita A, Hayakawa T. Role of pyruvate in ischaemia-like conditions on cultured neurons. Neurol Res 1994;16(6):460-4.

[12] Phillips JW, Jones ME, Berry MN. Implications of the simultaneous occurrence of hepatic glycolysis from glucose and gluconeogenesis from glycerol. Eur J Biochem 2002;269(3):792-7.

[13] Sumida KD, Crandall SC, Chadha PL, Qureshi T. Hepatic gluconeogenic capacity from various precursors in young versus old rats. Metabolism 2002;51(7):876-80.

[14] Tomizawa M, Toyama Y, Ito C, Toshimori K, Iwase K, Takiguchi M, Saisho H, Yokosuka O. Hepatoblast-like cells enriched from mouse embryonic stem cells in medium without glucose, pyruvate, arginine, and tyrosine. Cell Tissue Res 2008;333(1):17-27.

[15] Pierce GB, Jr., Dixon FJ, Jr., Verney EL. Teratocarcinogenic and tissue-forming potentials of the cell types comprising neoplastic embryoid bodies. Lab Invest 1960;9(583-602.

[16] Teramoto K, Hara Y, Kumashiro Y, Chinzei R, Tanaka Y, Shimizu-Saito K, Asahina K, Teraoka H, Arii S. Teratoma formation and hepatocyte differentiation in mouse liver transplanted with mouse embryonic stem cell-derived embryoid bodies. Transplant Proc 2005;37(1):285-6.

[17] Miura K, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, Nakagawa M, Koyanagi M, Tanabe K, Ohnuki M, Ogawa D, Ikeda E, Okano H, Yamanaka S. Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol 2009;27(8):743-5.

[18] Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an

RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 2009;85(8):348-62.

[19] Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. Science 2008;322(5903):949-53.

[20] Lin SL, Ying SY. Mechanism and method for generating tumor-free iPS cells using intronic microRNA miR-302 induction. Methods Mol Biol 2013;936(295-312.

[21] Yakubov E, Rechavi G, Rozenblatt S, Givol D. Reprogramming of human fibroblasts to pluripotent stem cells using mRNA of four transcription factors. Biochem Biophys Res Commun 2010;394(1):189-93.

[22] Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, Yamanaka S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol 2008;26(1):101-6.

[23] Abe K, Niwa H, Iwase K, Takiguchi M, Mori M, Abe SI, Abe K, Yamamura KI. Endoderm-specific gene expression in embryonic stem cells differentiated to embryoid bodies. Exp Cell Res 1996;229(1):27-34.

[24] Yanai H, Nakamura K, Hijioka S, Kamei A, Ikari T, Ishikawa Y, Shinozaki E, Mizunuma N, Hatake K, Miyajima A. Dlk-1, a cell surface antigen on foetal hepatic stem/progenitor cells, is expressed in hepatocellular, colon, pancreas and breast carcinomas at a high frequency. J Biochem 2010;148(1):85-92.

[25] Alekseenko LL, Zemelko VI, Zenin VV, Pugovkina NA, Kozhukharova IV, Kovaleva ZV, Grinchuk TM, Fridlyanskaya, II, Nikolsky NN. Heat shock induces apoptosis in human embryonic stem cells but a premature senescence phenotype in their differentiated progeny. Cell Cycle 2012;11(17):3260-9.

[26] Cheng F, Ke Q, Chen F, Cai B, Gao Y, Ye C, Wang D, Zhang L, Lahn BT, Li W, Xiang AP. Protecting against wayward human induced pluripotent stem cells with a suicide gene. Biomaterials 2012;33(11):3195-204.

[27] Conesa C, Doss MX, Antzelevitch C, Sachinidis A, Sancho J, Carrodeguas JA. Identification of specific pluripotent stem cell death--inducing small molecules by chemical screening. Stem Cell Rev 2012;8(1):116-27.

[28] Bieberich E, Silva J, Wang G, Krishnamurthy K, Condie BG. Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants. J Cell Biol 2004;167(4):723-34.

[29] Wheatley DN, Scott L, Lamb J, Smith S. Single amino acid (arginine) restriction: growth and death of cultured HeLa and human diploid fibroblasts. Cell Physiol Biochem 2000;10(1-2):37-55.

[30] Niwa A, Yamamoto K, Sorimachi K, Yasumura Y. Continuous culture of Reuber hepatoma cells in serum-free arginine-, glutamine-and tyrosine-deprived chemically defined medium. In Vitro 1980;16(11):987-93.

[31] McGee MM, Greengard O, Knox WE. The quantitative determination of phenylalanine hydroxylase in rat tissues. Its developmental formation in liver. Biochem J 1972;127(4):669-74.

[32] Kido J, Nakamura K, Mitsubuchi H, Ohura T, Takayanagi M, Matsuo M, Yoshino M, Shigematsu Y, Yorifuji T, Kasahara M, Horikawa R, Endo F. Long-term outcome and intervention of urea cycle disorders in Japan. J Inherit Metab Dis 2012;35(5):777-85.

[33] Ohira RH, Dipple KM, Zhang YH, McCabe ER. Human and murine glycerol kinase: influence of exon 18 alternative splicing on function. Biochem Biophys Res Commun 2005;331(1):239-46.

[34] Ai Y, Jenkins NA, Copeland NG, Gilbert DH, Bergsma DJ, Stambolian D. Mouse galactokinase: isolation, characterization, and location on chromosome 11. Genome Res 1995;5(1):53-9.

[35] Nakamura T, Teramoto H, Tomita Y, Ichihara A. L-proline is an essential amino acid for hepatocyte growth in culture. Biochem Biophys Res Commun 1984;122(3):884-91.

[36] Niwa H, Masui S, Chambers I, Smith AG, Miyazaki J. Phenotypic complementation establishes requirements for specific POU domain and generic transactivation function of Oct-3/4 in embryonic stem cells. Mol Cell Biol 2002;22(5):1526-36.

[37] Si-Tayeb K, Noto FK, Nagaoka M, Li J, Battle MA, Duris C, North PE, Dalton S, Duncan SA. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology 2010;51(1):297-305.

[38] Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, Zhang RR, Ueno Y, Zheng YW, Koike N, Aoyama S, Adachi Y, Taniguchi H. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013;499(7459): 481-4.

[39] Strom SC, Chowdhury JR, Fox IJ. Hepatocyte transplantation for the treatment of human disease. Semin Liver Dis 1999;19(1):39-48.

IntechOpen