Concise Review: Differentiation of Human Adult Stem Cells Into Hepatocyte-like Cells In vitro

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Adult stem cells (ASCs) are undifferentiated cells found throughout the body that divide to replenish dying cells and regenerate damaged tissues, which are the powerful sources for cell therapy and tissue engineering. Bone marrow-derived mesenchymal stem cells (BMSCs), adipose tissue-derived mesenchymal stem cells (ADSCs), and peripheral blood monocytes (PBMCs) are the common ASCs, and many studies indicated that ASCs isolated from various adult tissues could be induced to hepatocyte-like cells in vitro. However, the isolation, culture protocols, characterization of ASCs and hepatocyte-like cells are different. This review aims to describe the isolation and culture procedures for ASCs, to summarize the molecular characterization of ASCs, to characterize function of hepatocyte-like cells, and to discuss the future role of ASCs in cell therapy and tissue engineering.

Keywords: Adult stem cells, Bone marrow-derived mesenchymal stem cells, Adipose tissue-derived mesenchymal stem cells, Peripheral blood monocytes, Hepatocyte-like cells

Introduction

Currently, liver transplantation is the only definitive treatment for the end-stage liver diseases. However, its widely used was limited by the expensive costs, shortage of donor organs and invasive procedure (1). Hepatocyte transplantation has emerged as a feasible alternative to liver transplantation in some liver diseases (2), but it is limited by organ donors and the low cell quality of available liver tissues (3, 4). Thus, searching other sources of cells to replace mature hepatocytes is urgent.

Although human embryonic stem cells (hESCs) and umbilical cord blood stem cells (UCBSC) could be induced into hepatocyte-like cells in vitro (5), their widely used were limited by the low differentiation quality, ethics, and teratoma formation (6). Recently, the adult stem cells (ASCs) derived from various tissues, including bone marrow derived mesenchymal stem cells (BMSCs), adipose tissue derived mesenchymal stem cells (ADSCs), and peripheral blood mononuclear cells (PBMCs) have been developed as new cell sources contributing to liver regeneration for their high efficiency of hepatogenic differentiation using simple procedures and no ethnic issues (7-10).

In the present study, we investigated whether ASCs are the ideal seed cells for the liver regeneration from the followings: ASCs biology, including isolation, culture, differentiation to hepatocytes, and the further role of ASCs in cell therapy and tissue engineering.
Materials and Methods

Materials

In recent studies, samples of human bone marrow were obtained by Lumbar Puncture (LP), human adipose tissue were obtained from abdominal subcutaneous adipose tissue of gastric cancer patients or liposuction patients, human peripheral blood were obtained from patients with HBV or healthy adult blood donors, in accordance with the local ethics committee.

Isolation and expansion of BMSCs, ADSCs, and PBMCs

Most laboratory data showed that BMSCs could be prepared through the density degree of centrifuge, flow cytometry sorting, and sidewall sieve method (11-14). Side-wall sieve method has become popular one for its simple operation, lower cost, less injury to cell (15). Adipose tissue was minced with scissors and scalpels into less than 3mm pieces and isolation of ADSCs proceeded as previously described (16, 17). Generally, human peripheral blood monocytes isolated from donors were isolated by density gradient centrifugation and further purified by adherence separation. In order to obtain more PBSCs in shortly time, PBMCs were mobilized with recombinant G-CSF at 5~10 μg/kg/d, administered subcutaneously daily to mobilize PBMCs from bone marrow to peripheral blood (18, 19). Then PBMCs were collected by means of aphaeresis.

Characterization of ASCs

Flow Cytometer was used to identify the surface marker of the adult stem cells. Conget et al indicated that the BMSCs express many surface agents including CD13, CD44, CD29, CD105, but didn’t express CD1a, CD14, CD31, CD34, CD56, CD45 (20). ASCs derived from all three sources displayed no expression of hematopoietic markers (CD14, CD34, CD45), of the stem cell marker CD133, or the marker for endothelial cells CD144. More than 90% of MSCs derived from the three sources expressed the typical MSC marker proteins CD44, CD73, CD29, and CD90. However, the intensity of expression of CD90 of PBSCs was significantly below that of the other tissues. More than 90% of the ASCs derived from all three sources displayed no expression of hematopoietic markers (CD14, CD34, CD45), of the stem cell marker CD133, or the marker for endothelial cells CD144. More than 90% of MSCs derived from the three sources expressed the typical MSC marker proteins CD44, CD73, CD29, and CD90. However, the intensity of expression of CD90 of PBSCs was significantly below that of the other tissues. More than 90% of the ASCs derived from all three sources expressed the typical MSC marker proteins CD44, CD73, CD29, and CD90. However, the intensity of expression of CD90 of PBSCs was significantly below that of the other tissues. More than 90% of the ASCs derived from all three sources expressed the typical MSC marker proteins CD44, CD73, CD29, and CD90. However, the intensity of expression of CD90 of PBSCs was significantly below that of the other tissues. More than 90% of the ASCs derived from all three sources expressed the typical MSC marker proteins CD44, CD73, CD29, and CD90.

Table 1. Identification of different ASCs from gene expression

|                | BMSCs | ADSCs | PMSCs |
|----------------|-------|-------|-------|
| CD10           | +     | +     | +     |
| CD13           | +     | +     | +     |
| CD59           | +     | +     | +     |
| CD105          | +     | +     | +     |
| CD166          | +     | +     | +     |
| CD49d          | +     | +     | +     |
| SH3            | +     | +     | +     |
| CD29           | +     | +     | +     |
| CD44           | +     | +     | +     |
| CD71           | +     | +     | +     |
| CD90           | +     | +     | +     |
| CD106          | +     | +/-   | +/-   |
| CD120a         | +     | +     | +     |
| CD124          | +     | +     | +     |
| CD11b          | -     | -     | -     |
| CD14           | -     | +/-   | +     |
| CD31           | -     | +/-   | -     |
| CD34           | -     | -     | +     |
| CD45           | -     | -     | +     |
| CD48           | -     | -     | -     |
| CD135          | -     | -     | -     |
| CD117          | +     | +/-   | +/-   |

(27-32). ADSCs are characterized as CD45, CD34+, CD105+, CD31-(33) (Table 1).

Protocols of ASCs’ transdifferentiation

Some studies indicated that functional hepatocytes could be induced from ASCs by some cytokines or through coculture with other cell types. However, the potential of the ASCs’ transdifferentiation is generally low. Therefore, researchers are keen to explore new methods to induce ASCs differentiate into functional hepatocytes in vitro currently. The current protocols used in different ASCs are summarized in Table 2.

Functional analysis of hepatocyte-like cells from ASCs

The hepatocyte-like cells from ASCs were confirmed from the gene and protein expression. Gene expressions were identified by RT-PCR, using the common markers of hepatocytes, including ALB, AFP, CK18, CK19, and CYP3A4 (34). Protein expressions were usually identified by immunohistochemistry or immunofluorescence, western blot, from the expression of albumin, CK18, CYP3A4, CYP1A1, CYP2C9 and NADPH-P450 (35-38). To compare the potential of hepatogenic differentiation of the different adult stem cells in vitro, researches indicated ADSCs have a similar differentiation potential towards the hepatic lineage, similar to BMSCs. However, their longer culture period and proliferation capacity differ from the BMSCs (39-41).
Table 2. Overview of ASCs expansion, and hepatic differentiation

| Expansion Medium | Hepatic Differentiation Medium | Ref. |
|------------------|--------------------------------|------|
| BMSCs DMEM, 10% FBS | 500 ml Williams Medium E with-out L-glutamine, 50 mg/l L-glutamine, 100 IU/l penicillin/streptomycin, 20 mM HEPES, 20 mM sodium pyruvate, 5 mM Dex, 10 ng/ml EGF, 5 ng/ml HGF; 20 μl/ml insulin, 10% FBS, and 10% horse serum | 42 |
| DMEM, 10% FBS | 100 U/ml penicillinG, 100 μg/ml streptomycin, 50 ng/ml L-glutamine, 100 μg/ml aprotinin in William’s medium E. Medium A contained 10% FBS; medium. Medium B contained 1 nM insulin and 1 nM Dex in medium A and Coculture with Hepatocytes | 43 |
| DMEM, 5% FBS | 0.03 mM nicotinamide, 0.25 mM sodium-pyruvate and 1.623 mM glutamine, 10 ng/ml FGF-4, 20 ng/ml HGF, 1 x ITS and 20 lg/l Dex | 44 |
| 60% DMEM-LG/5% FBS, 40%MCDB -201, 1x ITS, 10-9 M Dex, 10-4M ascorbic acid 2-phosphate, 10 ng/ml EGF, 100 U penicillin, 1000 U streptomyycin | DMSO (0.1%), HGF (10 ng/ml), OSM (10 ng/ml) | 45 |
| ADSCs DMEM-LG,15% human serum, 50 μg/ml gentamicine | EGF (20 ng/ml), bFGF (10 ng/ml), HGF (20 ng/ml), nicotinamide (4.9 mmol/l), OSM (20 ng/ml), Dex (1 μmol/l), ITS (10 μmol/l), BSA (1.25 mg/ml), linoleic acid (190 μmol/l) | 46 |
| ADSCs DMEM, 10% FBS | Transferrin (5 μg/ml), hydrocortisone-21-hemisuccinate (10−6 M), BSA (0.5 mg/ml), ascorbic acid (2 mM), EGF (20 ng/ml), insulin (5 μg/ml), Dex (10−8 M), HGF (150 ng/ml), FGF1 (300 ng/ml), FGF4 (25 ng/ml), OSM (30 ng/ml), Dex (2×10−5 mol/l) | 47 |
| ADSCs DMEM, 10% FBS | Activin A (20 ng/ml), FGF4 (20 ng/ml), transferrin (5 μg/ml), hydrocortisone-21-hemisuccinate (10−6 mol/l), BSA (0.5 mg/ml), ascorbic acid (2 mmol/l), EGF (20 ng/ml), insulin (5 μg/ml), Dex (10−8 M), HGF (150 ng/ml), FGF1 (100 ng/ml), FGF4 (25 ng/ml), OSM (30 ng/ml), Dex (2×10−5 mol/l), 1x ITS, nicotinamide (0.05 mmol/l), DMSO (0.1%) | 48 |
| 60% DMEM, 40% MCDB, 5 mg/ml apotransferrin, 5 ng/ml selenous acid, 5 mg/ml linoleic acid, 5 mg/ml bovine insulin, 100 mM ascorbic acid 2-phosphate, 1 mM Dex, 10 ng/ml PDGF, 10 ng/ml EGF, 100 U penicillin, 10 mg/ml streptomyycin, 15% FCS | Dex (1 nM), ascorbic acid (100 μM), EGF (10 ng/ml), bFGF (10 ng/ml), HGF (10 ng/ml), OSM (10 ng/ml), DMSO (0.1%) | 49 |
| 60% DMEM-LG, 40% MCDB-201, 1x ITS, 1 nM Dex, 100 mM ascorbic acid 2-phosphate, 10 ng/ml EGF, 5% FBS | 5′ Azacytidine (20 μM), human hepatocyte maintenance medium, FCS (2%), HGF (40 ng/ml), EGF (20 ng/ml) | 50 |
| PMSCs RPMI 1640 medium,10% human ABserum, 2 mmol/l glutamine, 100 U/ml penicillin, and 100 μg/ml streptomyacin | RPMI 1640 medium,10% human ABserum, 2 mmol/l glutamine, 100 U/ml penicillin, and 100 μg/ml streptomyacin, 140 μmol/l β-mercaptoethanol, 5 ng/ml M-CSF, 0.4 ng/ml human IL-3, 3 ng/ml [FGF]-4 | 51 |

Discussion

In the present study, we described different ASCs could be induced into hepatocyte lineage cells in different culture systems in vitro. It is very safe and easy to acquire the enough ASCs, and then induce them into functional hepatocytes in vitro. Based on this progress, ASCs transplantation might be a novel therapy for the severe liver diseases, and also will be ideal seed cells for liver tissue engineering. However, which ASCs are better still needs us to investigate from their preparation, molecular characterization, and functional assay. This paper firstly pro-
vides such a concise review focused on ASCs’ biology and differentiates potential, indicating ASCs might be an ideal seed cells in cell transplant therapy and tissue engineering. We also believe in the future, some studies will show us the most appropriate ASCs for cell transplant or tissue engineering.

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Potential conflict of interest
The authors have no conflicting financial interest.

References

1. Dhawan A, Puppi J, Hughes RD, Mitry RR. Human hepatocyte transplantation: current experience and future challenges. Nat Rev Gastroenterol Hepatol 2010;7:288-298
2. Navarro-Alvarez N, Soto-Gutierrez A, Kobayashi N. Hepatocyte transplantation: a step forward. Curr Opin Organ Transplant 2007;12:652-658
3. Zaret KS, Grompe M. Generation and regeneration of cells of the liver and pancreas. Science 2008;322:1490-1494
4. Terry C, Hughes RD, Mitry RR, Lehec SC, Dhawan A. Cryopreservation-induced nonattachment of human hepatocytes: role of adhesion molecules. Cell Transplant 2007;16:639-647
5. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145-1147
6. Gallicano GI, Mishra L. Hepatocytes from induced pluripotent stem cells: a giant leap forward for hepatology. Hepatology 2010;51:20-22
7. Lee KD, Kuo TK, Whang-Peng J, Chung YF, Lin GT, Chou SH, Chen JR, Chen YP, Lee OK. In vitro hepatic differentiation of human mesenchymal stem cells. Hepatology 2004;40:1275-1284
8. Snykers S, Vanhaecke T, Papeleu P, Luttun A, Jiang Y, Chou SH, Chen JR, Chen YP, Lee OK. In vitro hepatic differentiation of human mesenchymal stem cells. Hepatology 2004;40:1275-1284
9. Snykers S, Vanhaecke T, Papeleu P, Luttun A, Jiang Y, Chou SH, Chen JR, Chen YP, Lee OK. In vitro hepatic differentiation of human mesenchymal stem cells. Hepatology 2004;40:1275-1284
10. Shin KS, Lee HJ, Jung J, Cha DH, Kim GJ. Culture and in vitro hepatogenic differentiation of placenta-derived stem cells, using placental extract as an alternative to serum. Cell Prolif 2010;43:435-444
11. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman I, Grompe M. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 2000;6:1229-1234
12. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Gund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41-49
13. Jang YK, Collector MI, Baylin SB, Diehl AM, Shkirski SJ. Hematopoietic stem cells convert into liver cells within days without fusion. Nat Cell Biol 2004;6:532-539
14. di Bonzo LV, Ferrero I, Cravanzola C, Mareshi K, Rustichelli D, Novo E, Sanavio F, Bizzotto S, Castiglione F, Colombatto S, Fagioli F, Parola M. Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential. Gut 2008;57:223-231
15. Chen Y, Dong XJ, Zhang GR, Shao JZ, Xiang LX. In vitro differentiation of mouse bone marrow stromal stem cells into hepatocytes induced by conditioned culture medium of hepatocytes. J Cell Biochem 2007;102:52-63
16. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benham P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001;7:211-228
17. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JJ, Mizuno H, Alfonso ZC, Fraser JK, Benham P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279-4295
18. Matsunaga T, Sakamaki S, Kohyo Y, Ohi S, Hirayama Y, Niiitsu Y. Recombinant human granulocyte colony-stimulating factor can mobilize sufficient amounts of peripheral blood stem cells in healthy volunteers for allogeneic transplantation. Bone Marrow Transplant 1993;11:103-108
19. Kasai M, Miyama Y, Kawamura M. Application of peripheral blood stem cells (PBSC) mobilized by recombinant human granulocyte colony stimulating factor for allogeneic PBSC transplantation and the comparison of allogeneic PBSC transplantation and bone marrow transplantation. Transfus Apher Sci 2002;26:121-127
20. Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. J Cell Physiol 1999;181:67-73
21. De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, Dragoo JL, Ashjian P, Thomas B, Benham P, Chen I, Fraser J, Hedrick MH. Comparison of multipotential cells from human adipose tissue and bone marrow. Cells Tissues Organs 2003;174:101-109
22. Wagner W, Wein F, Seekinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W, Ho AD. Comparative characteristics of mesenchymal stem
cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 2005;33:1402-1416
23. Lee RH, Kim B, Choi I, Kim H, Choi HS, Suh K, Bae YC, Jung JS. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell Physiol Biochem 2004;14:311-324
24. Katz AJ, Tholpady A, Tholpady SS, Shang H, Ogle RC. Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. Stem Cells 2005;23:412-423
25. Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 2001;189:54-63
26. Kern S, Eichler H, Steeoe J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006;24:1294-1301
27. Strem BM, Hicok KC, Zhu M, Wulur I, Alfonso Z, Schreiber RE, Fraser JK, Hedrick MH. Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med 2005;54:132-141
28. Dicker A, Le Blanc K, Aström G, van Harmelen V, Götherström C, Blomqvist L, Arner P, Rydén M. Functional studies of mesenchymal stem cells derived from adult human adipose tissue. Exp Cell Res 2005;308:282-290
29. Im GI, Shin YW, Lee KB. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? Osteoarthritis Cartilage 2005;13:845-853
30. Safford KM, Hicok KC, Safford SD, Halvorsen YD, Wilkison WO, Gimble JM, Rice HE. Neurogenic differentiation of murine and human adipose-derived stromal cells. Biochem Biophys Res Commun 2002;294:371-379
31. Cao Y, Sun Z, Liao L, Meng Y, Han Q, Zhao RC. Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. Biochem Biophys Res Commun 2005;323:370-379
32. Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. Biochem Biophys Res Commun 2005;328:258-264
33. Boquest AC, Shahdadfar A, Frønsdal K, Sigurjonsson O, Tunheim SH, Collas P, Brinchmann JE. Isolation and transcription profiling of purified uncultured human stromal stem cells: alteration of gene expression after in vitro cell culture. Mol Biol Cell 2005;16:1131-1141
34. Sgodd M, Aurich H, Kleist S, Aurich I, König S, Dollinger MM, Fleig WE, Christ B. Hepatocyte differentiation of mesenchymal stem cells from rat peritoneal adipose tissue in vitro and in vivo. Exp Cell Res 2007;313:2875-2886
35. Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest 2002;109:1291-1302
36. Lee KD, Kuo TK, Whang-Peng J, Chung YF, Lin CT, Chou SH, Chen JR, Chen YP, Lee OK. In vitro hepatic differentiation of human mesenchymal stem cells. Hepatology 2004;40:1275-1284
37. Anjos-Afonso F, Siapati EK, Bonnet D. In vivo contribution of murine mesenchymal stem cells into multiple cell-types under minimal damage conditions. J Cell Sci 2004;117:5655-5664
38. Aurich I, Mueller LP, Aurich H, Luetzkendorf J, Tisljar K, Dollinger MM, Schermann W, Wallhof J, Hengstler JG, Fleig WE, Christ B. Functional integration of hepatocytes derived from human mesenchymal stem cells into mouse livers. Gut 2007;56:405-415
39. Kern S, Eichler H, Steeoe J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006;24:1294-1301
40. Lee RH, Kim B, Choi I, Kim H, Choi HS, Suh K, Bae YC, Jung JS. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell Physiol Biochem 2004;14:311-324
41. Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. Biochem Biophys Res Commun 2005;328:258-264
42. Fiegel HC, Lioznov MV, Cortes-Dericka L, Lange C, Kluth D, Fehse B, Zander AR. Liver-specific gene expression in cultured human hematopoietic stem cells. Stem Cells 2003;21:98-104
43. Takeda M, Yamamoto M, Isoda K, Higashiyama S, Hiroe M, Ohgushi H, Kawase M, Yagi K. Availability of bone marrow stromal cells in three-dimensional coculture with hepatocytes and transplantation into liver-damaged mice. J Biosci Bioeng 2005;100:77-81
44. Snykers S, Vanhaecke T, Papeleu P, Luttun A, Jiang Y, Vander Heyden Y, Verfaillie C, Rogiers V. Sequential exposure to cytokines reflecting embryogenesis: the key for in vitro differentiation of adult bone marrow stem cells into functional hepatocyte-like cells. Toxicol Sci 2006;94:330-341
45. Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. Biochem Biophys Res Commun 2005;328:258-264
46. Talens-Visconti R, Bonora A, Joffer R, Mirabet V, Carbonell F, Castell JV, Gómez-Lechón MJ. Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells. World J Gastroenterol 2006;12:5834-5845
47. Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Quinn G, Okochi H, Ochiya T. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. Hepatology 2007;46:219-228
48. Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Osaki M, Kato T, Okochi H, Ochiya T. Rapid hepatic fate specification of adipose-derived stem cells and their therapeutic potential for liver failure. J Gastroenterol Hepatol 2009;24:70-77
49. Aurich H, Sgodd M, Kaltwasser P, Vetter M, Weise A, Lühr T, Brulport M, Hengstler JG, Dollinger MM, Fleig...
WE, Christ B. Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue in vitro promotes hepatic integration in vivo. Gut 2009;58:570-581

50. Okura H, Komoda H, Saga A, Kakuta-Yamamoto A, Hamada Y, Fumimoto Y, Lee CM, Ichinose A, Sawa Y, Matsuyama A. Properties of hepatocyte-like cell clusters from human adipose tissue-derived mesenchymal stem cells. Tissue Eng Part C Methods 2010;16:761-770

51. Ruhnke M, Ungefroren H, Nussler A, Martin F, Brulpport M, Schormann W, Hengstler JG, Klapper W, Ulrichs K, Hutchinson JA, Soria B, Parwaresch RM, Heeckt P, Kremer B, Fändrich F. Differentiation of in vitro-modified human peripheral blood monocytes into hepatocyte-like and pancreatic islet-like cells. Gastroenterology 2005;128:1774-1786