REFERENCES

1. Takebe T, Sekine K, Enomura M, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature*. 2013;499(7459):481–484.
2. Si-Tayeb K, Lemaigre FP, Duncan SA. Organogenesis and development of the liver. *Dev Cell*. 2010;18(2):175–189.
3. Lenhoff S, Olofsson T. Cytokine regulation of GM-CSF and G-CSF secretion by human umbilical cord vein endothelial cells (HUVEC). *Cytokine*. 1996;8(9):702–709.
4. Boomsma RA, Geenen DL. Mesenchymal stem cells secrete multiple cytokines that promote angiogenesis and have contrasting effects on chemotaxis and apoptosis. *PLoS One*. 2012;7(4):e35685.
5. Gouysse G, Couvelard A, Frachon S, et al. Relationship between vascular development and vascular differentiation during liver organogenesis in humans. *J Hepatol*. 2002;37(6):730–740.
6. Yusa K, Rashid ST, Strick-Marchand H, et al. Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. *Nature*. 2011;478(7369):391–394.
7. Rashid ST, Corbineau S, Hannan N, et al. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. *J Clin Invest*. 2010;120(9):3127–3136.
8. Hou P, Li Y, Zhang X, et al. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science*. 2013;341(6146):651–654.
9. Lee AS, Tang C, Rao MS, Weissman IL, Wu JC. Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. *Nat Med*. 2013;19(8):998–1004.
10. Suva ML, Riggi N, Bernstein BE. Epigenetic reprogramming in cancer. *Science*. 2013;339(6127):1567–1570.
11. Olausson M, Patil PB, Kuna VK, et al. Transplantation of an allogeneic vein bioengineered with autologous stem cells: a proof-of-concept study. *Lancet*. 2012;380(9838):230–237.
12. Gonfiotti A, Jaus MO, Barale D, et al. The first tissue-engineered airway transplantation: 5-year follow-up results. *Lancet*. 2014;383(9913):238–244.
13. Elliott MJ, De Coppi P, Speggiorin S, et al. Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study. *Lancet*. 2012;380(9846):994–1000.
14. Skrahin A, Ahmed RK, Ferrara G, et al. Autologous mesenchymal stromal cell infusion as adjunct treatment in patients with multidrug and extensively drug-resistant tuberculosis: an open-label phase 1 safety trial. *Lancet Respir Med*. 2014;2(2):108–122.
15. Uygun BE, Soto-Gutierrez A, Yagi H, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. *Nat Med*. 2010;16(7):814–820.

Regenerative Medicine

Shortcut Route for Generation of Functional Hepatocyte Cells from Human Skin Allogenically for Autologous Treatment of Chronic Liver Diseases

Abstract

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Abbreviations: iPS: induced pluripotent stem cells

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Transplantation of human adult hepatocyte cells have been used for treatment of both genetic and non genetic human liver diseases but with limited success because of several reasons such as poor quality of hepatocytes, inability to proliferation upon transplantation, chance of immunorejection, patient unmatched hepatocytes, difficult to cryopreservation, needed immunosuppressant drugs. Comparatively, human fetal hepatocyte cell has significantly higher proliferation potential than primary adult hepatocyte cells upon transplantation. Due to ethical issue, the human fetal hepatocyte option does not look realistic solution for liver patients. Very recently, induced pluripotent stem cell (iPS) technology together with chemical biology brings new golden opportunities to generate the human hepatocyte like cells from skin cell. Since introduction of iPS technology in 2006, it was thought that one has to make pluripotency state and then differentiate towards hepatocyte cells. But now researchers proved that it doesnot need to go to back to pluripotent state for generation of hepatocyte cells. The intermediate endodermal state is sufficient and appropriate step for generation of hepatocyte cells. They developed a short cut route via endodermal state and created hepatocytes cells. The clinical potential of these created hepatocyte cells were evaluated the functional and proliferation ability of in injured humanized mouse model. After nine month transplantation, they observed that the functional and proliferation ability of transplanted hepatocyte cells. In fact, most of conventional iPSC-derived hepatocyte cells unable to proliferate satisfactorily upon transplantation like adult human hepatocytes. Transplanted hepatocyte should be functional and

ability for proliferate quickly, are both main prerequisites for therapeutic applications. This short cut route is not only for generation of hepatocyte cells but also for creation of other human body cells. Such shortcut routes for generation of functional hepatocyte cells allogenically from human skin raise a new hope for regeneration of acute or chronic liver who are risk of developing liver failure.

COMMENTS

Since 2006, induced pluripotent stem cells (iPS) technology[1] has been revolutionizing preclinical research and clinical practice rapidly across the globe. The Nobel strategy of iPS technology is that patient's specific functional cells can be generating from their skin cells for their treatment of incurable diseases. Although the iPS technology is very initial stage in human clinical trial but it may eliminate the need of organ transplantation in future. In early 2013, the world first clinical trial of iPS technology for treatment of nearly blind peoples has been started in Japan. The generation of functional hepatocytes from human skin cells by utilizing iPS technology is unprecedented opportunities for surgeons as well as liver patients for transplantation to get allogenic functional hepatocyte cells to regenerate their liver without waiting for donor liver. The clinical applicability of such iPS derived hepatocytes has not been tested in human clinical trials because of the oncogenic concerns of iPS cell and other limitations. However, potential of iPS derived hepatocytes have been tested in various animal models of injured livers and found fascinating results about recovery of liver disorders. Previously, all hepatic differentiation methods utilize iPS cells for conversion to hepatocytes cells. Currently, it was thought that skin cell must go back to pluripotency state in order to make hepatocytes cells. Pluripotent state of iPS cell is associated with tumor formations. Very recently, Zhu et al[2] discovered the breakthrough invention that generation of functional hepatocytes is possible and advisable from endodermal state without going back to pluripotent state unlike current protocols. These researchers compared pluripotent state derived hepatocyte with endodermal state derived hepatocytes and found the endodermal state derived hepatocytes is much more superior than pluripotent state derived hepatocyte in term of proliferation capacity upon transplantation and hepatic functions. Since pluripotency is associated with tumor formation, it is a clinically innovative idea not to go back to pluripotent state of iPS cell in order to avoid the tumor formation. They reprogrammed skin cells to induced multipotent progenitor cell, which is much faster process than conventional reprogramming methods. Polygonal hepatocytes were generated from induced multipotent progenitor cell. These differentiated hepatocytes were occasionally binucleate but express hepatocyte markers such as HNF4-alfa, albumin synthesis, cytokeratin 18 and alpha-1 antitrypsin. Interestingly, glycogen storage, lipid uptake and urea production were observed in these differentiated hepatocytes. Zhu et al[2] reported that the gene expression analysis showed that induced multipotent progenitor cell derived hepatocyte cells were more similar with human fetal primary hepatocyte than mature adult primary hepatocytes. Virtually, trasnplated hepatocytes cell do not proliferate rapidly and survive for short time during hepatocyte transplantation. Hepatocyte cells are originally obtained from dead or donor tissue. The survival time of these hepatocyte average of week and negligible ability to multiply. Proliferation of transplanted cells in transplanted site and long term survival are main prerequisites for successful therapeutic applications for cell transplantation. Primary human fetal hepatocyte has extensive potential to meet **the both** prerequisites, proliferation of transplanted cells in transplanted site and long-term survival for successful therapeutic applications. Successful treatment of fulminant hepatic failure and clinical end-stage chronic liver failure by transplantation of human fetal hepatocyte was reported in human cases.[3,4] Gridelli and coauthors[3] reported the strong proliferative potential of human fetal cells. Successful treatment was done by transplantation of immortalized human fetal hepatocytes in a mouse model of acute liver failure induced by 90% hepatectomy[5] and rat acute liver model.[6] Although the human fetal hepatocyte has strong potential to cure liver diseases but ethical concern is an ongoing issue. But Zhu et al[2] discovered another shot cut way to create human fetal hepatocyte cells using skin cells without need of human fetal donor which is valuable milestone for cell therapies of liver diseases. First they transduced 10,000 cells human fibroblast cells with three pluripotent genes (OCT4, SOX2 and KLF4) for three days and then transferred these transduced cells into endodermal state using several growth factors and small molecule CHIR99021. Interestly, they observed the endodermal specific genes after 2 weeks. The idea behind that was whether it is essential to back to pluripotent state or endodermal state. The human liver itself is endogermal origin. After their exciting experiment, it seems that it is not essential to go back to pluripotent state to generate functional hepatocyte cells. After getting the endodermal state of transduced cells, Zhu et al[2] directed these cells towards hepatic differentiation by inhibiting biliary differentiation using some small molecules. Biopotential embryonic liver progenitor cells under goes biliary fate by transforming growth factors and Notch signaling molecules. Zhu et al[2] used very innovative approach to promote hepatic differentiation using two small molecules A83 and Notch inhibitors compound which inhibit biliary differentiation. They used a humanized Fah−/−/Rag2−/−/Il2rg−/− (FRG) mice[7], an immune-deficient mouse model of human tyrosinemia type 1 to evaluate the clinical potential and transplanted 10,000,000 induced multipotent progenitors derived