Preclinical Liver Bud Engineering towards Clinical Target for Liver Diseases

Abstract

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;499:481–484.

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Preclinical liver bud engineering opens a door for solving the never ending worldwide liver organ shortage problem in near future. It was previously thought that human hepatocyte transplantation, an alternative of orthotropic liver transplantation, was good choice for treatment of various diseases of liver patients. The clinical applicability of human hepatocyte transplantation has gained no widespread clinical acceptance due to concerns of quality and availability of human hepatoyctes and their immunogenic problems. The induce pluripotent technology could solve the existing limitation of human hepatocytes. Currently, a proof of concept experiment makes wave to hepatologists that it make more clinical sense to transplant of human hepatocyte along with mesenchymal stem cells and human umbilical vein endothelial cells instead of hepatocyte cells alone during hepatocytes transplantation to liver patients. This mixture strategy provides local microenvironments for liver bud formation which mimic the human fetal liver. Human umbilical vein endothelial cells may produce GM-CSF and G-CSF which is essential for neovascularization and stem cell mobilization while mesenchymal stem cells produce multiple cytokines including elevated levels of VEGF to promote angiogenesis. Upon transplantation in mice with live failure, liver buds became visualized by forming blood vessel formation by which liver bud facilities to get proper cytokines and growth factors for proper liver regeneration as liver itself has impressive potential of spontaneous regeneration upon appropriate micronenvioments. It is clinical message to all hepatoycte transplantation centers around the world to improve the clinical success.

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Abbreviations: VEGF: vascular endothelial growth factoriPSC: induced pluripotent stem cellsMDR: multidrug-resistantXDR: extensively drug-resistantGM-CSF: granulocyte-macrophage colony-stimulating factorG-CSF: granulocyte-colony stimulating factorFDA: food and drug administration

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COMMENTS

Millions of acute and chronic liver patients are dyeing during waiting time for donor organs. Liver diseases are an umbrella term, very complex to treat. Donor organ shortage is an unsolved worldwide problem. It is a great hope that never ending worldwide liver organ shortage problem could be solving in near future by utilizing induce pluripotent technology. Japanese researchers[1] proved that it is possible to generate 4 mm–5 mm liver bud *in vitro* in 2 days, upon mix of 1×10^6 human iPSC derived hepatic endoderm cells, $0.08–1 \times 10^5$ human umbilical vein endothelial cells and 2×10^5 mesenchymal stem cells in a conventional culture dish. These liver buds make vascularized network along with new blood vessel formation around transplanted site and perform liver functions when transplanted into mice with liver failure. Japanese researchers[1] believed that hundreds of such *in vitro* created liver buds could re-establish the 30% of hepatic function of patient's liver. Although hepatocyte transplantation has been gained clinical outcomes for some metabolic disorder, but it hasn't gained widespread clinical acceptance for restore the liver functions of liver patients. Hepatocyte may not make quick and enough vascularization network upon transplantation, finally without proper blood supply, most of transplanted hepatocytes unable to get appropriate growth factors and cytokines and therefore, unable to restore liver functions and finally die. This proof of concept experiment makes wave to hepatologists that it makes more clinical sense to transplant of hepatocyte along with mesenchymal stem cells and human umbilical vein endothelial cells instead of hepatocyte cells alone during hepatocytes transplantation to liver patients. This mixture strategy provides not only enhanced in *in vivo* like hepatic functions by forming liver bud but also make neovascularization which is essential to get proper cytokines and growth factors for proper liver regeneration as liver itself has impressive potential of regeneration upon appropriate microenvironments. The molecular and cellular mechanism for spontaneous self organization of these three cells to form tiny liver bud remains unclear. The authors[1] analyzed the 83 genes which are more active during liver development. It is more important to compare with all major adult liver genes those are expressed in healthy adult liver. During embryonic development for liver, the close association of biliary epithelium, portal mesenchymal and endothelial cells happens for liver bud formation.[2] A healthy liver contains approximately 10–100 billion hepatocytes and hepatocytes constitute 70% of liver mass. A liver patient's need at least 30% of total live mass. In order to make such high number of liver buds, an automated culture system is needed for upscaling of generation of such liver buds. Different size of liver buds could be generated by using different quality of human iPSC derived hepatic endoderm cells, human umbilical

vein endothelial cells and mesenchymal stem cells. Bioreactor based perfusion culture system is also needed for generation bigger liver bud. Bioreactor has key role to provide proper oxygenations to bigger liver bud. The mix-culture of human umbilical vein endothelial cells and mesenchymal stem cells with iPSC derived hepatic endoderm cells to form three dimensional and vascularized tissue formation has clinical implication not only to liver bud but also applicable to make other mini organ buds such as pancreas, kidney, retinal tissue, heart tissue, lung tissue and so on. Currently, it has became central focus for generating of clinical grades functional cells (retinal cells, renal cells, cardiomyocyte, insulin producing cells, neuronal cell and so on) from iPSC cells which is easy, quick, unethical and more convenient for all researcher, regulatory agencies, heath care industry, patients and doctors.

Transplanted conventional human iPSC mature hepatocyte produces much less albumin than liver bud, suggesting that either primary hepatocyte cell therapies or iPSC derived hepatocytes alone is not sufficient to recapitulate the qualitative *in vivo like* hepatic functions. It would be wise idea to mix with human umbilical vein endothelial cells and mesenchymal stem cells during transplantation of functional hepatocyte cells in order to make proper *in vivo* like hepatic functions. After making liver bud *in vitro*, they took from culture dish and transplanted in mouse liver model, surprisingly the liver bud hooked up and vascularized in 48 h. Human umbilical vein endothelial cells produce GM-CSF and G-CSF which is essential for neovascularization and stem cell mobilization.[3] Mesenchymal stem cells produce multiple cytokines including elevated levels of VEGF which not only promote angiogenesis but also project from chemotaxis and apoptosis.[4] Vascularization is important for organogenesis of liver.[5] The take home message of this proof of study is that mesenchymal stem cells and human umbilical vein endothelial cells have important role for vascularization and organoid formation.

Further, It was evaluated the drug metabolizing potential by using two drugs (ketoprofen and debrisoquine), found that these liver bud metabolized in similarly like *in vivo* human liver. This is an excellent milestone for drug discovery process and could solve the problem of drug attrition. Hepatotoxicity is the main reasons for post marketing withdrawal of approved drugs. Until now, more than 1100 approved market drugs cause liver toxicity to patients. Drug discovery process rely on nonhuman animal models which are not even little human. The average financial investment for drug development per drug is $800. It is estimate that 90% of drugs fail in clinical trials. There are something are missing in preclinical stage. There are many drugs are potential safe in animal model but cause adverse reaction to multiorgan failure in human, suggesting animal model is not reliable for drug discovery process. FDA estimated that 10% toxicity prediction in early stage would save the pharmaceutical industry $100 million per drug. Now, it is time to jump to liver bud engineering for preclinical evolution of drug candidates to make safer and effective drug.

The iPSC technology brings another milestone for curing genetic liver diseases like alpha 1-antitrypsin deficiency.[6,7] The incidence of this genetic disease is 1 in every 2000 Europeans. Defect of single A1AT gene makes more A1AT protein which could build up in liver and finally make cirrhosis. Such diseases could be cure by donor organ. Therefore, it is impossible to cure all A1AT patients. But iPSC technology brings a great hope to waiting liver patients without depending donor organs as well as without worry about rejection and immune problems. Patients need to give some skin fibroblast cells and it takes 4 weeks to generate liver cells from their skin fibroblast cells which genetically identical to them. Very interestingly, it is possible to display the pathological mechanism of A1AT disease *in vitro* which could be correct *in vitro* by giving proper drug candidates and might possible to give back to patient for curing A1AT disease. It is also possible screen different possible drugs *in vitro* for curing the pathological mechanism of A1AT disease. After screening process, it may possible to say which are the effective, it could be possible say to patients which drug should take for curing the A1AT disease.

Although Takebe et al[1] achieved impressive milestone by creating liver bud *in vitro* which mimic in *in vivo* liver physiology but there are some important concerns need to solve before jumping in clinical trials. First, iPSC causes tumorgencity, is a major hurdle for cell therapies.[8] The iPSC express several oncogenes[9] which makes another barrier for iPSC therapies in human clinical trails. The iPSC technology involves integration of oncogenes and impossible to remove the oncogenes from iPSC. However, luckily another achievement came up recently that creation of iPSC cell by using seven chemicals only.[10] But, it have tested in mouse somatic cell, several laboratories including us are trying making chemical human iPSC in order to overcome the existing limitation of reprogramming factors. Hopefully, safer human iPSC could generate in near future.

Decellularized organs have been used in organ replacement with outstanding successes in human patients. Decellularized organs have been recellularized with different types of cells. The mixture strategy of liver bud engineering may applicable during recellularization process of decellularized organs for clinical trials. Extrahepatic portal vein obstruction of a 10-year-old girl was successfully treatment by using autologous endothelial and smooth muscle cells from his 20 mL of bone marrow.[11] A 30-year-old woman was treated by autologous stem-cell-derived epithelial cells and chondrocytes in 2008 and

