REVIEW ARTICLE

# In Vitro Hepatic Differentiation of Adult, Embryonic, Induced Pluripotent and Perinatal Stem-Cells

***Quratulain Khan[1], Bushra Wasim[1], Shumaila Usman[2], Talat Mirza[2]***
*1Department of Anatomy, Ziauddin University, 2Department of Research, Ziauddin University, Karachi, Pakistan.*

**ABSTRACT**

Globally regenerative medicine is considered as one of the rapidly growing biomedical industry have objective to substitute damaged cells. Cell transplantation is less intrusive than whole-organ transplantation, and has been used to provide an alternative for patients to whole-organ transplantation. The End-stage liver disease comprises a subgroup of patients with cirrhosis who have signs of decompensation that is irreversible with medical treatment. The only restorative therapy for severe end-stage liver disease is orthotropic liver transplantation. However, liver transplantation has several limitations such as scarcity of organ donors, immunosuppressive drugs, and several postoperative complications. Thus, cell transplantation can be used for the treatment of end stage liver disorders to decrease the mortality in acute liver failure. Therefore, stem cells can be used for cellular therapy, development of liver disease models, and tissue-engineering applications. This review involved the studies conducted on the stem cells potential of hepatic differentiation, isolated from different sources. The PubMed and Google Scholar were searched for scientific studies reported the sources of stem cells based on their origin and their potential of hepatic differentiation in-vitro by using different tools of differentiation. All the research articles were selected in which solely hepatic differentiation in combination with different tools is reported.

**Keywords**: Adult Stem Cells, Embryonic Stem Cells, Induced Pluripotent Stem Cells, In-vitro, Mesenchymal Stem Cells.

***Corresponding Author:***
Dr. Quratulain Khan
Department of Anatomy,
Ziauddin University, Karachi, Pakistan.
Email: drquratkhalil786@gmail.com
doi.org/10.36283/PJMD9-4/013

## INTRODUCTION

The aim of regenerative medicine is not only to replace what is impaired, but also to provide the fundamentals required for in vivo repair, to create alternates that seamlessly interact with the living body, and to stimulate the body's inherent abilities for regeneration. Furthermore, it utilizes the combined knowledge of biomaterials, tissue engineering and contemporary cell therapy approaches that moves it beyond conventional transplantation and replace[1]. Nowadays there are various clinics around the world, working on stem cell-based interventions to treat severe chronic disease or terminal illness[2].

Liver disease comprises for 3.5% of all deaths globally almost two million deaths annually, half is due to complications of cirrhosis and another half is due to hepatitis and liver cancers[3]. In end stage liver disease, orthotropic liver transplantation was thought to be the final treatment option. About 5000 patients undergo liver transplantation annually around the world[4]. Despite outstanding progress in lifespan, liver transplant is considered as a complicated surgical procedure with noteworthy morbidity and mortality[5].

In order to overcome these hurdles, regenerative medicine emerges as aspiration for the treatment of degenerative disorders. In regenerative medicine and pharmacology researches, functional hepatocytes attain a therapeutic value and are an attractive substitute of orthotropic liver transplantation (OLT)[6]. Different types of cells have been exploited to produce donor free and expandable source of hepatocytes like cells[7]. Several groups documented the generation of hepatocytes like cells (HLCs) from

different types of cells by using stepwise protocols and combination of growth factors[8].

## DISCUSSION

This review explored the studies conducted on the recent sources of stem cells and their potential of hepatic differentiation by using different tools of differentiation. The searched for scientific studies reported (Figure 1) on the in vitro hepatic differentiation of cells and tools to generate HLCs.

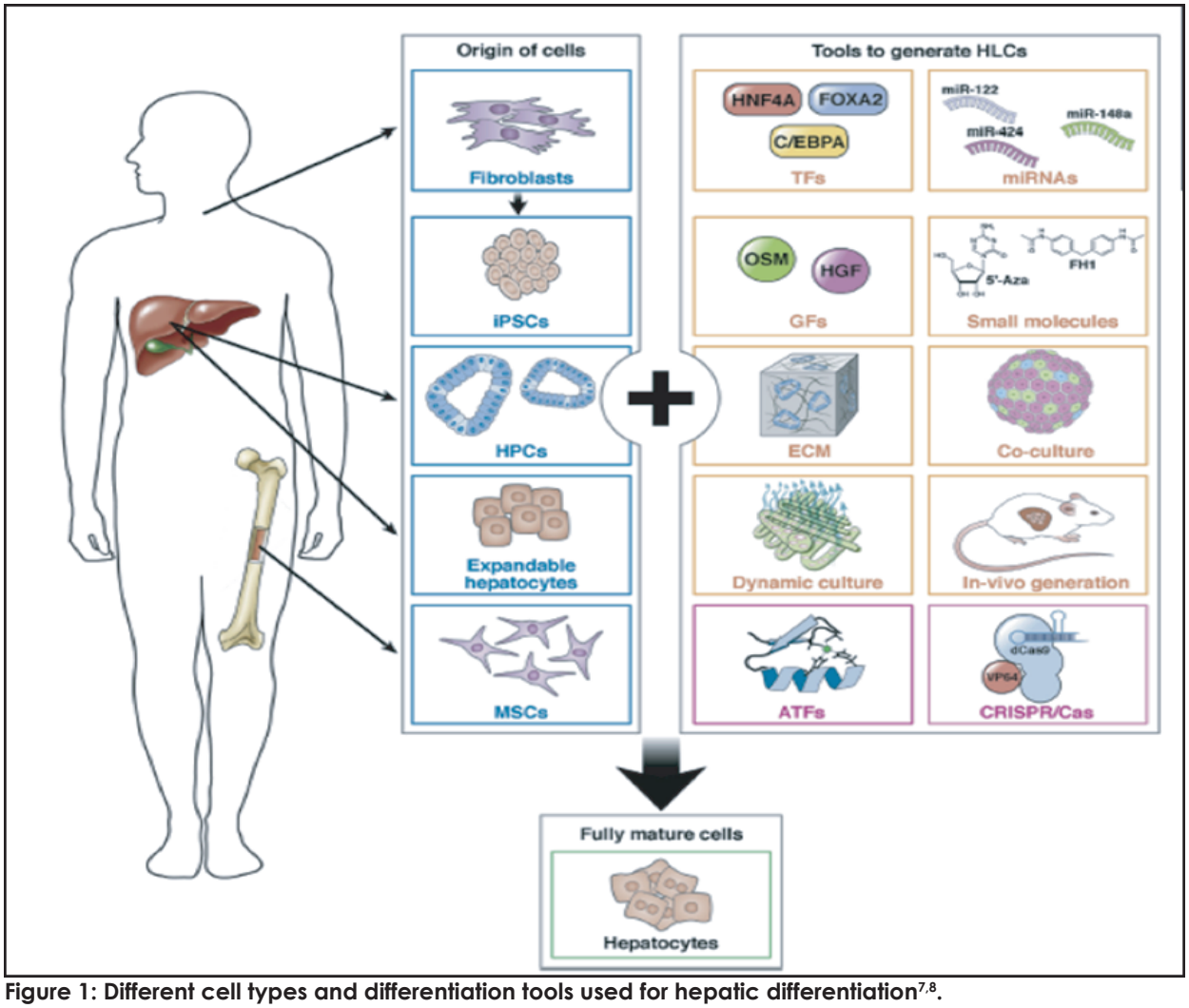


**Figure 1: Different cell types and differentiation tools used for hepatic differentiation[7,8].**

### Adult Stem Cells

The chief source of stem cells in tissue regeneration is Adult stem cells. They are multipotent and obtained easily from diverse sources such as liver, bone marrow, adipose tissue, dental pulp, periodontal ligament, synovial membrane, hair follicle, that can undergone in vitro differentiation[10,11]. Kern et al. and Wagner et al. showed that Adipose tissue derived MSCs had alike features to bone marrow mesenchymal stem cells (BMMSCs)[12,13]; these cells harvested from cosmetic liposuctions and were grown easily under traditional tissue culture settings. Both stem cells were likely to differentiate into hepatocytes[14,15]. Besides this, mesenchymal stem cells are characterized as having immunomodulatory effects by reducing the response of immunocompetent cells. Major limitations of these cells are having a carcinomatous potential and fibrotic effect thus they cannot be utilized for clinical purposes[16-18].

### In Vitro Hepatic Differentiation of Adult Stem Cells

Adult somatic tissues are the source of adult stem cells. Liver stem/progenitor cells reside in liver remain scanty in number, having specific markers and able to differentiate in vitro both in hepatocyte and biliary duct thus contribute in liver regeneration[10,11]. In 2006, Frédéric P Lemaigre concluded that Activin/TGFβ signaling pathway was responsible for liver progenitors (hepatoblasts) fate to differentiate into hepatocytes or biliary cells[19]. Mesenchymal stem cells differentiated into hepatocytes are a source for liver regeneration or tissue engineering[20]. Adult bone marrow mesenchy-

mal stem cells (BMMSCs) transformed into cells with similar structural and functional features of hepatocytes[21]. In a study BMMSCs were differentiated into hepatic like cells when cultured with the mixture of growth factors and small molecules as hepatocyte growth factor (HGF), insulin transferrin-selenium, nicotinamide, Wnt/β- catenin inhibitor (Hexachlorophene). Another study showed that dexamethasone also ameliorates the hepatic differentiation[22,23]. Similarly, using fibroblast growth factor (FGF-4) and hepatocyte growth factor (HGF) BMMSCs were differentiated into hepatocytes[24]. Spindle shaped adipose tissue derived MSCs (ATMSCs) can transform into polygonal hepatic like cells which showed hepatic markers, including albumin (ALB) was enhanced by the addition of trichostatin A (TSA)[14]. The hepatic differentiation of ATMSCs involved two-step protocol with sequential addition of growth factors[25]. Adult multipotent adipose-derived stem cells (M-ADSCs) transformed into hepatocyte like cells by using four-step nonviral protocol, also expressed hepatic markers[26]. Dental pulp derived MSCs (MSC-DP) altered their morphology into hepatic like cells as well as produced albumin and IGF-1 when cultured in the presence of HGF, dexamethasone, oncostatin and incubated with hydrogen sulphide[27]. In case of transplantation of MSCs, it appears to repair injured liver and excel its function[17].

**Embryonic Stem Cells (ESCs)**

The embryoblast is the rich source of embryonic stem cells and acquire the capability to develop into structures of three germ layers. However, application of ESCs has ethical limitation as they are obtained from the inner cell (embryoblast) of blastocyst stage of the embryo and can lead to death of the fetus[16, 28].

**In Vitro Hepatic Differentiation of Embryonic Stem Cells**

In vitro Hepatic differentiation from ESCs various tools of differentiation have been used on the basis signal pathways and hepatogenic growth factors. Such as Activin A, HGF, FGF, epidermal growth factor (EGF), bone morphogenetic protein (BMP), vascular endothelial growth factor (VEGF), oncostatin M (OSM) and touboul's maturation media although the cost of recombinant growth factors were high but hepatocytes generated successfully. Besides growth factors hepatic like cells were produced by using small molecule the cost-effective method, CHIR a GSK3 inhibitor, DMSO, dexamethasone, HGF receptor agonist N-hexanoic-Tyr, Ile-(6) aminohexanoic amide (dihexa) [29-32]. In a research, it was shown that mouse ESCs differentiated from immature to matured hepatocytes via sequential addition of valproic acid and cytokines[33]. Cai et al. successfully established a three-stage method of hepatic differentiation in serum free media on human ESCs lines by the utilization of Activin A, fibroblast growth factor-4 and BMP-2[34].

**Induced Pluripotent Stem Cells (iPSCs)**

Induced pluripotent stem cells have identical characteristics to ESCs, in addition to pluripotency. They can be generated in vitro from any cell of the body except the gametes. These cells have been widely used since the time of their first induction by Yamanaka in 2006, as an alternative to ESCs because of less ethical concerns. However, the propagation of iPSCs is hindered in skills of reprogramming the cells, which require external source of retrovirus transfection, plasmid and direct protein introducing mostly viral vector[16,35].

**In Vitro Hepatic Differentiation of Induced Pluripotent Stem Cells**

After their emergence, iPSCs had been used by many authors reported hepatic like cells (HLCs) generation. Growth factors as Activin A, FGF-4, HGF, BMP-2, and OSM were used to differentiate mouse iPSCs into HLCs, which can perform main liver functions e.g. albumin secretion, glycogen storage, cytochrome P450 activity and indocyanine green uptake and release[36]. Transformation of iPSCs into hepatocytes become successful by utilization of growth factors during in vitro differentiation but the problem of increase cost of growth factors and cytokines would reduce their usage to great extent. Small molecule method, provides the affordable and efficient platform of in vitro hepatic differentiation having great potential in future clinical application[6,36,37]. Yuki Kondo et al. explained the effects of histone deacetylase inhibitor, valproic acid on hepatic transformation of iPSCs[38]. In another study small molecule, Glycogen synthase kinase (GSK) inhibitor such as CHIR99021 utilized in the formation of definitive endoderm (DE) instead of growth factor, this is a cost-effective step toward hepatic differentiation. Further DMSO and Dexamethasone (Figure 2) were used for hepatic fate and maturation[39]. Schematic diagram is representing the stepwise differentiation of hepatocytes.

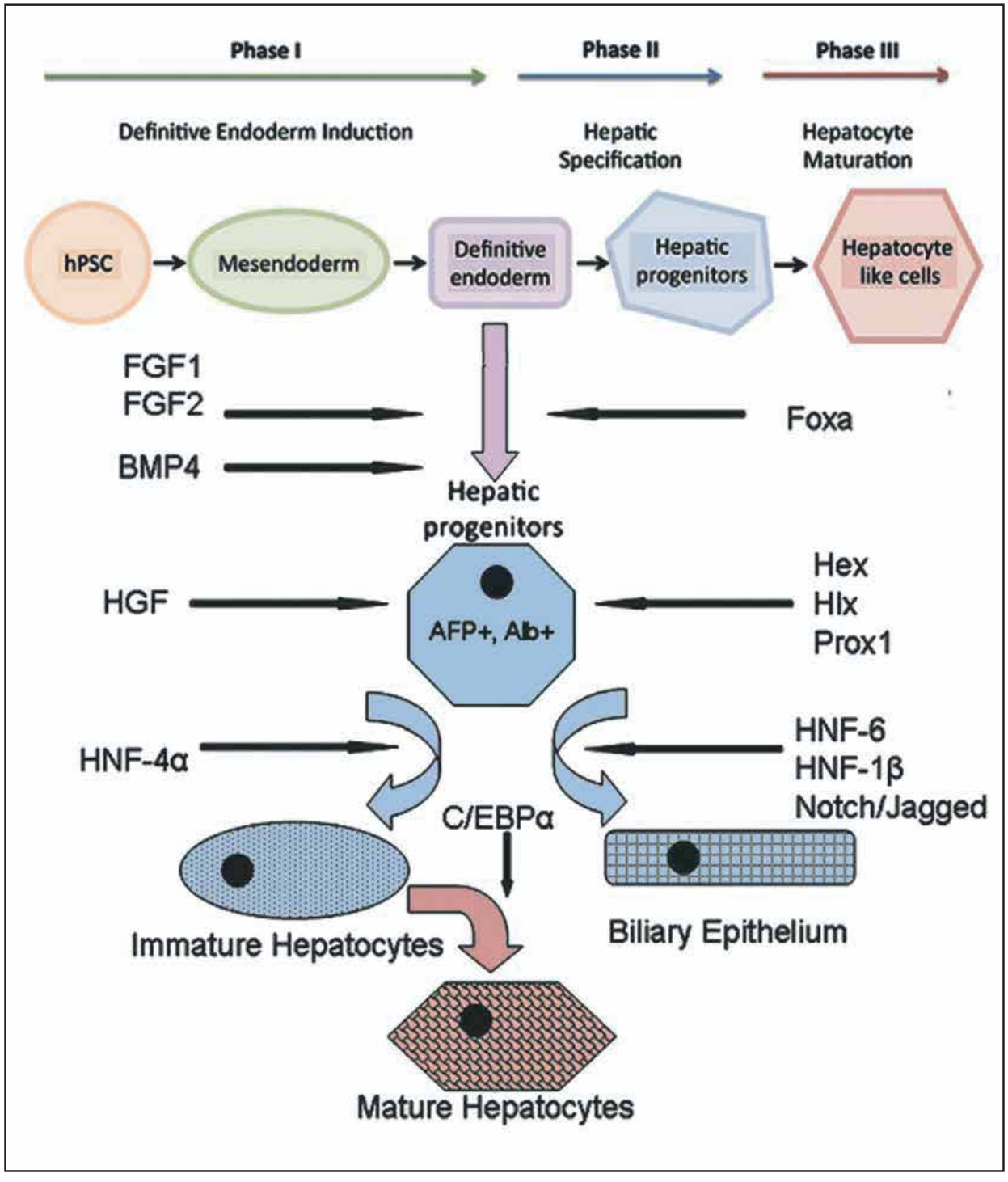


**Figure 2: The phases of differentiation of hepatocytes[39, 40].**

**Perinatal Stem Cells**

Medical wastes after birth such as umbilical cord, placental membrane and amniotic fluid contain plenty of perinatal stem cells, which can be effortlessly, harvested, with low immunogenicity and have no ethical issue. Due to these characteristics after differentiation, they can be transplanted in effective manner. Perinatal stem cells can be divided into characterized and less characterized stem cells. Among them hematological stem (cord blood MSCs) cells first isolated in 1974, used in many hematological diseases are best characterized while the less characterized stem cells retrieve from the placenta, amniotic fluid and from the tissue surrounding the umbilical cord vessels—i.e. Wharton's jelly. Placental derived mesenchymal stem cells (PDMSCs) and Umbilical

cord mesenchymal stem cells (UCMSCs) are enormously populated in fetal membranes and umbilical cord respectively. They can be isolated without invasive procedure, proliferate rapidly due to short doubling time also have trilineage differentiation potential[9,40,41]. Human amniotic epithelial cells (hAECs) are retrieved through noninvasive technique from amniotic membranes of placenta. They are ethically indisputable, anti-fibrotic and immunologically naïve. To keep the transplantation process secure hAECs avoid rejection as they are deficient in telomerase as well as HLA class I and II antigen and do not form teratoma as they express the tumor rejection antigen. (1-60)[42-44].

**In Vitro Hepatic Differentiation of Perinatal Stem Cells**

Placental derived mesenchymal stem cells (PDMSCs) have higher in vitro hepatic differentiation potential than BMMSCs and ATMSCs. Differentiated (HLCs) acquire hepatic phenotype as well as store glycogen, uptake lipoproteins and inclusion of rifampicin enhanced the expression of CYP3A4 [45,46]. UCMSCs avail same culture conditions as BMMSCs to differentiate into HLCs by addition of HGF and FGF-4 but umbilical cord mesenchymal stem cells did not develop into mature hepatocytes, as they did not express HepPar1 or hepatocyte nuclear factor 4[47]. In another study both UCMSCs and BMMSCs were induced in hepatic differentiation media for four weeks and examined for specific hepatic markers i.e. albumin and CYP3A4, UCMSCs shows increased levels of hepatic markers than BMMSCs[48]. In addition, Wharton's jelly MSCs (WJ-MSCs) derived from umbilical cord extracellular matrix can be differentiated into HLCs within 18 days by using hepatocyte growth factor and combination of small molecules such as nicotinamide, insulin transferrin selenium (ITS) and dexamethasone which is the simple, highly efficient and time consuming process[49,50]. In a study, small molecule encouraged hepatic transformation of UCMSCs as valproic acid, induced AKT and ERK signal activation and transform UCMSCs into HLCs both morphologically and functionally[51]. According to latest study, amniotic fluid mesenchymal stem cells (AF-MSCs) are as genetically stable as BMMSCs, a preclinical trial was done and undifferentiated human AF-MSCs transplanted into immunocompetent mice, which lessen hepatic apoptosis, and reduced inflammation and fibrosis of liver[52]. Human amniotic epithelial cells (hAECs) retrieve from human amnion differentiated into hepatic like cells and expressed hepatic markers, such as albumin, alpha-fetoprotein, andalpha1-antitrypsin[44,53]. In a study hAECs were differentiated into other liver cell types, such as hepatic sinusoidal endothelial cells (HSECs) [54]. Hepatic differentiation of hAECs utilized the mixture of growth factors as FGF and HGF and cytokines as Activin-A. Differentiated hAECs expressed markers at levels comparable to those of fetal hepatocytes[43]. Human AECs differentiated into hepatocytes and transplanted in acute liver failure mice, which increased the existence rate of mice and repaired the hepatic damage[55].

## CONCLUSION

Liver transplantation has several limitations; thus, researchers discovered a substitute treatment in the form of "stem cells" which have regenerative and differentiation ability. By investigating stem cells behavior in proliferation, survival, differentiation, and regenerative potential, it would be able to assess their reliable healing potential in different degenerative disorders and bring its application closer to reality. Thus, there is a dire need of a cell type i.e., perinatal stem cells with non-tumorigenic potential, anti-inflammatory and immunomodulatory properties for hepatocytes regeneration. However, the future direction for scientific work is ameliorate the practice of liver regeneration, hepatic like cells that can be culture and multiply repeatedly both in vitro and in vivo that will be able to be used in cell transplantation to achieve the needs of compromised livers for regeneration and for complete replacement of liver transplantation.

## ACKNOWLEDGEMENTS

The authors like to thanks Dr. Rehan Imad and Dr. Ambrina Khatoon for their valuable contribution towards reviewing this article.

## CONFLICT OF INTEREST

There is no conflict of interest among the authors.

## AUTHORS' CONTRIBUTION

QK did the conceptualization, literature search and wrote the draft. SU revised, critically reviewed and scientifically arranged the content. BW and TM did the proof reading of the review article.

## REFERENCES

1. Greenwood HL, Singer PA, Downey GP, Martin DK, Thorsteinsdottir H, Daar AS. Regenerative medicine and the developing world. PLoS Med. 2006;3(9):1-5.
2. Kristin Mattews BK, Keri Sprung. Increased access to stem Cell Interventions or an increased in unproven treatments. Stem Cells Dev. 2018;27(21):1463-1465.
3. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol. 2019;70(1):151-171.
4. Cao Y, Zhang B, Lin R, Wang Q, Wang J, Shen F. Mesenchymal stem cell transplantation for liver cell failure: a new direction and option. Gastroenterol Res Pract. 2018;2018:1-10.
5. Horvat N, Marcelino AS, Horvat JV, Yamanari TR, Batista Araújo-Filho JD, Panizza P, *et a*l. Pediatric liver

transplant: techniques and complications. Radiographics. 2017;37(6):1612-1631.
6. Du C, Feng Y, Qiu D, Xu Y, Pang M, Cai N, *et al.* Highly efficient and expedited hepatic differentiation from human pluripotent stem cells by pure small-molecule cocktails. Stem Cell Res Ther. 2018;9(1):1-5.
7. Hosseini V, Maroufi NF, Saghati S, Asadi N, Darabi M, Ahmad SN, *et al.* Current progress in hepatic tissue regeneration by tissue engineering. J Transl Med. 2019;17(1):1-24.
8. Sauer V, Tchaikovskaya T, Wang X, Li Y, Zhang W, Tar K, *et al.* Human urinary epithelial cells as a source of engraftable hepatocyte-like cells using stem cell technology. Cell Transplant. 2016;25(12):2221-2243.
9. Gaggi G, Izzicupo P, Di Credico A, Sancilio S, Di Baldassarre A, Ghinassi B. Spare parts from discarded materials: fetal annexes in regenerative medicine. Int J Mol Sci. 2019;20(7):1-18.
10. Liu WH, Ren LN, Wang T, Navarro-Alvarez N, Tang LJ. The involving roles of intrahepatic and extrahepatic stem/progenitor cells (SPCs) to liver regeneration. Int J Biol Sci. 2016;12(8):954-963.
11. Liu S, Zhou J, Zhang X, Liu Y, Chen J, Hu B, *et al.* Strategies to optimize adult stem cell therapy for tissue regeneration. Int J Mol Sci. 2016;17(6):1-16.
12. Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, *et al.* Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol. 2005;33(11):1402-1416.
13. Kern S EH, Stoeve 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.
14. Yin L, Zhu Y, Yang J, Ni Y, Zhou Z, Chen Y, *et al.* Adipose tissue-derived mesenchymal stem cells differentiated into hepatocyte-like cells in vivo and in vitro. Mol Med Rep. 2015;11(3):1722-1732.
15. Taléns-Visconti R, Bonora A, Jover R, Mirabet V, Carbonell F, Castell JV, *et al.* Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells. World J Gastroenterol. 2006;12(36): 5834-5845.
16. Nicolas C, Wang Y, Luebke-Wheeler J, Nyberg SL. Stem cell therapies for treatment of liver disease. Biomed. 2016;4(1): p.2.
17. Meier RP, Muller YD, Morel P, Gonelle-Gispert C, Buhler LH. Transplantation of mesenchymal stem cells for the treatment of liver diseases, is there enough evidence? Stem Cell Res. 2013;11(3):1348-1364.
18. Snykers S, De Kock J, Rogiers V, Vanhaecke T. In vitro differentiation of embryonic and adult stem cells into hepatocytes: state of the art. Stem Cells. 2009;27 (3):577-605.
19. Clotman F, Lemaigre FP. Control of hepatic differentiation by activin/TGFbeta signaling. Cell Cycle. 2006;5(2): 168-171.
20. Wu X-B, Tao R. Hepatocyte differentiation of mesenchymal stem cells. Hepatobiliary Pancreat Dis Int. 2012;11(4):360-371.
21. Pilat N, Unger L, Berlakovich GA. Implication for bone marrow derived stem cells in hepatocyte regeneration after orthotopic liver transplantation. Int J Hepatol. 2013;2013:1-7.
22. Chivu M, Dima SO, Stancu CI, Dobrea C, Uscatescu V, Necula LG, *et al.* In vitro hepatic differentiation of human bone marrow mesenchymal stem cells under differential exposure to liver-specific factors. Transl Res. 2009;154(3):122-132.
23. Itaba N, Sakabe T, Kanki K, Azumi J, Shimizu H, Kono Y, *et al.* Identification of the small molecule compound which induces hepatic differentiation of human mesenchymal stem cells. Regen Ther. 2015;2:32-41.
24. Al Ghrbawy NM, Afify RA, Dyaa N, El Sayed AA. Differentiation of bone marrow: derived mesenchymal stem cells into hepatocyte-like cells. Indian J Hematol Blood Transfus. 2016;32(3):276-283.
25. Puglisi MA, Saulnier N, Piscaglia AC, Tondi P, Agnes S, Gasbarrini A. Adipose tissue-derived mesenchymal stem cells and hepatic differentiation: old concepts and future perspectives. Eur Rev Med Pharmacol Sci. 2011;15(4):355-364.
26. Li H, Zhu L, Chen H, Li T, Han Q, Wang S, *et al.* Generation of functional hepatocytes from human adipose-derived MYC(+) KLF4(+) GMNN(+) stem cells analyzed by single-cell RNA-SEQ profiling. Stem Cells Transl Med. 2018;7(11):792-805.
27. Ohkoshi S, Hara H, Hirono H, Watanabe K, Hasegawa K. Regenerative medicine using dental pulp stem cells for liver diseases. World J Gastrointest Pharmacol Ther. 2017;8(1):1-6.
28. Hay DC, Fletcher J, Payne C, Terrace JD, Gallagher RC, Snoeys J, *et al.* Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. Proc Nat Acad Sci. 2008;105(34):12301-12306.
29. Varghese DS, Alawathugoda TT, Ansari SA. Fine tuning of hepatocyte differentiation from human embryonic stem cells: growth factor vs. small molecule-based approaches. Stem Cells Int. 2019;2019:1-18.
30. Siller R, Greenhough S, Naumovska E, Sullivan GJ. Small-molecule-driven hepatocyte differentiation of human pluripotent stem cells. Stem Cell Reports. 2015;4(5):939-952.
31. Touboul T, Chen S, To CC, Mora-Castilla S, Sabatini K, Tukey RH, *et al.* Stage-specific regulation of the WNT/beta-catenin pathway enhances differentiation of hESCs into hepatocytes. J Hepatol. 2016;64(6):1315-1326.
32. Dong XJ, Zhang GR, Zhou QJ, Pan RL, Chen Y, Xiang LX, *et al.* Direct hepatic differentiation of mouse embryonic stem cells induced by valproic acid and cytokines. World J Gastroenterol. 2009;15(41):5165-5175.
33. Cai J, Zhao Y, Liu Y, Ye F, Song Z, Qin H, *et al.* Directed differentiation of human embryonic stem cells into functional hepatic cells. Hepatol. 2007;45(5):1229-1239.
34. Iwamuro M, Komaki T, Kubota Y, Seita M, Kawamoto H, Yuasa T, *et al.* Hepatic differentiation of mouse iPS cells in vitro. Cell Transplant. 2010;19(6):841-847.
35. Wu YM, Huang YJ, Chen P, Hsu YC, Lin SW, Lai HS, *et al.* Hepatocyte-like cells derived from mouse

induced pluripotent stem cells produce functional coagulation factor IX in a hemophilia b mouse model. Cell Transplant. 2016;25(7):1237-1246.
36. Kondo Y, Iwao T, Yoshihashi S, Mimori K, Ogihara R, Nagata K, *et al.* Histone deacetylase inhibitor valproic acid promotes the differentiation of human induced pluripotent stem cells into hepatocyte-like cells. PLoS One. 2014;9(8):1-11.
37. Asumda FZ, Hatzistergos KE, Dykxhoorn DM, Jakubski S, Edwards J, Thomas E, *et al.* Differentiation of hepatocyte-like cells from human pluripotent stem cells using small molecules. Differentiation. 2018;101:16-24.
38. Semenov OV, Koestenbauer S, Riegel M, Zech N, Zimmermann R, Zisch AH, *et al.* Multipotent mesenchymal stem cells from human placenta: critical parameters for isolation and maintenance of stemness after isolation. Am J Obstet Gynecol. 2010;202(2):e1-e13.
39. Hu C, Li L. In vitro and in vivo hepatic differentiation of adult somatic stem cells and extraembryonic stem cells for treating end stage liver diseases. Stem Cells Int. 2015;2015:1-11.
40. Pratama G, Vaghjiani V, Tee JY, Liu YH, Chan J, Tan C, *et al.* Changes in culture expanded human amniotic epithelial cells: implications for potential therapeutic applications. PLoS One. 2011;6(11):1-12.
41. Marongiu F, Gramignoli R, Dorko K, Miki T, Ranade AR, Paola Serra M, *et al.* Hepatic differentiation of amniotic epithelial cells. Hepatology. 2011;53(5):1719-1729.
42. Maymo JL, Riedel R, Perez-Perez A, Magatti M, Maskin B, Duenas JL, *et al.* Proliferation and survival of human amniotic epithelial cells during their hepatic differentiation. PLoS One. 2018;13(1):1-28.
43. Lee KD, Kuo TK, Whang-Peng J, Chung YF, Lin CT, Chou SH, *et al.* In vitro hepatic differentiation of human mesenchymal stem cells. Hepatol. 2004;40(6):1275-1284.
44. Lee HJ, Jung J, Cho KJ, Lee CK, Hwang SG, Kim GJ. Comparison of in vitro hepatogenic differentiation potential between various placenta-derived stem cells and other adult stem cells as an alternative source of functional hepatocytes. Differentiation. 2012;84(3):223-231.
45. MarcSokal DAL. Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. Sci Direct. 2008; 134(3): 833-848.
46. Yu YB, Song Y, Chen Y, Zhang F, Qi FZ. Differentiation of umbilical cord mesenchymal stem cells into hepatocytes in comparison with bone marrow mesenchymal stem cells. Mol Med Rep. 2018;18(2):2009-2016.
47. Prasajak P, Leeanansaksiri W. Developing a new two-step protocol to generate functional hepatocytes from wharton's jelly-derived mesenchymal stem cells under hypoxic condition. Stem Cells Int. 2013;2013:1-11.
48. Varaa N, Azandeh S, Khodabandeh Z, Gharravi AM. Wharton's jelly mesenchymal stem cell: various protocols for isolation and differentiation of hepatocyte-like cells; narrative review. Iran J Med Sci. 2019;44(6):437-448.
49. An SY, Han J, Lim HJ, Park SY, Kim JH, Do BR, *et al.* Valproic acid promotes differentiation of hepatocyte-like cells from whole human umbilical cord-derived mesenchymal stem cells. Tissue Cell. 2014;46(2):127-135.
50. Liu H, Liu DQ, Li BW, Guan LD, Yan ZF, Li YL, *et al.* Human amniotic fluid-derived stem cells can differentiate into hepatocyte-like cells in vitro and in vivo. In Vitro Cell Dev Biol Anim. 2011;47(9):601-608.
51. Miki T. Stem cell characteristics and the therapeutic potential of amniotic epithelial cells. Am J Reprod Immunol. 2018;80(4):1-10.
52. Serra M, Marongiu M, Contini A, Miki T, Cadoni E, Laconi E, *et al.* Evidence of amniotic epithelial cell differentiation toward hepatic sinusoidal endothelial cells. Cell Transplant. 2018;27(1):23-30.
53. Liu QW, Liu QY, Li JY, Wei L, Ren KK, Zhang XC, *et al.* Therapeutic efficiency of human amniotic epithelial stem cell-derived functional hepatocyte-like cells in mice with acute hepatic failure. Stem Cell Res Ther. 2018;9(1):1-15.
54. Monga kN-BaSPS. Wnt/beta-catenin signaling in hepatic organogenesis. Biosci. 2008;4 (2):92-99.
55. Siller R, Greenhough S, Naumovska E, Sullivan GJ. Small-molecule-driven hepatocyte differentiation of human pluripotent stem cells. Stem Cell Rep. 2015;4(5):939-952.