DOI: 10.11844/cjcb.2013.08.0078

不同途径与策略获取成熟肝(样)细胞的研究进展

樊晋宇[1,2] 王 妍[1,2] 李丽丽[1,2] 徐存拴[1,2]*

([1]河南省–科技部共建细胞分化调控国家重点实验室培育基地, 新乡 453007;
[2]河南师范大学生命科学学院, 新乡 453007)

摘要 肝细胞是肝脏生理功能的主要执行者, 在肝脏疾病的细胞治疗与药物研究和开发中具有重要的理论与应用价值。直接分离的原代肝细胞很难在体外增殖培养, 因此从其他途径获取大量成熟的肝细胞一直是备受关注的研究热点。该文简要综述了采用不同策略从肝干细胞、胚胎干细胞、其他成体祖/干细胞以及其他成熟体细胞诱导为成熟肝细胞或肝样细胞的研究成果与最新研究进展, 并对该研究领域进行了小结与展望。

关键词 肝细胞; 肝干细胞; 胚胎干细胞; 诱导多能干细胞; 多能成体祖细胞; 诱导分化; 转分化

Research Progress in Generation of Mature Hepatocytes or Hepatocyte-like Cells by Different Ways and Strategies

Fan Jinyu[1,2], Wang Yan[1,2], Li Lili[1,2], Xu Cunshuan[1,2]*

([1]*Key Laboratory for Cell Differentiation Regulation, Xinxiang 453007, China*; [2]*College of Life Science, Henan Normal University, Xinxiang 453007, China*)

Abstract Hepatocytes perform most of the liver's functions and are very important for the cellular therapy of liver disease as well as in drug research and development. It is difficult for primary cultured hepatocytes to expand and maintain for a long period *in vitro*, so how to get a large number of mature, fully differentiated hepatocytes has always been regarded as one of the most important research problem. In this review, we are going to summarize the results and the latest research progress of derivation of mature hepatocytes or hepatocyte-like cells from liver stem cells, embryonic stem cells, adult progenitor or stem cells as well as other adult cells via different strategies, with the expectation of directions for future research.

Key words hepatocytes; liver stem cells; embryonic stem cells; induced pluripotent stem cells; pluripotent adult progenitor cells; induced differentiation; transdifferentiation

1 引言

肝细胞是肝脏生命活动的主要执行者, 占整个肝脏细胞总数的65%和总肝量的70%~80%。许多理化因素、生物因素等都能引起肝细胞损伤, 导致肝脏疾病的发生[1]。肝细胞移植是治疗肝脏相关疾病, 如急性肝衰竭、遗传代谢性肝病以及病毒等导致的慢性肝衰竭疾病等的一个较好的替代方案[2]。肝细胞移植能弥补或克服原位肝移植治疗方案中的很多缺陷, 如供体肝脏的短缺、原位肝移植的免疫排斥等, 因此越来越受到国内外学者的关注[3-4]。此外, 由于肝细胞是化合物的主要代谢场所, 在药物开发中, 原代肝细胞常被用于细胞水平上药物吸收代谢

收稿日期: 2013-03-26 接受日期: 2013-04-27
河南省基础与前沿技术研究项目(批准号: 092300410015、082300450560)资助的课题
*通讯作者。Tel: 0373-3326001, E-mail: xucs@x263.net
Received: March 26, 2013 Accepted: April 27, 2013
This work was supported by the Basic and Frontier Technology Research Program of Henan province (Grant No.092300410015, 082300450560)
*Corresponding author. Tel: +86-373-3326001, E-mail: xucs@x263.net
网络出版时间: 2013-07-18 16:03 URL: http://www.cnki.net/kcms/detail/31.2035.Q.20130718.1603.002.html

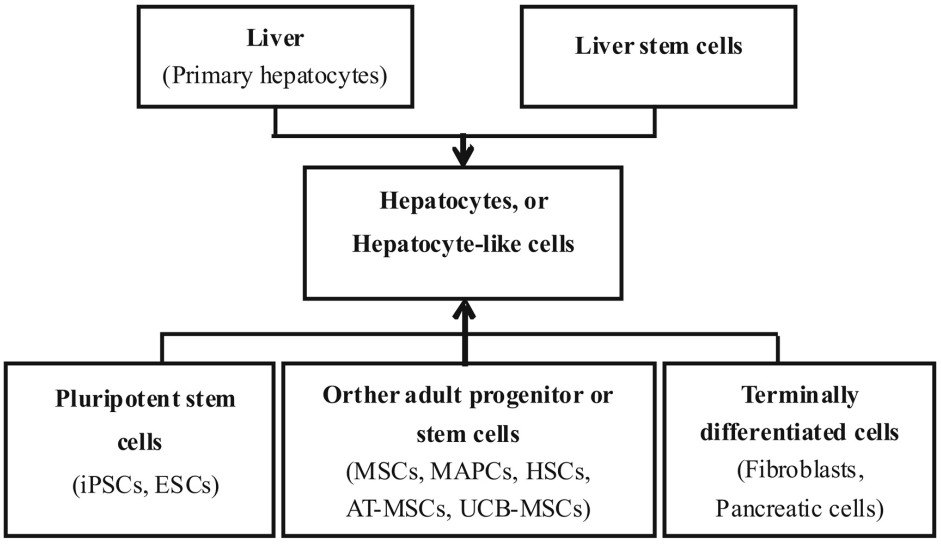

图1 从多种途径获取肝(样)细胞

Fig.1 Different ways to generate hepatocytes or hepatocyte-like cells

检测, 毒理学研究以及安全性评价等[5-6]。

自1969年Berry等[7]首次发明两步灌流法分离肝细胞以来, 许多科研人员对其不断改进[8-9], 形成了非常成熟可靠的原代肝细胞分离方法。两步灌流法辅以密度梯度离心[10]或磁珠分离法[11]即可获得纯度和活性均较高的原代肝细胞。原代肝细胞的培养对其附着基质要求较高, 通常培养在胶原蛋白或明胶包被的培养瓶或三维材料上, 还需在基础培养基中添加胰岛素(insulin)、地塞米松(dexamethasone, Dex)、转铁蛋白(transferrin, Trf)、肝细胞生长因子(hepatocyte growth factor, HGF)、表皮生长因子(epidermal growth factor, EGF)等刺激细胞增殖[12]。尽管如此, 原代肝细胞仅在培养初期能维持较好的形态和功能, 在持续培养中, 肝细胞特异的功能逐渐消失, 并且很难增殖、扩大培养[13-14]。尤其对于具有临床应用价值的人源原代肝细胞, 由于其来源较少且扩大培养较难等问题, 极大地限制了肝病细胞疗法的研究与应用。因此, 如何通过其他途径获取成熟肝细胞成为多年来生命科学领域备受关注的热点问题[15]。目前, 该领域的研究已取得了显著的进展(图1)。本文简要综述了采用不同策略从不同来源细胞诱导获取肝(样)细胞的研究成果与进展。

2 源于肝干细胞途径

肝干细胞(liver stem cells, LSCs或hepatic stem cells, HSCs, 本文采用前一个简称)是肝脏内能定向分化为肝细胞或胆管上皮细胞的一类成体干细胞(adult stem cells, ASCs)[16], 在啮齿目动物中常称之为卵圆细胞(oval cells, OVCs)[17]。当肝脏受药物危害慢性损伤或肝细胞分裂被抑制时, LSCs被激活增殖、更新, 并分化为肝细胞和胆管上皮细胞, 参与肝损伤的修复和再生[1,18], 这也被认为是肝干细胞介导的再生模型。不同文献显示的LSCs标志物蛋白仍有争议, 但目前认为比较确切的标志物有上皮细胞黏附分子(epithelial cellular adhesion molecule, EpCAM)[19]、分化抗原群133(cluster of differentiation, CD133)[16]、性别决定区Y框9[sex determining region Y (SRY) box 9, Sox9][20-21]、富含亮氨酸重复序列的G蛋白偶联受体5(leucine-rich-repeat-containing G-protein-coupled receptor 5, Lgr5)[22]等。

LSCs具有一般成体干细胞的特征, 能在体内定向分化为肝细胞或胆管上皮细胞, 因此在细胞治疗中通常直接移植LSCs以补偿受损肝细胞[23-24]。LSCs极易在体外进行增殖, 并保持其自我更新与分化潜能。通过添加HGF与成纤维生长因子4(fibroblast growth factor 4, FGF4), LSCs能在体外定向分化为肝细胞, 表达肝细胞的标志蛋白, 并具有肝细胞的大多数功能[16,25]。虽然LSCs能在体外扩增培养, 并能诱导为成熟肝细胞, 然而其来源仍然有很大局限性, 通常是从慢性肝病损伤患者[26-28]分离, 或从药物损伤诱导及2/3肝切除的模型动物分离[17,23,29]。

3 源于胚胎干细胞

胚胎干细胞(embryonic stem cells, ESCs)在去除饲养层细胞或白血病抑制因子(leukemia inhibitory factor, LIF)后, 能自发分化形成包括三个胚层细胞的胚状体(embryoid bodies, EBs)[30]。在1996年, Abe等[31]发现在培养小鼠胚胎干细胞过程中, ESCs聚集形成EBs后, 能够表达内胚层特异的肝谱系基因甲胎蛋白(alpha-fetoprotein, AFP)、白蛋白(albumin, ALB)、转甲状腺素蛋白(transthyretin, TTR)、肝细胞核因子1(hepatocyte nuclear factor 1, HNF1)、肝细胞核因子-3β(hepatocyte nuclear factor-3β, HNF-3β)等。该实验从分子水平检测到ESCs自发形成的EBs包含内胚层肝谱系细胞, 为胚胎干细胞能发育成肝细胞提供了依据。

2001年, Hamazaki等[32]研究了小鼠ESCs分化为肝细胞的情况。ESCs在去除LIF自发分化形成EBs后, 在持续培养的第6天表达*Afp*和α-抗胰蛋白酶(alpha antitrypsin, AAT); 第12天表达*Alb*; 此后, 继续培养的细胞始终未检测到成熟肝细胞的标志物葡萄糖-6-磷酸酶(glucose-6-phosphatase, G6P)和酪氨酸氨基转移酶(tyrosine aminotransferase, TAT)。若EBs在培养的第9~12天中加入酸性成纤维细胞生长因子(acidic fibroblast growth factor, aFGF); 第12~18天中加入HGF; 第15~18天同时加入制瘤素M(oncostatin M, OSM)、地塞米松、胰岛素和转铁蛋白及亚硒酸盐组合(insulin plus transferring plus selenium acid, ITS), 则EBs细胞逐步向肝细胞方向分化, 形成肝样细胞, 并表达*Afp*、*Aat*、*Alb*、*G6p*和*Tat*等肝特异性基因。该实验说明EBs的自发分化虽然包括肝谱系细胞, 但是不会自发形成成熟的肝细胞, 只有加入外源诱导因子后才可能形成成熟肝细胞。Cai等[33]和Agarwal等[34]利用类似的细胞因子组合激活素A(activin A)、ITS、FGF4、骨形态发生蛋白2(bone morphogenetic protein 2, BMP2)、HGF、Dex、aFGF、碱性成纤维细胞生长因子(basic fibroblast growth factor, bFGF)及骨形态发生蛋白4(bone morphogenetic protein 4, BMP4)分别将人的ESCs逐步诱导为肝样细胞。Baharvand等[35]采用类似的诱导方式, 建立了从ESCs到肝细胞分化的三维培养体系。相较于二维培养系统, 三维培养条件诱导的细胞形态结构更接近肝细胞, 能更早检测到*Alb*和*G6p*的表达, 诱导形成的肝样细胞具有更高的尿素合成能力和*Afp*表达水平。电子显微镜结果显示, 三维培养的肝样细胞具有类似肝细胞的显微结构, 包括线粒体、糖原、内质网、细胞核、核仁以及脂肪颗粒等。

Takayama等[36]和Inamura等[37]则发现, 细胞因子SOX17(Sry-related HMG box 17, SOX17)和造血干细胞同源表达框因子(hematopoietically expressed homeobox, HEX)能将胚胎干细胞诱导分化为内胚层细胞及肝谱系细胞, 在加入肝细胞核因子4α(hepatocyte

表1 胚胎干细胞来源的肝样细胞

Table 1 ES cell-derived hepatocyte-like cells

来源细胞 Original cells	诱导因子 Induction factors	肝细胞特异基因表达 Hepatocytes-specific genes expression	肝细胞功能 Hepatocytes function	参考文献 References
mES	aFGF, HGF, OSM, Dex and ITS	*Afp*, *Alb*, *Aat*, *Ttr*, *G6p*, *Tat*	JNK activity	[32]
mES	aFGF HGF, OSM, ITS, bFGF, and Dex	*Alb*, *Afp*, *Tat*	Albumin secretion, urea production	[38]
hES	aFGF, HGF, OSM, Dex and ITS	*HNF3β*, *AFP*, *TTR*, *AAT*, *CK-8*, *CK-18*, *CK-19*, *TDO*, *ALB*, *CYP7a1*, *G6P*, *TAT*	Urea production, albumin secretion, glycogen storage, ICG uptake	[35]
hES	Activin A, FGF4, BMP2, HGF, OSM and Dex	*AFP*, *ALB*, *CK8*, *CK18*, *G6P*, *AAT*, *HNF4α*, *PEPCK*, *TDO2*, *CYP7a1*, *TAT*, *CYP3a4*, *CYP2b6*	Albumin secretion, cytochrome p450 activity, glycogen storage, ICG uptake, LDL uptake	[33]
hES	Activin A, FGF4, HGF, OSM and Dex.	*GATA4*, *HNF4α*, *AFP*, *ALB*, *AAT*, *CYP3a4*, *CYP7a1*	Albumin secretion, cytochrome p450 activity, glycogen storage, ICG up take	[34]
hES	Activin A, NaB, DMSO, HGF and OSM	*HNF4α*, *TTR*, *TO*, *CAR*, *APOF*, *CYP3a4*, *CYP3a7*, *CYP2d6*, *CYP2c9*, *AFP*, *ALB*, *TAT*, *CYP2c19*	Cytochrome p450 activity, glycogen storage, produce and export plasma proteins (albumin, A2M fibrinogen, fibronectin)	[39]

m: 来源于小鼠; r: 来源于大鼠; h: 来源于人。

m means mouse; r means rat; h means human.

nuclear factor 4α, Hnf4α)后, ESCs能诱导为成熟肝样细胞, 表达肝细胞特异基因, 具有成熟肝细胞的多种功能。

迄今, 通过不同的细胞因子组合, 多个种属的ESCs细胞被成功地诱导分化为形态和功能上与肝细胞类似的肝样细胞(表1)。然而, 由于ESCs的获取需要破坏胚胎, 存在伦理问题; 此外, 当用ESCs来源的肝样细胞做肝病的细胞移植治疗时, 由于肝样细胞的主要组织相容性复合体(major histocompatibility complex, MHC)与受体的不同, 往往存在免疫排斥。

4 源于诱导性多能干细胞

诱导性多能干细胞(induced pluripotent stem cells, iPSCs)是在形态、基因表达、表观遗传修饰状态、细胞自我更新、以及分化潜能等方面与胚胎干细胞相似的一类细胞。Takahashi等[40]发现, 通过四个转录因子组合(*Oct4*、*Sox2*、*Klf4*和*c-Myc*)可将小鼠成纤维细胞逆分化为iPSCs。此后, 各个实验室又相继将人[41]、大鼠[42]等不同种属的体细胞转化成iPSCs。

2009年, Song等[43]首次将人源iPSCs诱导分化成为肝样细胞。他们模拟肝细胞的发育过程, 采取类似于ESCs逐步诱导分化为肝细胞的方法, 分别在诱导的0~3, 3~7, 7~13, 13~21天用Activin A和成纤维生长因子(fibroblast growth factor, FGF)组合、BMP2和HGF组合、成纤维生长因子7(fibroblast growth factor 7, FGF7)和OSM组合, 以及Dex分阶段诱导。结果显示, 在内胚层诱导阶段有其特异性基因*Sox17*、*Foxa2*(Forkhead box a2)等表达, 在肝细胞特异分化阶段有*Afp*、*Alb*、*Ck18*(cytokeratin18, CK18)、*Ck19*(cytokeratin19, Ck19)、*Pepck*(phosphoenolpyruvate carboxykinase)等表达。iPSCs的形态也从最开始的胚胎干细胞样变成了类似上皮样细胞的肝样细胞。诱导形成的肝样细胞能够储存糖原、合成尿素、分泌白蛋白, 有细胞色素P450的活性表达。此后, 多个实验室采用类似的方法将人源或小鼠的iPSCs诱导形成了肝样细胞(表2)[44-47]。

从iPSCs诱导肝样细胞与从ESCs诱导肝样细胞类似。该途径诱导肝样细胞避开了胚胎干细胞所涉及的伦理道德问题, 实验重复性好, 细胞来源广泛, 而且还可通过iPSCs途径诱导出源自患者自身的肝样细胞, 在细胞移植时能避开免疫排斥问题, 是获取大量肝样细胞的一大进步。然而, 目前常用的iPSCs诱导方法中存有潜在的不安全因素, 如慢病毒载体、原癌基因(如*c-Myc*)等。在iPSCs分化过程中有较高的癌细胞转化倾向, 并且iPSCs常残留有来源体细胞的遗传记忆。

表2 诱导性多能干细胞来源的肝样细胞

Table 2 iPSCs cell-derived hepatocyte-like cells

来源细胞 Original cells	诱导因子 Induction factors	肝细胞特异基因表达 Hepatocytes-specific genes expression	肝细胞功能 Hepatocytes function	参考文献 References
miPSCs	Activin A, Wnt3a, HGF, DMSO, OSM, Dex and ITS	*Afp*, *Hnf4*, *Alb*, *Ck18*, *G6p*, *Tdo2*, *Tat*, *Cyp3a4*, *Cyp7a1*	Cytochrome p450 activity, glycogen storage, LDL uptake	[45]
hiPSCs	Activin A, BMP2, FGF4, HGF, FGF7, OSM, Dex and N2B27	*CYP2a6*, *CYP3a4*, *CK19*, *AAT*, *TDO2*, *AFP*, *ALB*, *CK8*, *CK18*	Urea production, albumin secretion, cytochrome p450 activity, glycogen storage	[43]
hiPSCs	CDM, Activin, FGF, BMP4, Ly, RPMI, HGF, OSM	*ALB*, *AFP*, *AAT*, *PEPCK*, *G6P*	Urea production, albumin secretion, cytochrome p450 activity, glycogen storage, LDL uptake	[44]
hiPSCs	Activin A, bFGF, BMP4, FGF4, HGF, FGF1, FGF4, FGF10 , OSM , Dex, FOXA2 , HNF1a	*AAT*, *CYP3a4*, *ALB*, *CYP2d6*, *CYP3a4*, *CYP7a1*, *TO*, *CYP3a7*, *CYP2c9*, *CYP2c19*	Urea production, albumin secretion, cytochrome p450 activity, glycogen storage, ICG uptake, LDL uptake	[47]
hiPSCs	Activin A, Wnt3a, HGF, DMSO, OSM, Dex and ITS	*AFP*, *HNF4*, *ALB*, *CK18*, *G6P*, *TDO2*, *TAT*, *CYP3A4*, *CYP7a1*	CYP3A4 enzyme activity, glycogen storage, LDL uptake, urea production	[46]

表3 成体祖/干细胞来源的肝样细胞

Table 3 Adult progenitor/stem cells-derived hepatocyte-like cells

来源细胞 Original cells	诱导因子 Induction factors	肝细胞特异基因表达 Hepatocytes-specific genes expression	肝细胞功能 Hepatocytes function	参考文献 References
r/m/hMAPC	FGF4, HGF	*Hnf3β*, *Gata4*, *Ck19*, *Afp*, *Ck18*, *Ttr*, *Hnf-4*, *Hnf1α*, *Alb*, *Cyp2b9*, *Cyp2b13*, *Cyp1b1*	Urea production, albumin secretion, cytochrome p450 activity, LDL uptake, glycogen storage	[51]
rMSC/HSCs	FN, Dex, EGF, ITS, aFGF, bFGF and HGF	*Ck18*, *Alb*, *Afp*	Albumin secretion	[52]
rMSC	HGF and FGF4	*Afp*, *Alb*	AFP and albumin secretion, urea production, glycogen storage	[53]
rBMSC	FGF4, HGF, ITS and Dex	*Afp*, *Hnf3β*, *Alb*, *Ck18 and Hnf1α*	Albumin secretion, urea production, glycogen storage	[61]
rMAPC	Activin, Wnt3a, bFGF, BMP4, FGF8b, aFGF, FGF4, HGF and FN	*Afp*, *Ttr*, *Alb*, *Aat*, *Tat*, *G6p*, *Mgst1*, *Factor V*, *Arginase1*, *Hnf4α*	Albumin secretion, urea production	[62]
rMAPC	Activin A, Wnt3a, BMP4, FGF2, FGFs(FGF1, FGF4 and FGF8), HGF, FN	*Afp*, *Ttr*, *Krt19*, *Alb*, *Aat*, *Tat*, *G6p*, *Cyp1a2* and *Factor V*	Urea production, albumin secretion, GST activity, cytochrome p450 activity	[60]
mHSCs	SCF, IL-6, FLT3, OSM, EGF, FGF4, TGF-α	*Alb*, *Ck18*, *Tdo*, *Tat*	Cytochrome p450 activity	[63]
hUCB	FGF1, FGF2, LIF, SCF and HGF	*ALB*, *GS*, *CK18*, *CK19*, *AFP*	Albumin secretion	[54]
hBMSCs	HGF, bFGF, OSM, Dex and ITS	*AFP*, *HNF3β*, *ALB*, *CK18*, *CYP2b6*, *TAT*, *TO*, *G6P*, *HNF4* and *HNF1α*	Albumin secretion, cytochrome p450 activity, glycogen storage, LDL uptake, Urea production	[64]
hUCB- MSCs	Dex, ITS, HGF, ITS and OSM	*ALB*, *AFP*, *CK18*, *GS*, *TAT*, *HGF*, *C-MET*, *PEPCK*, *CPS*	Albumin secretion, LDL uptake	[55]
hAT-MSCs	FGF1, FGF4, HGF, OSM, Dex	*ALB*, *AFP*, *TTR*, *TDO2*, *CYP7a1*, *HNF4α*	Albumin secretion, urea production, LDL uptake	[58]

5 源于其他多能成体祖/干细胞

源于内胚层的肝细胞不仅能从同胚层的肝干细胞诱导而来, 也能从具多潜能的ESCs或iPSCs细胞诱导而来, 还能从其他胚层的成体祖/干细胞诱导分化而来(表3)。研究最早最多的是骨髓中的各种非肝源成体祖/干细胞。骨髓是一个成体祖/干细胞的细胞库, 包含多种成体祖/干细胞, 如造血干细胞(hematopoietic stem cells, HSCs), 间充质干细胞(mesenchymal stem cells, MSCs), 成体祖细胞 (multipotent adult progenitor cells, MAPCs)等[48]。

1999年, Petersen等[49]首次提出, 在肝再生的干细胞增殖模型中, 增殖的肝细胞可能来源于骨髓中的某种细胞。他们通过实验确证了这个观点, 即用致死剂量辐射照射的雌性受体大鼠接受雄性大鼠的骨髓移植, 再将受体大鼠用2-乙酰氨基芴(2-acetylaminofluoren, 2-AAF)处理, 终止其肝细胞的增殖后, 用CCl_4或2/3肝切除启动肝再生, 结果检测到受体大鼠中肝细胞的增殖部分来源于供体大鼠的骨髓细胞。该实验虽然未能确定何种骨髓细胞转变成肝细胞, 但开启了其他成体祖细胞向肝细胞诱导分化的研究。

2000年, Lagasse等[50]进一步确证了骨髓中的HSCs能转变成肝细胞。实验以延胡索乙酰水解酶基因缺陷(fumarylacetoacetate hydrolase, $Fah^{-/-}$)小鼠为受体, 用致死辐射照射后, 将供体野生型$Fah^{+/+}$小鼠的HSCs移植到受体小鼠, 结果受体小鼠的肝功能逐渐恢复, 并在肝脏中检测到来自供体的$Fah^{+/+}$肝细胞, 说明HSCs在体内能转变成肝细胞。2002年, Schwartz等[51]在体外将骨髓中的多能成体祖细胞MAPCs诱导分化为肝样细胞。实验以人、小鼠和大鼠来源的MAPCs用含FGF4和HGF的培养基培养, 7天后检测到肝细胞谱系的标志基因*Hnf-*

3β、*Gata4*、*Ck19*、*Ttr*和*Afp*; 14~21天检测到*Alb*、*Ck18*、*Hnf-4*(hepatocyte nuclear factor-4, Hnf-4)和*Hnf-1α*(hepatocyte nuclear factor-1α, Hnf-1α)。诱导的肝样细胞能分泌白蛋白、尿素, 能够储存糖原, 吸收低密度脂蛋白, 有苯巴比妥诱导的细胞色素P450活性。Shu等[52]和Kang等[53]则分别从体内和体外将骨髓中的另一种成体干细胞MSCs诱导为肝样细胞。

除了骨髓中的成体祖/干细胞能在体内外诱导分化为肝样细外, 其他来源的多种间充质干细胞也已被诱导为肝样细胞, 如来源于脐带血的间充质干细胞(umbilical cord blood-derived mesenchymal stem cells, UCB-MSCs)[54-56]、来自胎儿肺的间充质干细胞[57]、来自脂肪组织的间充质干细胞(adipose tissue-derived mesenchymal stem cells, AT-MSCs)[58-59]等。

从多种非肝脏来源的成体祖/干细胞诱导形成肝样细胞的研究, 提供了更多的肝样细胞获取途径。然而该途径的肝样细胞诱导分化效率还较低, 且中胚层细胞等杂细胞较多, 因此在实际应用中依然有较大的限制[49,60]。

6 从其他体细胞直接转分化

早在1981年, Scarpelli等[65]就发现在金色叙利亚仓鼠(Syrian golden hamster)的胰腺细胞再生增殖时, 加入胰腺细胞癌诱导剂N-亚硝基-双[2-氧丙烷基]胺(N-nitrosobis(2-oxopropyl) amine, BOP)后, 部分胰腺细胞能在体内转分化为肝样细胞。而Dabeva等[66]发现胰腺上皮祖细胞(pancreatic epithelial progenitor cells, PEPCs)移植到肝脏后, 也能在体内转分化为肝样细胞, 表达肝细胞的特异基因。2002年, Tosh等[67]首次在体外, 用Dex将胰腺细胞系AR42J-B13诱导转分化为肝样细胞, 并表达肝细胞的标志蛋白谷氨酰胺合成酶(glutamine synthetase, GS)、氨甲酰磷酸合成酶I(carbamoylphosphate synthase I, CPS I)、葡萄糖-6-磷酸酶、转甲状腺素蛋白、白蛋白及转铁蛋白等。

2011年, Huang[68]等首次利用$p19^{Arf-/-}$($p19^{Arf}$由*Cdkn2a*编码, 即细胞周期蛋白依赖性激酶抑制剂2a, cyclin-dependent kinase inhibitor 2a, Cdkn2a)小鼠模型的鼠尾成纤维细胞, 从14个与肝脏发育相关的特异转录因子中筛选出3个转录因子的组合, 即*Gata4*、*Hnf1a*和*Foxa3*(Forkhead box a3)能将小鼠鼠尾成纤维细胞直接诱导转分化成肝样细胞(induced hepatocyte-like cells, iHep)。iHep细胞具有典型的上皮样细胞形态, 能表达肝细胞特异基因如*Alb*、*Ttr*、*Aat*、*Trf*、*Ck8*、*Ck18*等, 并且具有成熟肝细胞的功能, 如尿素合成、糖原储存、吲哚菁绿(indocyanine green, ICG)与低密度脂蛋白(low-densitylipoprotein, LDL)吸收以及细胞色素p450活性等。iHep细胞移植到$Fah^{-/-}$小鼠后, 近一半的小鼠肝脏功能得到恢复而存活下来。同年, Sekiya等[69]从12个与肝细胞发育相关的转录因子中, 筛选出另外3个转录因子组合, 即*Hnf4α*与*Foxa1*(Forkhead box a1), *Hnf4α*与*Foxa2*或*Hnf4α*与*Foxa3*均能将小鼠胚胎成纤维细胞(mouse embryonic fibroblasts, MEFs)或成体成纤维细胞直接转分化为iHep。形成的iHep表达成熟肝细胞的特异基因, 具有成熟肝细胞功能, 也同样能将$Fah^{-/-}$小鼠

表4 成体细胞来源的肝样细胞

Table 4 Adult cells-derived hepatocyte-like cells

来源细胞 Original cells	诱导因子 Induction factors	肝细胞特异基因表达 Hepatocytes-specific genes expression	肝细胞功能 Hepatocytes function	参考文献 References
rPancreatic cells	Dex, OSM	ND	Albumin secretion, expression of marker enzymes (G6Pase, CPS I, GS *et al*)	[67]
mPancreatic cells	BOP	ND	Glycogen storage, albumin secretion	[65]
mFibroblasts	Gata4, Hnf1a and Foxa3	*Alb*, *Ttr*, *Aat*, *Transferrin*, *Pah*, *Ck8*, *Ck18*, *Gjb1*, *Cldn2*, *Gata4*, *Foxa2*, *Foxa3*, *Hnf1a*, *Hnf4a*, *Col1a1*, *Pdgfrb*, *Postn*, *Fsp1*, *E-cadherin*	Urea production, cytochrome p450 activity, glycogen storage, ICG uptake, LDL uptake	[68]
mFibroblasts	Hnf4 a plus Foxa1, Foxa2 or Foxa3	*Comt1*, *Nat2*, *Nat1*, *MaoA*, *MaoB*, *Tpmt*, *Gsta4*, *Gstt1*, *Ugt1a1*, *Sult1a1*, *Fmo1*, *Mgst1*, *Aat*, *Fmo3*, *Fmo5*, *Hnmt*, *Nnmt*, *Gs*	Albumin secretion, urea production, glycogen storage, cytochrome P450 activity, ICG uptake, drugs metabolization	[69]

ND: 表示未检测。

ND: means no detection.

的肝功能恢复。

体细胞直接转分化为肝样细胞, 越过了细胞重编程至多潜能干细胞的状态, 减少了获取肝样细胞的时间及成本, 并极大地降低了细胞转化过程中癌变的风险。然而, 迄今成熟体细胞直接转分化为肝样细胞的研究结果还较少, 仅在两类细胞中取得了成功, 且转分化的效率还比较低, 转化程度参差不齐(表4)。

7 结语与展望

肝细胞在基础研究、临床疾病治疗以及药物研究与开发中具有重要的价值。直接分离的原代肝细胞虽然较完整地保留了肝细胞的遗传特征与功能, 但肝细胞的多种生理功能易在体外培养中消失, 并且很难分裂增殖。这也促进了人们采用不同策略从其他途经获取肝细胞的研究, 如从肝祖/干细胞、胚胎干细胞或诱导性多能干细胞、各种成体祖细胞以及其他体细胞的直接转分化。然而从这些途径获取的肝细胞或肝样细胞与原代分离的肝细胞还有较大差异, 距离临床或药物开发等应用仍有较长的路需探索。理想的体外培养肝细胞应该具有这样的特征: 诱导形成的肝(样)细胞能保持肝细胞特征, 具有肝脏特异性的代谢、解毒、合成、分泌等功能, 并且来源充足、纯净, 可体外扩增培养, 在肝病的细胞治疗中没有形成肿瘤的风险。因此, 在诱导肝(样)细胞的研究中, 最主要的是研究肝细胞定向分化中最关键的因素是什么, 若能找出更多的决定肝细胞分化命运的关键基因或化合物, 将极大地提高肝细胞的诱导效率, 获得更接近于肝细胞特征与功能的肝样细胞。此外, 起始细胞选择也至关重要, 如从iPSCs细胞或其他易获得的体细胞诱导获得肝细胞, 在再生医学与未来的个性化诊疗研究中显示出更加诱人的前景。相信在未来的研究中, 这些领域将取得更大进步, 人们能从理想的途径获取用于肝脏疾病治疗、药物开发、基础研究等的肝细胞或肝样细胞。

参考文献 (References)

1 Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol 2004; 5(10): 836-47.

2 Ohashi K, Park F, Kay MA. Hepatocyte transplantation: Clinical and experimental application. J Mol Med 2001; 79(11): 617-30.

3 Yu CH, Chang MH, Chien CS, Chen YH, Chang MF, Chen HL. Hepatocyte transplantation and the differentiation fate of host oval cells in acute severe hepatic injury. Cell Transplant 2010; 19(2): 231-43.

4 Skvorak KJ, Paul HS, Dorko K, Marongiu F, Ellis E, Chace D, *et al*. Hepatocyte transplantation improves phenotype and extends survival in a murine model of intermediate maple syrup urine disease. Mol Ther 2009; 17(7): 1266-73.

5 LeCluyse EL. Human hepatocyte culture systems for the *in vitro* evaluation of cytochrome P450 expression and regulation. Eur J Pharm Sci 2001; 13(4): 343-68.

6 Guguen-Guillouzo C, Guillouzo A. General review on *in vitro* hepatocyte models and their applications. Methods Mol Biol 2010; 640: 1-40.

7 Berry MN, Friend DS. High-yield preparation of isolated rat liver parenchymal cells: A biochemical and fine structural study. J Cell Biol 1969; 43(3): 506-20.

8 Bhogal RH, Hodson J, Bartlett DC, Weston CJ, Curbishley SM, Haughton E, *et al*. Isolation of primary human hepatocytes from normal and diseased liver tissue: A one hundred liver experience. PLoS One 2011; 6(3): e18222.

9 Zhang ZG, Li XB, Gao L, Liu GW, Kong T, Li YF, *et al*. An updated method for the isolation and culture of primary calf hepatocytes. Vet J 2012; 191(3): 323-6.

10 Dalet C, Fehlmann M, Debey P. Use of Percoll density gradient centrifugation for preparing isolated rat hepatocytes having long-term viability. Anal Biochem 1982; 122(1): 119-23.

11 Gailhouste L. Isolation and purification method of mouse fetal hepatoblasts. Methods Mol Biol 2012; 826: 33-47.

12 Shulman M, Nahmias Y. Long-term culture and coculture of primary rat and human hepatocytes. Methods Mol Biol 2013; 945: 287-302.

13 Tan GD, Toh GW, Birgersson E, Robens J, van Noort D, Leo HL. A thin-walled polydimethylsiloxane bioreactor for high-density hepatocyte sandwich culture. Biotechnol Bioeng 2013;110(6): 1663-73.

14 Yamamoto N, Wu J, Zhang Y, Catana AM, Cai H, Strom S, *et al*. An optimal culture condition maintains human hepatocyte phenotype after long-term culture. Hepatol Res 2006; 35(3): 169-77.

15 Ji S, Zhang L, Hui L. Cell fate conversion: Direct induction of hepatocyte-like cells from fibroblasts. J Cell Biochem 2013; 114 (2): 256-65.

16 Herrera MB, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, *et al*. Isolation and characterization of a stem cell population from adult human liver. Stem Cells 2006; 24(12): 2840-50.

17 Yovchev MI, Grozdanov PN, Joseph B, Gupta S, Dabeva MD. Novel hepatic progenitor cell surface markers in the adult rat liver. Hepatology 2007; 45(1): 139-49.

18 Zhao Q, Ren H, Zhu D, Han Z. Stem/progenitor cells in liver injury repair and regeneration. Biol Cell 2009; 101(10): 557-71.

19 Turner R, Lozoya O, Wang Y, Cardinale V, Gaudio E, Alpini G, *et al*. Human hepatic stem cell and maturational liver lineage biology. Hepatology 2011; 53(3): 1035-45.

20 Dorrell C, Erker L, Lanxon-Cookson KM, Abraham SL, Victoroff T, Ro S, *et al*. Surface markers for the murine oval cell response. Hepatology 2008; 48(4): 1282-91.

21 Dorrell C, Erker L, Schug J, Kopp JL, Canaday PS, Fox AJ, *et al*. Prospective isolation of a bipotential clonogenic liver progenitor cell in adult mice. Genes Dev 2011; 25(11): 1193-203.

22 Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, *et al*. *In vitro* expansion of single Lgr5+ liver stem cells induced

by Wnt-driven regeneration. Nature 2013; 494(7436): 247-50.
23 Yovchev MI, Grozdanov PN, Zhou H, Racherla H, Guha C, Dabeva MD. Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. Hepatology 2008; 47(2): 636-47.
24 Li Z, Chen J, Li L, Ran JH, Li XH, Liu ZH, *et al*. Human hepatocyte growth factor (hHGF)-modified hepatic oval cells improve liver transplant survival. PLoS One 2012; 7 (9): e44805.
25 Duret C, Gerbal-Chaloin S, Ramos J, Fabre JM, Jacquet E, Navarro F, *et al*. Isolation, characterization, and differentiation to hepatocyte-like cells of nonparenchymal epithelial cells from adult human liver. Stem Cells 2007; 25(7): 1779-90.
26 Roskams T, de Vos R, van Eyken P, Myazaki H, van Damme B, Desmet V. Hepatic OV-6 expression in human liver disease and rat experiments: Evidence for hepatic progenitor cells in man. J Hepatol 1998; 29(3): 455-63.
27 Tan J, Hytiroglou P, Wieczorek R, Park YN, Thung SN, Arias B, *et al*. Immunohistochemical evidence for hepatic progenitor cells in liver diseases. Liver 2002; 22(5): 365-73.
28 Selden C, Chalmers SA, Jones C, Standish R, Quaglia A, Rolando N, *et al*. Epithelial colonies cultured from human explanted liver in subacute hepatic failure exhibit hepatocyte, biliary epithelial, and stem cell phenotypic markers. Stem Cells 2003; 21(6): 624-31.
29 Ichinohe N, Tanimizu N, Ooe H, Nakamura Y, Mizuguchi T, Kon J, *et al*. Differentiation capacity of hepatic stem/progenitor cells isolated from D-galactosamine-treated rat livers. Hepatology 2013; 57(3): 1192-202.
30 Itskovitz-Eldor J, Schuldiner M, Karsenti D, Eden A, Yanuka O, Amit M, *et al*. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med 2000; 6 (2): 88-95.
31 Abe K, Niwa H, Iwase K, Takiguchi M, Mori M, Abe SI, *et al*. Endoderm-specific gene expression in embryonic stem cells differentiated to embryoid bodies. Exp Cell Res 1996; 229(1): 27-34.
32 Hamazaki T, Iiboshi Y, Oka M, Papst PJ, Meacham AM, Zon LI, *et al*. Hepatic maturation in differentiating embryonic stem cells *in vitro*. FEBS Lett 2001; 497(1): 15-9.
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. Hepatology 2007; 45(5): 1229-39.
34 Agarwal S, Holton KL, Lanza R. Efficient differentiation of functional hepatocytes from human embryonic stem cells. Stem Cells 2008; 26(5): 1117-27.
35 Baharvand H, Hashemi SM, Kazemi Ashtiani S, Farrokhi A. Differentiation of human embryonic stem cells into hepatocytes in 2D and 3D culture systems *in vitro*. Int J Dev Biol 2006; 50(7): 645-52.
36 Takayama K, Inamura M, Kawabata K, Katayama K, Higuchi M, Tashiro K, *et al*. Efficient generation of functional hepatocytes from human embryonic stem cells and induced pluripotent stem cells by HNF4alpha transduction. Mol Ther 2012; 20(1): 127-37.
37 Inamura M, Kawabata K, Takayama K, Tashiro K, Sakurai F, Katayama K, *et al*. Efficient generation of hepatoblasts from human ES cells and iPS cells by transient overexpression of homeobox gene HEX. Mol Ther 2011; 19(2): 400-7.
38 Chinzei R, Tanaka Y, Shimizu-Saito K, Hara Y, Kakinuma S, Watanabe M, *et al*. Embryoid-body cells derived from a mouse embryonic stem cell line show differentiation into functional hepatocytes. Hepatology 2002; 36(1): 22-9.
39 Hay DC, Zhao D, Fletcher J, Hewitt ZA, McLean D, Urruticoechea-Uriguen A, *et al*. Efficient differentiation of hepatocytes from human embryonic stem cells exhibiting markers recapitulating liver development *in vivo*. Stem Cells 2008; 26(4): 894-902.
40 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126(4): 663-76.
41 Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, *et al*. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007; 318(5858): 1917-20.
42 Liao J, Cui C, Chen S, Ren J, Chen J, Gao Y, *et al*. Generation of induced pluripotent stem cell lines from adult rat cells. Cell Stem Cell 2009; 4(1): 11-5.
43 Song Z, Cai J, Liu Y, Zhao D, Yong J, Duo S, *et al*. Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. Cell Res 2009; 19(11): 1233-42.
44 Rashid ST, Corbineau S, Hannan N, Marciniak SJ, Miranda E, Alexander G, *et al*. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J Clin Invest 2010; 120(9): 3127-36.
45 Li HY, Chien Y, Chen YJ, Chen SF, Chang YL, Chiang CH, *et al*. Reprogramming induced pluripotent stem cells in the absence of c-Myc for differentiation into hepatocyte-like cells. Biomaterials 2011; 32(26): 5994-6005.
46 Chen YF, Tseng CY, Wang HW, Kuo HC, Yang VW, Lee OK. Rapid generation of mature hepatocyte-like cells from human induced pluripotent stem cells by an efficient three-step protocol. Hepatology 2012; 55(4): 1193-203.
47 Takayama K, Inamura M, Kawabata K, Sugawara M, Kikuchi K, Higuchi M, *et al*. Generation of metabolically functioning hepatocytes from human pluripotent stem cells by FOXA2 and HNF1alpha transduction. J Hepatol 2012; 57(3): 628-36.
48 Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, *et al*. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 2010; 466(7308): 829-34.
49 Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, *et al*. Bone marrow as a potential source of hepatic oval cells. Science 1999; 284(5417): 1168-70.
50 Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, *et al*. Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. Nat Med 2000; 6(11): 1229-34.
51 Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, *et al*. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest 2002; 109(10): 1291-302.
52 Shu SN, Wei L, Wang JH, Zhan YT, Chen HS, Wang Y. Hepatic differentiation capability of rat bone marrow-derived mesenchymal stem cells and hematopoietic stem cells. World J Gastroenterol 2004; 10(19): 2818-22.
53 Kang XQ, Zang WJ, Song TS, Xu XL, Yu XJ, Li DL, *et al*. Rat bone marrow mesenchymal stem cells differentiate into hepatocytes *in vitro*. World J Gastroenterol 2005; 11(22): 3479-84.
54 Kakinuma S, Tanaka Y, Chinzei R, Watanabe M, Shimizu-Saito

K, Hara Y, *et al*. Human umbilical cord blood as a source of transplantable hepatic progenitor cells. Stem Cells 2003; 21(2): 217-27.

55 Hong SH, Gang EJ, Jeong JA, Ahn C, Hwang SH, Yang IH, *et al*. *In vitro* differentiation of human umbilical cord blood-derived mesenchymal stem cells into hepatocyte-like cells. Biochem Biophys Res Commun 2005; 330 (4): 1153-61.

56 Tang XP, Zhang M, Yang X, Chen LM, Zeng Y. Differentiation of human umbilical cord blood stem cells into hepatocytes *in vivo* and *in vitro*. World J Gastroenterol 2006; 12(25): 4014-9.

57 Ling L, Ni Y, Wang Q, Wang H, Hao S, Hu Y, *et al*. Transdifferentiation of mesenchymal stem cells derived from human fetal lung to hepatocyte-like cells. Cell Biol Int 2008; 32(9): 1091-8.

58 Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Quinn G, *et al*. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. Hepatology 2007; 46(1): 219-28.

59 Aurich H, Sgodda M, Kaltwasser P, Vetter M, Weise A, Liehr T, *et al*. Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue *in vitro* promotes hepatic integration *in vivo*. Gut 2009; 58(4): 570-81.

60 Roelandt P, Sancho-Bru P, Pauwelyn K, Verfaillie C. Differentiation of rat multipotent adult progenitor cells to functional hepatocyte-like cells by mimicking embryonic liver development. Nat Protoc 2010; 5(7): 1324-36.

61 Snykers S, Vanhaecke T, Papeleu P, Luttun A, Jiang Y, Vander Heyden Y, *et al*. 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(2): 330-41; discussion 235-9.

62 Park Y, Subramanian K, Verfaillie CM, Hu WS. Expansion and hepatic differentiation of rat multipotent adult progenitor cells in microcarrier suspension culture. J Biotechnol 2010; 150(1): 131-9.

63 Khurana S, Mukhopadhyay A. *In vitro* transdifferentiation of adult hematopoietic stem cells: An alternative source of engraftable hepatocytes. J Hepatol 2008; 49(6): 998-1007.

64 Lee KD, Kuo TK, Whang-Peng J, Chung YF, Lin CT, Chou SH, *et al*. *In vitro* hepatic differentiation of human mesenchymal stem cells. Hepatology 2004; 40(6): 1275-84.

65 Scarpelli DG, Rao MS. Differentiation of regenerating pancreatic cells into hepatocyte-like cells. Proc Natl Acad Sci USA 1981; 78(4): 2577-81.

66 Dabeva MD, Hwang SG, Vasa SR, Hurston E, Novikoff PM, Hixson DC, *et al*. Differentiation of pancreatic epithelial progenitor cells into hepatocytes following transplantation into rat liver. Proc Natl Acad Sci USA 1997; 94(14): 7356-61.

67 Tosh D, Shen CN, Slack JM. Differentiated properties of hepatocytes induced from pancreatic cells. Hepatology 2002; 36(3): 534-43.

68 Huang P, He Z, Ji S, Sun H, Xiang D, Liu C, *et al*. Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. Nature 2011; 475(7356): 386-9.

69 Sekiya S, Suzuki A. Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. Nature 2011; 475(7356): 390-3.