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In Vitro Differentiation of Embryonic and Adult Stem Cells into Hepatocytes: State of the Art

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ABSTRACT

Stem cells are a unique source of self-renewing cells within the human body. Before the end of the last millennium, adult stem cells, in contrast to their embryonic counterparts, were considered to be lineage-restricted cells or incapable of crossing lineage boundaries. However, the unique breakthrough of muscle and liver regeneration by adult bone marrow stem cells at the end of the 1990s ended this long-standing paradigm. Since then, the number of articles reporting the existence of multipotent stem cells in skin, neuronal tissue, adipose tissue, and bone marrow has escalated, giving rise, both in vivo and in vitro, to cell types other than their tissue of origin. The phenomenon of fate reprogrammation and phenotypic diversification remains, though, an enigmatic and rare process. Understanding how to control both proliferation and differentiation of stem cells and their progeny is a challenge in many fields, going from preclinical drug discovery and development to clinical therapy. In this review, we focus on current strategies to differentiate embryonic, mesenchymal(-like), and liver stem/progenitor cells into hepatocytes in vitro. Special attention is paid to intracellular and extracellular signaling, genetic modification, and cell-cell and cell-matrix interactions. In addition, some recommendations are proposed to standardize, optimize, and enrich the in vitro production of hepatocyte-like cells out of stem/progenitor cells. STEM CELLS *2009;27:577–605*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION: THE STEM CELL MICROENVIRONMENT

The totipotent fertilized egg is the ultimate stem cell that gives rise to all tissues of the developing embryo. In the adult, "multipotent" stem/progenitor cells reside for a nearly infinite term at restricted locations to allow continuation of the cycle of life [1–3]. These so-called stem cell niches have been identified in the bone marrow [4], brain [5], skin [6], intestinal crypt [7], and liver [1, 8]. The original idea of a stem cell "niche" evolved from the concept that stem/progenitor cells inhabit tissues within an "inductive microenvironment" that directs their self-renewal, differentiation, and cell fate in both normal physiology and disease [1, 3, 9]. Many developmental regulatory signaling molecules, including Wnts, bone morphogenic proteins (BMP), fibroblast growth factors (FGFs), Notch, and others, may play a role [1, 7, 8]. In addition to stem/progenitor cells, the niche microenvironment comprises nonstem niche cells (e.g., stromal cells, periductular fibroblasts, and stellate cells), parasympathetic nerve endings and specialized extracellular matrix (Fig. 1) [1, 2, 10, 11]. Other cell-cell interactions have also been hypothesized. The coordinated signaling between component cells and scaffold, (in)direct cell-cell contacts, and integration of stem cell-autonomous properties represent an interactive and dynamic system, organized to facilitate cell fate decisions in a proper spatiotemporal manner [1, 2, 8].

Historically, the developmental paradigm was that adult stem cells were, in contrast to their embryonic counterparts, subjected to "cell fate determinism." Nowadays, new insights on stem cell potency have challenged the latter canonical developmental hierarchy [12]. Nevertheless, "adult stem cell plasticity" still remains an obscure and rather rare phenomenon. The finding that at least some transitions may be ascribed to cellular fusion events have underpinned true plastic phenomena [13, 14] and has led to an outbreak of raw headlines, utterly questioning adult stem cell versatility, for example, "Adult Stem Cell Plasticity—Fact or Artifact?" [15], "Recipes for Adult Stem Cell Plasticity: Fusion Cuisine or Readymade?" [16], "Adult Stem Cell Plasticity—Fact or Fiction" [17], and "Stem Cell Fusion Confusion" [18].

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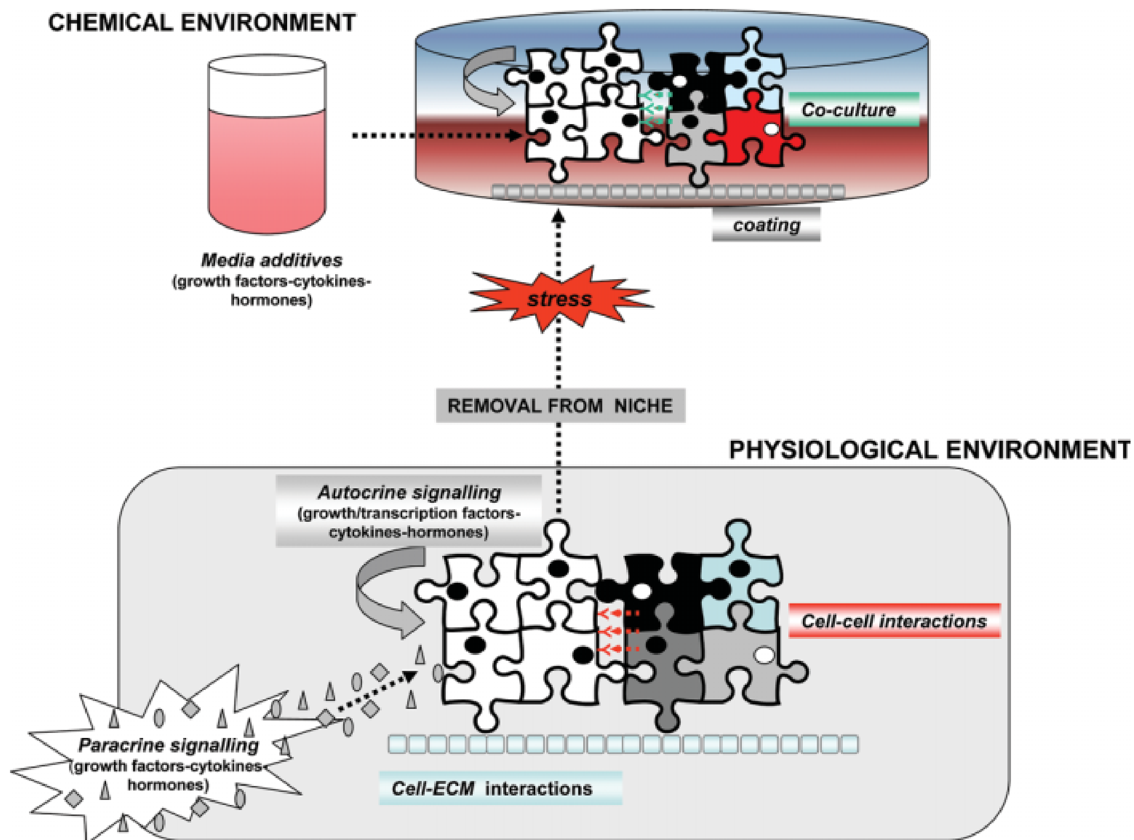

Figure 1. Adult stem/progenitor cell environment in vivo and ex vivo. The balance between cell growth/differentiation of adult stem/progenitor cells is regulated by a complex cross-talking network of paracrine and autocrine signals and cell-cell and cell-extracellular matrix interactions. Abbreviation: ECM, extracellular matrix.

Hitherto, the answer remained an open question. The fact is that not all cellular "redirections," no matter how rare their occurrence, might be ascribed to simple fusion events [12, 16, 19, 20]. In vitro, spontaneous fusion only occurs in coculture models, and, even so, the frequency rate is limited to about one fusion event per 10^3–10^6 cocultured cells [12, 20, 21]. In addition, in vivo, regular natural fusion of stem cells with other cell types seems unlikely because, with the exception of the liver and the pancreas, healthy organs lack substantial complements of polyploid cells [16]. This brings us back to the essence of fate reprogrammation of stem/progenitor cells: the stem cell microenvironment. In vivo, an injured environment seems most favorable for tissue replenishment by stem/progenitor cells [16, 22, 23], although extracellular cues provided by the transplanted stem/progenitor cells (cf. the "bystander" effect) also may be partly accountable for "recovery" of the recipient [23, 24]. In vitro, the highest success rates of phenotypic "diversification" were gained upon mimicking the microenvironment (Fig. 1). It is now well recognized that identification of the in vivo signaling patterns —the lineage-specific growth factors/cytokines and their (relative) dose and rank of application [8]—is crucial for eliciting distinct responses from cultured stem/progenitor cells and directing lineage-specific cell growth and differentiation in vitro. Apart from the latter cues, intrinsic cellular stress signals, executed by removal of stem/progenitor cells from their physiological niche, may also facilitate alterations in cellular architecture and phenotype via mechanisms of "cytoskeleton collapse" (Fig. 1; see also Need for Standardization, Optimization, and Enrichment) [25, 26].

In this survey, we provide an up-to-date overview on the wide variety of experimental conditions that have been applied thus far to trigger cultured pluripotent embryonic stem (ES) cells, multipotent mesenchymal(-like) stem/progenitor cells (MSCs), and bipotent liver progenitor cells (LPCs) into (functional) hepatocytes (Tables 1, 2,, and 3). In principle, most approaches are based on reconstructing the in vivo microenvironment via (a) addition of soluble medium factors and (b) reconstitution of cell-matrix, and (c) cell-cell interactions. Recently, (d) interest has also increased in chromatin modulation as a strategy to manipulate cell fate. Constitutive overexpression of liver-enriched transcription factor (LETF) genes might be an alternative but has a downside too.

From Stem Cells to Hepatocytes: Hepatogenesis In Vivo

The microenvironment of developing hepatocytes is a continuously changing process of successively occurring

Table 1. Strategies for in vitro differentiation of ES cells into hepatocyte-like cells including their molecular and functional endpoints

		Hepatic differentiation conditions					Hepatic features		
Origin	**Marker-based selection**	**Cell density**	**Cell-matrix/cell-cell interaction**	**Serum**	**Growth factors-cytokines/nonepigenetic additives**	**Differentiation-inducing agents**	**RNA + protein level**	**Functionality level**	**Refs**
EMBRYONIC STEM CELLS									
Successful transdifferentiation									
Spontaneous									
Monkey ES		3.6×10^6 cells/cm^2	NS	10% FCS	/		Oct3/4, AFP, ALB α1AT, HNF4α		[49]
mES		NS	Collagen type I	20% FCS	/		+: HNF3β, Oct4 Low: AFP, ALB −: C/EBPα		[50]
mES		NS	Gelatin	NS	/		AFP, ALB, TAT		[34]
mES		NS	(a) Gelatin (b) Suspension	20% FBS	/		(a) High CYP7A1 (b) Low Cyp7A1		[36]
Growth factors/cytokines/nonepigenetic modifiers									
mES		NS	Gelatin	NS	*D6*: 20 ng/ml aFGF, 10 ng/ml bFGF *D10*: 10 ng/ml HGF *D16*: ITS, dex 10 ng/ml OSM		AFP, ALB, TAT	Urea production	[34]
RESC		NS	Matrigel	/	3 ng/ml FGF4		AFP, ALB, α1AT, CK18		[41]
ES	Bry$^+$	2 EBs/cm^2	Matrigel	*2D upon differentiation* 15% SR *D5-13*: 15% SR *From D13*: 15% FCS	*2D upon differentiation*: 100 ng/ml Activin *D5-13*: Activin free *From D13*: dex		Sox17, Hhex, HNF4, Ipf1		[38]
Monkey ES		NS	Collagen type I	NS	20 ng/ml aFGF		+: AFP, TTR, ALB −: TAT		[42]
mE14-1 ES		NS	Gelatin	NS	*D2*: 100 ng/ml aFGF *D4*: 20 ng/ml HGF *D6*: 10 ng/ml OSM nicotinamide, dex, ITS		CYP2B10, CYP2C29, CYP2D9, CYP3A11, CYP7A1	Testosterone metabolism, PB induced 6β-OHT, 16β-OHT	[43]
mE14-1 ES		NS	Gelatin	NS	*D2*: 100 ng/ml aFGF *D4*: 20 ng/ml HGF *D6*: 10 ng/ml OSM nicotinamide, dex, ITS		Mixed culture (blood/ sinusoid vascular-like + hepatocyte layers)	ALB production, ammonia degradation	[44]
hES		NS	Collagen type I	/	FGF4, HGF		HNF3β, GATA4, HNF1	Urea and ALB production, PB-induced CYP450, ICG uptake	[53]

Table 1. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions: Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/nonepigenetic additives	Differentiation-inducing agents	Hepatic features: RNA + protein level	Functionality level	Refs
hES		NS	Collagen scaffold 3D culture system	NS	(a) *D9-12*: 100 ng/ml aFGF *D12-20*: 20 ng/ml HGF *D15-20*: dex, ITS, 10 ng/ml OSM		(a) CK19 (↓), CK18, CK8, TTR, ALB, α1AT, TO, TAT G6P, CYP7A1	(1) AFP, ALB and urea production, ICG uptake	[175]
					(b) No GF		(b) +: AFP, CK19, TTR, α1AT, CK8, CK18, ALB −: G6P, TAT	(2) Low AFP, ALB urea production	
mE14 ES		NS	NS	NS	aFGF, HGF, OSM		TTR, α1AT, AFP, ALB G6P, TAT	ICG and glycogen uptake	[176]
mES		1.6-2 EBs/cm^2	Matrigel	10% FBS	20 ng/ml HGF, 50 ng/ml aFGF, 10 ng/ml OSM, ITS, dex		GATA4, AFP, ALB, TTR, CK18, GST, TO	Glycogen storage	[177]
hES		NS	Gelatin	NS	(a) 20 ng/ml HGF, 50 ng/ml β-NGF, separately/combined		(a) AFP, ALB, α1AT, TTR G6P, HNF4, SEK1		[35]
					(b) 10^{-7} M RA		(b) Only TTR		
mES		NS	Gelatin	NS	(a) *D7-11*: 100 ng/ml aFGF *D7-19*: 20 ng/ml TGF, 20 ng/ml AFP *D11-19*: 20 ng/ml EGF, 20 ng/ml KGF, 20 ng/ml HGF *D15-19*: dex, ITS, 10 ng/ml OSM		(a) AFP, ALB, CK7, CK8, CK18, CK9 CK19, GGT		[178]
					(b) No GF		(b) CK7, CK19, GGT but later in time		
mES (pALB-EGFP)		0.32 × 10^6 cells/cm^2	(1) *D0-3*: 3D on gelatin, no feeder cells (2) *D3-8*: 3.2 × 10^5/cm^2 on gelatin (3) *D8-10*: 3.2 × 10^5/cm^2 on collagen	NS	(1) *D0-3*: 10^8 M RA, 100 U/ml LIF (2) *D3-8*: 100 ng/ml FGF1, 20 ng/ml FGF4, 50 ng/ml HGF (3) *D8-10*: 10 ng/ml OSM (4) *From D10*: TF, hydrocortisone, BSA, insulin		HNF3β, AFP, ALB, TTR, CK8, TAT, TO, G6P, LST1, CPS1, PEPCK, CYP1A1, HNF4	Urea and glucose synthesis	[179]
mCD4-Foxa2 ES		High density	*D6*: clusters formed are replated on gelatin	/	*D2-4*: 50 ng/ml activin *D4-6*: 50 ng/ml BMP4, 10 ng/ml bFGF, 50 ng/ml activin, 10 ng/ml VEGF	*D4*: selection of GFP-Bry^+/CD4-$Foxa2^{high}$/$cKit^{high}$	*D4*:Sox17, Shh, Hhex *D5-6*: AFP, CD4-Foxa2 cKit↓ *Upon D6*: +: AFP, ALB, CD4-Foxa2, CD31, CK19, Ecadherin, TAT,	ALB secretion, glycogen storage	[180]

Table 1. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions					Hepatic features		Refs
		Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/nonepigenetic additives	Differentiation-inducing agents	RNA + protein level	Functionality level	
					Upon D6: dex, 10 ng/ml EGF, 10 ng/ml bFGF, 20 ng/ml HGF, 20 ng/ml TGFα, 10 ng/ml VEGF		CPS1, CYP7A1, CYP3A11 −: cKit		
Monkey ES		3.6×10^{6} cells/cm^{2}	NS	NS	(1) *D0-3*: 100 U/ml LIF 10^{-8} mol/L RA (2) *D3-8*: 10 mg/l FGF2, 20 mg/l FGF4, 25 mg/l HGF, 10 mg/l OSM (3) dex, nicotinamide		AFP, ALB, α1AT, HNF4, Oct3/4		[49]
mES		NS	Gelatin	NS	(1) *D7-11*: 100 ng/ml aFGF (2) *D7-19*: 20 ng/ml TGF, 20 ng/ml AFP (3) *D11-19*: 20 ng/ml HGF (4) *D15-19*: ITS, 10 ng/ml OSM, dex (5) Isolation hepatic clusters and replate		AFP, TTR, ALB, HNF3β, G6P, TAT, CK8, CK18	ALB and urea secretion	[46]
mES (pALB-EGFP)		NS	(2) Gelatin (3) Collagen	NS	(1) *D0-3*:100 U/ml LIF, 10^{-8} mol/l RA (2) *D3-8*: 50 ng/ml HGF, 100 ng/ml FGF1, 20 ng/ml FGF4 (3) *D8-10*: 10 ng/ml OSM		HNF1β↑, HNF3α/β↑, HNF4↑, HNF6↑. C/EBPβ↑, ALB↑, TTR↑, AFP, TO, CYP2E1↑, CYP2D10↑, ADH↑, Oct3/4↓, Nanog↓		[55]
mES (pALB-EGFP)		NS	Collagen type I	NS	*D0-2*: TF, insulin, BSA, hydrocortisone *From D2*: niacinamide, dex		ALB, α1AT, TO, TTR	Glucose production, ammonia metabolism	[54]
hES		Differentiation onset upon 80% confluence *D5*: upon confluence 1/1 subculture	*From D5*: collagen	*D0-D3*: 0.5% FBS *D3-D5*: 2% KOSR *D5-D8*: 2% KOSR *D8-D11*: 0.5 mg/ml BSA	*D0-D5*: 100 ng/ml Activin A *D5-D11*: 10 ng/ml FGF-4, 10 ng/ml HGF *D11-D20*: dex, 10 ng/ml FGF4, 10 ng/ml HGF, 10 ng/ml OSM		*D11-D20*: HNF4, AFP, ALB, DPPIV, α1AT *D20*: CYP7A1, CYP3A4	Glycogen storage, ICG uptake, ALB secretion	[47]

Table 1. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions: Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/nonepigenetic additives	Differentiation-inducing agents	Hepatic features: RNA + protein level	Functionality level	Refs
mES (SK7, Pdx (1)		Preinduction: coculture with 10^4 M 15/cm^2 Differentiation: 2.0–2.5 $\times 10^3$ ES/cm^2		D0-D4: 10% FBS D4-D60: 10% SR	*D0-D4*: 4.5 g/l glucose, 20 ng/ml activin, 50 ng/ml bFGF *D4-D60*: 2 g/l glucose D8: +/− dex, 10 ng/ml HGF, OSM		D10-D30: ↑ALB, ↑CK19, ↑CYP7A1, ↑CYP2B10, ↑CYP3A11, ↑CYP3A13, ↑Sult2A1, ↑UGT1A1, ↑ABCB11 +dex, HGF, OSM, ↓CXCR4, ↓E-cadherin, ↑AFP (80%!)	CYP activity, glycogen storage, ALB secretion	[48]
hES (KhES-1)		Preinduction: coculture with 10^4 M 15/cm^2 Differentiation: 8.3-10 $\times 10^3$ ES/cm^2		*D0-D50*: 10% KSR	*D0-D50*: 4.5 g/l glucose *D0-D10*: 20 ng/ml activin, 10 μM LY294002 *D10-D50*: dex, 10 ng/ml HGF		D18: Sox17, 80% AFP!, 9% ALB, CK18/19, CYP7A1 D18-50: ↑ALB, CYP3A4, OATP1B1	Glycogen storage	[48]
Nonepigenetic modifiers									
mBALB/CES		NS	Gelatin	20% cow serum	dex, ITS		AFP↑, ALB↑		[181]
m, hES		NS	Collagen type I	20% FBS	dex, ITS		TTR↑, ALB	ALB secretion↑	[56]
m (EB5)		NS	Gelatin	10% FCS	+: dex, insulin, glycerol, ornithine, galactose, phenylalanine −: arginine, tyrosine, glucose, pyruvate		+: ALB, TTR, AFP, CK18 −: TAT, α1AT, G6P, PPECK	ICG uptake	[182]
Growth factors/cytokines/epigenetic modifiers									
					Hepatic progenitor cells				
mES cells (D3)		NS	Gelatin	15% FBS	(3) *D11-17/23*: 10 ng/ml HGF until confluent	(1) *D0-4*: 0.8% DMSO (2) *D4-10*: 2.5 mM SB	AFP, α1AT, CK18/19, GGT, HNF3β, DPPIV		[43]
					Hepatocytes				
mES cells (D3)		NS	Collagen type I	10% FBS	(4) *D17/23-23/29*: dex insulin, nicotinamide 20 ng/ml EGF 10 ng/ml HGF (5) *from D23/29*: 10 ng/ml OSM, dex		ALB, G6P↑, TAT↑	Glycogen storage ALB secretion	[43]
hES		NS	PAU coating, nonwoven PTFE	NS	*D0-3*: 100 ng/ml bFGF *D3-11*: 100 ng/ml HGF *D11-14*: dex	*D3*-11: 1% DMSO	ALB	Lidocaine metabolism, ALB and urea production	[183]
hEBs			Matrigel	20% FBS		5 mM SB	−: AFP +: ALB, α1AT, CK8/18	Glycogen storage, inducible CYP450 activity	[65]

Table 1. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions					Hepatic features		Refs
		Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/nonepigenetic additives	Differentiation-inducing agents	RNA + protein level	Functionality level	
mES(D3)		1 × 10^4 cells/cm^2	*D0-D10*: gelatin, collagen type I, polystyrene	*D0-10*: 20% FBS		*D0-4*: 1% DMSO *D4-10*: 2.5 mM SB	+: ALB, K18, DPPIV, ADH, CYP3A13, CYP27A1 −: AFP	Glycolysis, glycogen storage, urea production, CYP activity	[57]
		1 × 10^4 cells/cm^2 *D11*: subculture at 15 × 10^3 cells/cm^2	*D0-D10*: Gelatin From D11: 5% polyacrylamide	*D0-10*: 20% FBS From D11: /		*D0-4*: 1% DMSO *D4-10*: 2.5 mM SB From D11: /		Glycogen storage, ↑urea production ↑ALB secretion	[184]
		Phase I: pre-differentiation = no confluence	Gelatin	15% FBS	(3) *From D10 until confluence*: 10 ng/ml HGF	(*1*) *D0-4*: 0.8% DMSO (2) *D4-10*: 2.5 mM SB	+: CK19, ALB, α1AT, HNF3*β* −: SSEA1, AFP		
mES (D3)		Phase II: differentiation upon confluence and subculture	Collagen type I	10% FBS	(4) *Upon subculture: D0-D6*: insulin, dex, nicotinamide, 20 ng/ml EGF, 10 ng/ml HGF (5) *From D6-12/18*: 10 ng/ml OSM, dex		+: AFP, ALB, CK18, α1AT, HNF3*β*, HNF4, TAT −: CK19	Glycogen storage, ALB secretion	[43]
hES		Differentiation onset upon 50–70% confluence D3/5: 1/2 split	Matrigel	*D0-D3/5*:/*D3/5-D10/12*: SR *From D10/12*: 8.3% FBS	*D0-D3/5*: 100 ng/ml activin A *From D10/12*: insulin, hydrocortisone, 10 ng/ml HGF, 20 ng/ml OSM	*D0-D1/2*: 1 mM SB *D1/2-D3/5*: 0.5 mM SB *D3/5-D10/12*: 1% DMSO	*D0-D3/5*: CXCR4, HNF3*β*, Sox17 *D3/5-D10/12*: HNF4α↑, HNF1↑, TTR↑, AFP *From D10/12*: ALB, ApoF, CAR, TO, TAT↑, CYP3A4/7, CYP2C9/19	Glycogen storage, ALB/fibrinogen/fibronectin/A2M secretion, inducible CYP activity	[51]
mES, monkey ES		mES: 10^9 cells/cm^3 lumen V Monkey ES: 4 × 10^8 cells/cm^3 lumen V	Organoid culture in hollow fibers	20% FBS		*D9*: 1 mM SB	+: CPS↑	ALB secretion, ammonia removal	[185]

Table 1. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions: Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/nonepigenetic additives	Differentiation-inducing agents	Hepatic features: RNA + protein level	Functionality level	Refs
Growth factors/cytokines/LETFs overexpression									
mES		NS	(3D)	10% FBS	*D0-7*: 20 ng/ml FGF2, sodium pyruvate	HNF3β mRNA overexpression	OATP1, MRP1, MRP2, MRP3, UGT1a1	Triacylglycerol, urea, glycogen, ALB synthesis	[63]
mES		NS	Gelatin	10% FBS	20 ng/ml FGF2	HNF3β mRNA overexpression	TTR, AFP, ALB, α1AT, TO, PEPCK	ALB production	[64]
mES		NS	(3D)	10% FBS	50 ng/ml FGF2, dex, ascorbic phosphate, nicotinamide	HNF3β mRNA overexpression,	CK18, C3, CYP450, PEPCK, PXMP1-L	Urea, glycogen, triacylglycerol, ALB synthesis	[62]
Growth factors/cytokines/cell surface marker-based selection									
hES	(2) CXCR4+	NS	NS	0/0.2/2% FBS	(1) 100 ng/ml activin A		SOX17↑, FOXA2↑ (80% homogeneity)		[40]
Growth factors/cytokines/coculture									
mES	AFP expression	2×10^4 cells/cm^2	Collagen type I (a) Coculture ES with $Thy1^+$, $CD49f^{+/-}$, $CD45^-$ mouse fetal liver cells Collagen type I (b) No coculture		*D0-1*: 1000 U/ml LIF, 10 μM RA *D2-6*: 20 ng/ml bFGF, 20 ng/ml HGF *D7*: 10 ng/ml OSM		(a) AFP, Foxa2, ALB, TAT, TO, G6P (b) AFP, Foxa2, ALB	(a) Glycogen storage Ammonia clearance↑ (b)/	[59]
mES		1×10^4 ES/cm^2	Coculture with embryonic chick cardiac mesoderm	10% FBS	10 ng/ml EGF, dex, ITS, nicotinamide	1% DMSO	sox17α, HNF3β, GATA4, AFP↑, ALB↑		[61]
Coculture									
Monkey ES		3.6×10^6 ES/cm^2 1×10^5 MFLCs/cm^2	Co-culture with MFLCs	NS			+: AFP, ALB↑, α1AT, HNF4, CYP7A1 −: Oct3/4	Urea synthesis, glycogen storage	[49]
3D System									
ES (D3; P10-22)		NS	Alginate, poly-L-lysine, microencapsulation	20% FBS	NS			Urea and ALB secretion	[186]
mES		NS	3D collagen scaffold	NS	Exogenous GF hormones		+: ALB −: CK18		[58]

(a), (b) and (1), (2), (3) indicate different protocols applied and order of serial steps, respectively. ↓, downregulation; ↑, upregulation; −, negative, +, positive).
Abbreviations: α1AT, α1-antitrypsin; ADH, alcohol dehydrogenase; aFGF, acidic fibroblast growth factors; AFP, α-fetoprotein; ALB, albumin; A2M, α_2-macroglobulin; ApoF, apolipoprotein factor; bFGF, basic FGF; BMPs, bone morphogenic proteins; BRY, brachyury; BSA, bovine serum albumin; C, complement; CAR, constitutive androstane receptor; CD, cluster of differentiation; C/EBP, CCAAT enhancer binding protein; CK, cytokeratin; CPS, carbamoyl phosphate synthetase; CYP, cytochrome P450-dependent monooxygenases; 3D, three dimensional; dex, dexamethasone; DMSO, dimethyl sulfoxide; DPPIV, dipeptidylpeptidase IV; E, embryonic day; EBs, embryoid bodies; E-cadherin, epithelial-cadherin; EGF, epidermal growth factor; EGFP, enhanced green fluorescent protein; ES, embryonic stem cells; FBS, fetal bovine serum; FCS, fetal calf serum; FGF, fibroblast growth factors; GF, growth factors; GGT, γ-glutamyltransferase; G6P, glucose-6-phosphatase; GST, glutathione *S*-transferase; h, human; HGF, hepatocyte growth factor; Hhex, homeobox gene; HNF, hepatocyte nuclear factor; ICG, indocyanine green; IPF, insulin promoter factor; ITS, insulin-transferrin-selenious acid; KGF, keratinocyte growth factor; K(O)SR, knockout serum replacement; LIF, leukemia inhibitory factor; LST1, liver-specific organic anion transporter; m, mouse/murine; M15, mesonephric cell line; MFLCs, mouse fetal liver-derived cells; MRP, multidrug resistance protein; NGF, nerve growth factor; NS, not specified; OATP, organic anion transport polypeptide; 6/16β-OHT, 6/16 β-hydroxylase; OSM, oncostatin M; P, passage; PAU, poly-amino-urethane; PB, phenobarbital; PEPCK, phosphoenolpyruvate carboxykinase; PTFE, polytetrafluoroethylene; PXMP1-L, peroxisomal membrane protein 1-like protein; r, rat; RA, retinoic acid/all-*trans*-retinoic acid; RESC, rat embryonic stem cell-like cell line; SB, sodium butyrate; SEK1, Stress-signaling kinase; Shh, sonic hedgehog; Sox17, Sry-related HMG box transcription factor; SR, serum replacement; SSEA, stage-specific embryonic antigen; Sult, sulfotransferase; TAT, tyrosine aminotransferase; TF, transferrin; TGF, transforming growth factor; TO, tryptophan-2,3-dioxygenase; TTR, transthyretin; UGT, UDP-glucuronosyltransferase; VEGF, vascular endothelial growth factor.

biological events [27]. Each step of cell growth and differentiation is tightly regulated by intra- and extracellular communication, as well as cell autonomous mechanisms (Fig. 2). Nodal (activin), FGFs, BMP, hepatocyte growth factor (HGF), and oncostatin M (OSM) are herein the most essential extracellular signals [2, 27–30]. At the intracellular level, the liver-enriched transcription factors hepatocyte nuclear factor (HNF) 3α,β, HNF4α, HNF1α,β, HNF6, and CCAAT enhancer binding protein (C/EBP) α,β act consecutively, in essence, in a cross-regulatory manner, at specific developmental stages to regulate liver-specific gene expression [27–29, 31, 32] (Fig. 2).

In brief, at the onset of liver ontogeny (approximately rodent embryonic day [E] 8.5), specification from endodermal stem cells toward the hepatic epithelial lineages requires, next to HNF3β and activin A signaling, signaling from two adjacent mesodermal cell types: FGFs (FGF1 and basic bFGF) from the cardiogenic mesoderm cells, and BMPs (BMP2, BMP4, BMP5, and BMP7) from the septum transversum mesenchyme [27–29] (Fig. 2). Then (approximately rodent E9.0-9.5), cells start to massively proliferate and bud into the stromal environment of the septum transversum mesenchyme. The hepatic epithelial specified cells are now referred to as bipotent hepatoblasts (GATA4$^+$, HNF4α^+, HNF6$^+$, hepatic α-fetoprotein [AFP]$^+$/albumin [ALB]$^+$, and biliary cytokeratin [CK 17$^+$/CK19$^+$) [2, 27–29]. At rodent E11-12, the liver primarily becomes a primarily hematopoietic organ. Hematopoietic stem cells (HSCs) originating from the extrahepatic organ colonize the liver bud, thereby emitting a growth signal for the liver [28, 29]. Consequently, hepatoblasts continue to proliferate and start expressing placental alkaline phosphatase, intermediate filament proteins (CK14, CK8, and CK18), γ-glutamyltransferase, and later also α_1-antitrypsin, glutathione *S*-transferase P, C/EBPα, lactate dehydrogenase, and muscle pyruvate kinase [2, 28, 29]. As commitment progresses, three distinct cell populations are distinguished: (a) hepatocyte-committed cells that exclusively express hepatocyte markers, such as AFP and ALB, (b) cholangiocyte-committed progenitor cells, expressing biliary cell markers such as CK19, and (c) a bipotential hepatoblast population, expressing both hepatic and biliary markers. The latter population develops into biliary or hepatic cell lines and is therefore considered to be the fetal source of hepatic progenitor cells [2, 27, 29]. Differentiation along the cholangiocytic lineage is promoted by Notch signaling pathways [27, 29, 30]. HGF, excreted by mesenchymal cells or nonparenchymal liver cells, antagonizes the latter process, resulting in support of growth and differentiation of the fetal hepatocytes. The hormone insulin synergistically promotes this effect [2, 28, 33]. Subsequently, cooperative action of OSM, mostly produced by HSC, and glucocorticoids induces partial hepatic maturation and suppression of embryonic hematopoiesis [2, 27, 28, 33] (Fig. 2). OSM alone fails to induce differentiated liver phenotypes, implying the essence of glucocorticoids as triggers for hepatic maturation [28]. Upon progression of the differentiation stage, the gene expression profile of fetal liver epithelial cells acquires a more mature phenotype. In parallel, the percentage of bipotent cells is markedly reduced. At this point, although cells continue to proliferate, most of them are unipotent and irreversibly committed to either the hepatocytic or cholangiocytic lineage [2, 27, 29]. Complete functional hepatic maturation ultimately takes place after birth upon coassistance of HGF, produced by the surrounding nonparenchymal liver cells (sinusoidal, stellate, and endothelial cells) [33].

Table 2. Strategies for in vitro differentiation of MSCs into hepatocyte-like cells including their molecular and functional endpoints

		Hepatic differentiation conditions					Hepatic features		
Origin	Marker-based selection	Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/ nonepigenetic additives	Differentiation-inducing agents	RNA + protein level	Functionality level	Refs
MESENCHYMAL (-LIKE) STEM/PROGENITOR CELLS									
Successful transdifferentiation									
Growth factors/cytokines									
BM (femoral heads, 60–69-year-old healthy human adults; P2)		NS	NS	/	*2D preinduction*: 20 ng/ml EGF, 10 ng/ml bFGF *D0-7*: 20 ng/ml HGF, 10 ng/ml bFGF, nicotinamide *D7-21*: 20 ng/ml OSM, dex, ITS, BSA, linoleic acid		CK18, CK19, Thy1↓, HNF4α↑, C/EBPβ↑, ALB↑ CYP2E1, CYP3A4		[77]
BM (tibias + femora, 3–week-old C57BL/6 mice)		5×10^5 cells/cm^2	FN	10% FCS	ITS, dex, 40 ng/ml HGF, 20 ng/ml FGF4 10 ng/ml EGF, 10 ng/ml OSM		HNF3β, AFP, TTR, ALB, CK18, G6P, TAT	Glycogen storage, urea production	[72]
BM (tibias + femora, 6- to 7-week-old SD rats; P3)		2×10^4 cells/cm^2	FN	5% FBS	ITS, dex, 10 ng/ml EGF, 20 ng/ml aFGF, 10 ng/ml bFGF, 20 ng/ml HGF		*FIC* MSC: CK18 *Upon induction*: ALB, CK18		[71]
BM (tibias + femora, SD rats)		2×10^4 cells/cm^2 Differentiation onset upon 70% confluence	NS	1% FBS	20 ng/ml HGF, 10 ng/ml FGF4		AFP↑, ALB↑	Glycogen storage, urea production	[69]
BM (femora, 1-month-old Wistar rats)		NS	Polylysine	(a) 10% FBS + 5% cholestatic serum	(a) Niacinamide, dex, ITS		(a) + (b) AFP, CK18		[73]
				(b) 10% FBS	(b) Niacinamide, dex, ITS, 0.5 mg/ml HGF				
BM (healthy human; P4-5)		22×10^3 MSC/cm^2 Differentiation onset upon 100% confluence	Collagen type I	*Predifferentiation*: 2% FBS	*D0-2*: 10 ng/ml FGF4 *D3-5*: 20 ng/ml HGF *from D6 on*: ITS, dex, 20 ng/ml HGF		CK18	CYP activity	[84]
UCB (P3)		Differentiation onset upon 70% confluence	NS	1% FBS	20 ng/ml HGF 10 ng/ml FGF4		AFP, ALB, CK18	Glycogen storage, urea production	[138]
UCB (P5)		10^6 cells/cm^2 Differentiation onset upon 60% confluence	NS	10% FBS	*D0-14*: 50 ng/ml HGF, ITS, dex *D14-28*: 50 ng/ml OSM, ITS, dex		Thy1, cKit, Flt3, AFP, CK18/19, ALB, HGF, PEPCK, cMet, TAT, CPS	LDL uptake	[74]
ADSC (lipoaspirates, 38–49-year-old healthy human adults; P2)		NS	NS	/	*2D preinduction*: 20 ng/ml EGF, 10 ng/ml bFGF *D0-7*: 20 ng/ml HGF, 10 ng/ml bFGF, nicotinamide *D7-21*: 20 ng/ml OSM, dex, ITS, BSA, linoleic acid		CK19, CK18, Thy1↓, HNF4α↑, C/EBPβ↑, ALB↑, CYP2E1, CYP3A4		[77]

Table 2. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions: Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/ nonepigenetic additives	Differentiation inducing agents	Hepatic features: RNA + protein level	Functionality level	Refs
Placenta (P5)		NS	FN or poly-L-lysine		*0-24 h*: expansion media *24–40 h*: ITS, dex, 10 ng/ml EGF, 10 ng/ml PDGF *From 40 h*: 20 ng/ml HGF, 10 ng/ml FGF-4		Undifferentiated: Weak +: AFP, cMet −: CK18, ALB Differentiation: poly-L-lysine > FN +: ALB, CK18, TAT	Differentiation: LDL uptake, glycogen storage	[187]
BM (iliac crests, human, 19–32 years, P3)		5×10^5 cells/cm^2	NS	15% FBS or 20% HPR	*D0-D7*: 20 ng/ml HGF, dex *D7-D21*: 10 ng/ml OSM		HPR > FBS: ALB, AFP, CK18	HPR > FBS: urea production	[188]
UCB (P2-6)		1.5×10^4 cells/cm^2	Collagen type I	*D0-D1*: 10% FBS	*D1-3*: 20 ng/ml EGF, 10 ng/ml Bfgf *D3-13*: 20 ng/ml HGF, 10 ng/ml bFGF, ITS *D13-D23*: 20 ng/ml OSM, dex, ITS		Undifferentiated UCB: +: ALB, AFP, Cx32, DPPIV, CK8/18/19, PEPCK, TO, α1AT −: CYP2B6, CYP3A4, HNF4, HepPar1 Differentiation: +: ALB, AFP, Cx32, DPPIV, CK8/18/19, PEPCK, TO↑, TAT↑ CYP2B6, CYP3A4 −: HNF4, HepPar1	Differentiation: +: Glycogen storage, ↑G6P activity, urea production, inducible CYP3A4 activity −: ALB secretion	[189]
BM (iliac crests + vertebrae, postmortem human, 8–67 years, P1-9) Skin fibroblasts (human skin biopsy, 8–35 years, P1-3)		1.5×10^4 cells/cm^2	Collagen type I		*D0-D10*: ITS, dex, 10 ng/ml FGF-4, 20 ng/ml HGF *D10-D30*: ITS, dex, 20 ng/ml OSM		+: ALB, AFP, DPPIV, E-cadherin, Cx32, G6P, α1AT, PEPCK, TAT, TO −: CYP3A4, CYP2B6, HNF4, CK8/18, HepPar1	+: Glycogen storage, urea secretion −: ALB secretion, gluconeogenesis	[143]
BM (tibias + femora, C57BL/6 mice, P4)		5×10^5 cells/cm^2	FN	10% FCS	*D0-D21*: ITS, dex, 0–60 ng/ml HGF, 0–60 ng/ml FGF-4, 0–30 ng/ml EGF, 0–30 ng/ml OSM		35 ng/ml FGF + 30 ng/ml OSM > 40 ng/ml HGF + 40 ng/ml FGF + 30 ng/ml OSM > 60 ng/ml HGF + 20 ng/ml FGF + 30 ng/ml EGF + 20 ng/ml OSM: ALB, CK18, TTR, AFP	Urea production, ALB secretion	[190]

Table 2. (*Continued*).

Origin	Marker-based selection	Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/nonepigenetic additives	Differentiation inducing agents	RNA + protein level	Functionality level	Refs
		Hepatic differentiation conditions					Hepatic features		
Growth factors/cytokines/cell surface markers									
BM human	$CD45^-$, $GlyA^-$				20 ng/ml HGF, 10 ng/ml FGF4		AFP↓, ALB↑, CK18↑		[70]
BM (iliac crest, healthy human; P5-13)	$CD3^-$, $CD14^-$, $CD19^-$, $CD38^-$, $CD66b^-$, $GlyA^-$	1.2×10^4 cells/cm^2 Differentiation onset upon 60% confluence	NS	/	*2D prestimulation*: 20 ng/ml EGF, 10 ng/ml bFGF *D0-7*: nicotinamide, 20 ng/ml HGF, 10 ng/ml bFGF *From D7*: dex, ITS, 20 ng/ml OSM		AFP, CK18, ALB, HNF4, G6P, TAT, TO, inducible CYP2B6	ALB production, glycogen storage, urea secretion, LDL uptake	[75]
UCB (P5-13)	$CD3^-$, $CD14^-$, $CD19^-$, $CD38^-$, $CD66b^-$, $GlyA^-$	1.2×10^4 cells/cm^2 Differentiation onset upon 60% confluence	NS	/	*2D prestimulation*: 20 ng/ml EGF, 10 ng/ml FGF *D0-7*: nicotinamide, 20 ng/ml HGF, 10 ng/ml FGF *From D7*: dex, ITS, 20 ng/ml OSM		AFP, CK18, ALB, HNF4, G6P, TAT, TO, inducible CYP2B6	ALB production, glycogen storage, urea secretion, LDL uptake	[75]
ADSC (subcutaneous, 36–55 years, gastric cancer male/female patients, P5-9)	$CD105^+$	7×10^3–1×10^4 cells/cm^2	Collagen	*D1-21*: 0.5 mg/ml BSA	*D1-D21*: hydrocortisone, insulin, dex, 20 ng/ml EGF, 150 ng/ml HGF, 300 ng/ml aFGF, 25 ng/ml FGF-4 *D21-35*: dex 30 ng/ml OSM *D35-D70*: /		ALB, HNF4, TTR, CK18, TO, CYP7A1, CYP1A1, CYP2C9, CYP3A4	Glycogen storage, LDL uptake, ALB secretion, ammonia removal	[142]
ADSCs (subcutaneous, 55 years, gastric cancer male patient)							ALB, HNF3β, HNF6, TTR, CK18, UGTs, ADH, G6P, TO, CYP2A6, CYP2C8 CYP3A4, MDR1	Glycogen storage	[145]
Growth factors/cytokines/epigenetic modifiers									
BM (tibias + femora, C57/BL6 mice)		1×10^4 MSCs/cm^2 + 5.2×10^4 liver cells/cm^2	Coculture with nonparenchymal liver cells on collagen	>Coculture: 20% FBS Upon coculture: 5% liver failure patients sera	*12 h before coculture*: dx *Upon coculture*: nicotinamide, insulin, dex, 50 ng/ml HGF, 20 ng/ml OSM	*12 h before coculture*: 5 mM 5-AzaC *Upon coculture*: 1% DMSO	ALB, CK18/8/19, TAT		[90]
ADSC (abdominoplasty, 19–55 years human adults; P3-5)		2.5–3×10^4 cells/cm^2	FN	/	10 ng/ml HGF, 10 ng/ml OSM, ITS, 10 ng/ml EGF, dex	*From D10 on*: supplement of 0.1% DMSO	AFP, ALB	LDL uptake, urea production	[79]
BM (iliac crest, human adults)	100–200 cells/cm^2 Differentiation onset upon 100% confluence	NS		2% FCS	*D1*: 40 ng/ml HGF, 20 ng/ml EGF	*D0*: preincubation with 20 μM 5-AzaC	Cx32, HepPar1, CYP3A4, CPS, CK18, ALB, PEPCK	Glycogen storage, urea secretion	[82]
ADSC (subcutaneous/peritoneal, female donor)							Cx32, CYP3A4, CPS, CK18, ALB, PEPCK, DPPIV	Glycogen storage, urea secretion	[89]

Table 2. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions: Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/nonepigenetic additives	Differentiation inducing agents	Hepatic features: RNA + protein level	Functionality level	Refs
BM (iliac crest and femora, human)									
BM (healthy human; P4-5)		22 × 10^3 MSC/cm^2 Differentiation onset upon 100% confluence	Collagen type I	*Predifferentiation*: 2% FBS	*D0-2*: 10 ng/ml FGF4 *D3-5*: 20 ng/ml HGF *From D6 on*: ITS, dex, 20 ng/ml HGF	*From D6 on*: 1 μM TSA	ALB↑, CK18↑, HNF1α↑, MRP2↑ C/EBPα↑	ALB secretion↑, inducible CYP activity, urea secretion	[84]
ADSC (peritoneal, Fisher 344 rats)		200–300 cells/cm^2 Differentiation onset: upon 95% confluence —:	FN		(2) *D1*: hepatocyte growth medium	*D0*: preincubation with 20 μM 5-AzaC	AFP, ALB, CK18/19, CYP1A1, HepPar1, Cx32, DPPIV, PCK1	Glycogen storage, urea production	[87]
UCB hTERT (retrovirus infected)		2.1 × 10^3 cells/cm^2	NS	*D1-D22*: 10% FBS	*D1-D22*: 10 ng/ml FGF2, 20 ng/ml HGF, 20 ng/ml OSM	*D0*: preincubation with 1 μM 5-AzaC	ALB, C/EBPα/β↑, CYP1A1/2, PEPCK↑, wnt↓	Glycogen storage, urea secretion	[88]
BM (tibias + femora, SD rats)		22 × 10^3 MSC/cm^2 Differentiation onset upon 100% confluence	Collagen type I	*Predifferentiation*: 2% FBS	*D0-2*: 10 ng/ml FGF4 *D3-5*: 20 ng/ml HGF *From D6 on*: ITS, dex 20 ng/ml HGF	*From D6 on*: supplement of 1 μM TSA	AFP↑, ALB↑, CK18↑, HNF1α↑, HNF3β	ALB secretion↑	[91]
LEFTs overexpression									
ADSCs (lipoaspirates, 38–49 years, healthy human adults; P2)		NS	NS	/	*2D preinduction*: 20 ng/ml EGF, 10 ng/ml bFGF *D0-7*: nicotinamide, 20 ng/ml HGF, 10 ng/ml bFGF	*D7*: C/EBPα,β, mRNA overexpression	C/EBPβ, Thy1↓, CYP3A4↑		[77]
Growth factors/cytokines/coculture									
Direct coculture BM (femora + tibias, Lewis rats; ≥P9)		2.4 × 10^4 MSC/cm^2 + 1.6 × 10^4 hepatocytes/cm^2	Coculture with rat hepatocytes on FN	/	Dex, 100 ng/ml SCF, 20 ng/ml HGF, 50 ng/ml EGF, 10 ng/ml FGF4		Coculture: AFP, CK19/18, ALB		[78]
Coculture									
Indirect coculture BM (femora + tibias, 2- to 3-month-old SD rats; P1 + 3)		On top: MSCs On bottom: SD rat normal or cirrhotic liver tissue		10% FBS	/		AFP, ALB in cocultures with normal/injured liver		[83]
Conditioned culture medium BM (tibias, 2–3 month-old SD rats; P4)		NS	150 mg/l damaged liver tissue extract	NS	/		+: M2-PK, GST-p, ALB Wnt-1↓, Frizzled1↓, DSH↓, GSK-3β↓		[191]
Conditioned culture medium BM (femora + tibias, 8–10-week-old ICR mice; P3)		5 × 10^4 MSC/cm^2	Collagen type I, 50% hepatocyte conditioned media	10% FBS	/		AFP, HNF3β, CK19, CK18, ALB TAT, G6P	Glycogen storage, urea secretion	[192]

Table 2. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions					Hepatic features		Refs
		Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/ nonepigenetic additives	Differentiation inducing agents	RNA + protein level	Functionality level	
Growth factors/cytokines/specific ECM									
MSC (human; P6)			(a) Spherical cell pellet (b) Spherical pellet + SIS	10% FBS	*D0-14*: 50 ng/ml HGF, 10 ng/ml bFGF, ITS, dex, nicotinamide *D14-28*: 50 ng/ml OSM nicotinamide, ITS, dex		ALB, inducible CYP3A4 mRNA (a) > (b)	ALB and urea secretion, glycogen storage (a) > (b)	[76]
Failed transdifferentiation									
Growth factors/cytokines/cell surface markers									
BM (femora + tibias, Lewis.1WR2 rats; ≥P9)		2.4×10^4 MSC/cm^2	FN	/	100 ng/ml SCF, 20 ng/ml HGF, 50 ng/ml EGF, 10 ng/ml FGF4, dex		No expression of hepatic markers		[78]
BM (healthy human; P4-5)		22×10^3 MSC/cm^2 Differentiation onset upon 100% confluence	Collagen type 1	*Predifferentiation*: 2% FBS	10 ng/ml FGF4, dex, 20 ng/ml HGF, ITS		No expression of hepatic markers		[84]

(a), (b) and (1), (2), (3) indicate different protocols applied and order of serial steps, respectively. ↓, downregulation; ↑, upregulation; −, negative, +, positive).

Abbreviations: α1AT, α1-antitrypsin; ADH, alcohol dehydrogenase; ADSC, adipose tissue-derived stem cell; aFGF, acidic fibroblast growth factors; AFP, α-fetoprotein; ALB, albumin; 5-AzaC, 5-azacitidine; bFGF, basic FGF; BM, bone marrow; BSA, bovine serum albumin; CD, cluster of differentiation; C/EBP, CCAAT enhancer binding protein; CK, cytokeratin; CPS, carbamoyl phosphate synthase; Cx, connexin; CYP, cytochrome P450-dependent monooxygenases; dex, dexamethasone; DMSO, dimethylsulfoxide; DPPIV, dipeptidylpeptidase IV; DSH, dishevelled; ECM, extracellular matrix; EGF, epidermal growth factor; FBS, fetal bovine serum; FCS, fetal calf serum; FGF, fibroblast growth factor; FIC, freshly isolated cells; Flt3, FMS-like tyrosine kinase; FN, fibronectin; GlyA, glycophorin, A; G6P, glucose-6-phosphatase; GSK, glycogen synthase kinase; GST, glutathione *S*-transferase; m, months; HepPar1, hepatocyte paraffin 1; HGF, hepatocyte growth factor; HNF, hepatocyte nuclear factor; HPR, human platelet releasate; ITS, insulin-transferrin-selenious acid; LDL, low-density lipoprotein; MDR/MRP, multidrug resistance protein; M2PK, M_2-isozyme of pyruvate kinase; MSC, mesenchymal stem cell; NS, not specified; OSM, oncostatin M; P, passage; PDGF, platelet-derived growth factor; PEPCK, phosphoenolpyruvate carboxykinase; SCF, stem cell factor; SD, Sprague-Dawley; SIS, small intestine submucosa; TO, tryptophan-2,3-dioxygenase; TAT, tyrosine aminotransferase; TSA, trichostatin, A; TTR, transthyretin; UCB, umbilical cord blood; UGT, UDP-glucuronosyltransferase; w, weeks.

Table 3. Strategies for in vitro differentiation of LPCs into hepatocyte-like cells including their molecular and functional endpoints.

Origin	Marker-based selection	Hepatic differentiation conditions: Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/ nonepigenetic additives	Differentiation-inducing agents	Hepatic features: RNA + protein level	Functionality level	Ref.
LIVER PROGENITOR CELLS									
Successful transdifferentiation									
Spontaneous									
OC/CDE cell line from adult rat injury model		6.4×10^4 OC/cm^2	/	10% FCS	Insulin, hydrocortisone[a]		*Basal media*: −: GGT +: ALB (transient), LDH, G6P		[106]
LPC cell lines from allyl alcohol adult rat injury model		NS	*Expansion*: STO feeder layers *Differentiation*: removal of feeder layer	10% FBS			*Expanded LPCs on feeder layers*: (1) CK14$^+$, cKit$^+$, ALB$^-$, AFP$^+$; (2) CK14$^+$, cKit$^+$, ALB$^+$, AFP$^+$; (3) cKit$^+$, AFP$^+$, ALB$^+$, CK14$^-$; (4) AFP$^+$, ALB$^+$, CK14$^+$, cKit-1$^-$; (4) CD34$^+$, Thy1.1$^+$, CD45$^+$ *Spontaneous differentiation without feeder layers*: − or ↓: CD34, Thy1.1, OC10, CD45, CK14, cKit, OV1, OV6 +: CYP1A2		[193]
(1) Oval cells from 2-AAF/70% PH rat liver injury model (2) hepatoblasts from rat embryos	Dlk$^+$		Immunosorbent surface	10% FBS			(1) + (2) *FIC + in vitro culture*: AFP, Pref1$^+$, FA1$^+$		[194]
Growth factors/cytokines									
NPEC from adult human subacute injured livers		1.25×10^5 cells/cm^2	/	10% FBS	Insulin, hydrocortisone, 10 ng/ml EGF, 20 ng/ml HGF, nicotinamide, selenite, LA-BSA		CK7, CK19, CK18, ALB, α1AT, cMet, TGFβ receptor type II, GGT, biliary glycoprotein	AFP and α1AT secretion	[100]
LPC from E16, E18, E20 rat liver	Thy1$^{+/-}$ Ox43$^-$, Ox44$^-$	6.25×10^4 cm^2	Collagen type I	10% FCS	10 ng/ml EGF, insulin, dex, BSA		*Thy*$^{+/-}$: CK18, ALB, AFP Growth rate Thy$^+$ > Thy$^-$		[96]
Growth factors/cytokines/cell surface markers									
LPC from E13.5 mice liver	cMet$^+$, CD49f$^{+/low}$, cKit$^-$, CD45$^-$, TER119$^-$	1×10^3 cells/cm^2	Noncoated laminin, FN, type IV collagen, type I collagen	/	HGF, bFGF, OSM		*FIH*: HNF6, ALB HGF: ALB, α1AT C/EBPα↑, C/EBPβ↓ OSM: TO, G6P Hepatic effect of ECM < GF		[195]
Liver epithelial cells from E14.5 mouse liver	Dlk-1$^+$	Low density	Collagen	10% FBS	EGF, HGF		*FIC*: ALB *Upon in vitro induction*: ALB, CK19		[94]
		NS	gelatin		ITS, dex, 10 ng/ml OSM		TAT, G6P, CPS		
LPCs from adult mouse liver	CD45$^-$, TER119$^-$, side-scatter^{low}	1×10^3 cells/ cm^2	Collagen type I	10% FBS	Insulin, dex, nicotinamide, ascorbic acid, 20 μg/l HGF		*FIC*: integrin-α6$^+$, AFP$^+$, integrin-β1$^+$, cKit$^-$, Thy1.1$^-$ *Upon in vitro hepatic induction*: AFP, ALB		[101]

Table 3. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions: Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/nonepigenetic additives	Differentiation-inducing agents	Hepatic features: RNA + protein level	Functionality level	Ref.
LPC from 11 dpc mouse liver	cKit^{low}, CD45^{-}, TER119^{-}	2–4 × 10^5 cells/cm^2	Collagen type I	10% FCS	10 ng/ml EGF, dex, insulin + combinations of 40 ng/ml HGF, 10 ng/ml OSM, 20 ng/ml SCF, 50 ng/ml FGF-1		*FIC*: Moderate: HNF4, cKit High: ALB, AFP, TTR, HGF, OSMR, c-Met *Upon in vitro induction*: HGF: TTR, ALB, α1AT OSM: G6P, TAT HGF + OSM: TTR, ALB, α1AT, G6P, TAT	Glycogen storage	[97]
BEC from human healthy + tumor liver of adults and children	HEA^{+}	Subculture at 90% confluence	Collagen	5% FBS	10 ng/ml EGF, 10 ng/ml HGF, insulin, hydrocortisone		*While proliferating*[a]: +: CK8, CK18, CK7, CK19 Rare: AFP, ALB, α1AT, cKit −: vimentin, desmin, factor VIII		[102]
HPC from E13.5 mouse liver	(1) CD49f^{+}, Thy1, CD45^{-} (2) CD49f$^{+/-}$, Thy1^{+}, CD45^{-}	1 × 10^4 cells/cm^2	- Collagen type I - Coculture of CD49f^{+} and Thy1^{+} cells (1) direct (1/1); (2) indirect using inserts	10% FCS	Insulin, dex, nicotinamide, 20 μg/l HGF		*CD49f*$^{+}$: ALB, AFP, CK19 *Thy1*$^{+}$: +: smooth muscle actin, desmin, vimentin, −: AFP, ALB, or CK19 Coculture: TAT, TO	Coculture: glycogen storage	[95]
LPC from human fetal liver	−: CD2, CD3, CD14, CD16, CD19, CD24, CD36, CD38, CD45RA, CD56, CD66b, GlyA	NS	Collagen type I	10% FCS	50 ng/ml HGF, 20 ng/ml EGF, 10 ng/ml bFGF From D3: coexposure to 20% conditioned media		*FIH*: CD117^{+}, CD34^{+}, Lin^{-} In culture: ALB^{+}CK19^{+}/ALB^{+}/CK19^{+}/ALB^{-}CK19^{-}		[137]
SP cells from human cadaveric liver	Hoechst 33342^{-}, CD45$^{+/-}$	6 × 10^3 cells/cm^2	FN	10% FCS	dex, insulin, 20 ng/ml HGF, 10 ng/ml EGF		CD45$^{+/-}$: HepPar, CK8, ALB, CK18, α1AT, CYP2B6		[196]
Epithelial cells, hepatoblasts from E12.5 murine liver	TER119^{-}, CD45^{-}, cKit^{-}, CD31^{-}, Liv2^{+}, E-cadherin+, Dlk-1^{+}, Sca1^{+}	2 × 10^4 cells/cm^2	Collagen type I	10% FBS	dex, 10 ng/ml EGF, 10 ng/ml bFGF, 20 ng/ml TGFα		*FIC*: AFP^{+}, ALB^{+}, CKPan^{+}, CK19^{-} *Upon induction*: +: CK19, TAT, G6P −: TO		[136]
FLMPCs[a] from human fetal liver		1 × 10^4 cells/cm^2	Collagen	NS	10 ng/ml OSM		Undifferentiated FLMPC[a]: CD34^{+}, CD90^{+}, cKit^{+}, EPCAM^{+}, cMet^{+}, SSEA-4^{+}, CK18^{+}, CK19^{+}, ALB^{-}, AFP^{-}, CD44h^{+}, vimentin^{+} *Upon induction*; ALB, AFP, α1AT, HNF1α, HNF3β, HNF4	ALB secretion, glycogen storage, CYP activity	[98]
HLSC	+: CD29, CD73, CD44, CD90 −: CD34, CD45, CD117, CD133	NS	NS	NS	HGF, FGF4		*FIC*: CK8, CK18, vimentin, nestin, ALB, AFP *Upon induction*: AFP↓, CK8, CK18	*Upon induction*: CYP activity, ALB and urea production	[197]
Growth factors/cytokines/epigenetic modifiers									
LPC from E12 rat liver		NS	FN	Serum	Insulin, dex	DMSO	E12: AFP^{+}, ALB^{+}, HES6^{-} E15: HES6+, CK52−		[105]
						DMSO+ SB	+: BDS7		

Table 3. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions: Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/nonepigenetic additives	Differentiation-inducing agents	Hepatic features: RNA + protein level	Functionality level	Ref.
OC/CDE cell line from adult rat injury model					Insulin, hydrocortisone[a]		Basal media: −: GGT +: ALB (transient), LDH, G6P		
				10% FCS	Insulin, hydrocortisone, 2% DMSO/ 3.75 mM SB[a]		+: ALB (sustained), G6P↑		[106]
					Insulin, hydrocortisone, 3.75 mM SB[a]		+: GGT, AP↑		
WB F344		NS	NS	NS	dex	3.75 mM SB		TAT activity	[109]
LE/2, LE/6 oval cell line from CDE adult rat injury model		Subculture at 80–90% confluence	Irradiated 3T3 feeder layers on 3D collagen-gel sandwich	NS		1% DMSO	−: CK19, HES6, H.4, DPPIV, GGT +: CK8, CK18, ALB Long-term hepatic differentiation		[104]
FNRL from rat		4×10^{-4} cells/cm^2	NS	NS		5 mM SB for 24 h	*FIC*: −: ALB, TF, G6P, H.4, AFP, GGT, CK19 +: OV6 *Upon induction*: AFP, ALB		[110]
CF-PH from human liver		4×10^3 PH/cm^2	Coculture with 3T3 cells on collagen type I	10% human serum	5 ng/ml EGF, dex, nicotinamide, ascorbic acid	*D4*: 1% DMSO	*Nonconfluence*: CK7, CK19, CK8, CK18, ALB Confluence: −: CK7, CK19 +: ALB, HNF4, CYP450		[108]
LEPC from mouse liver		NS	NS	NS		SB	ALB, TO, ADH		[111]
HepaRG from HCV infected human liver		2.7×10^4 cells/cm^2 Purification by subculture		10% FBS	*D0-D14*: insulin, hydrocortisone	*D14-D28*: 2% DMSO	CYP3A4, CK18/19, CD49a, HNF3β↓, HNF4↑, HNF1		[113]
MSC from human fetal liver		NS		1% Matrigel	*From 12 h*: 10 μg/l HGF, 10 μg/l FGF-4	*0–10/12 h*: 2.5 mM 5-AzaC	+: AFP, GATA4, CK18, ALB, HNF1, GST −: other HNFs	ALB secretion Glycogen storage	[114]
MSC from healthy SD rat liver, 2, 4, 20, 28 months		2×10^4 cells/slide	NS	NS	/	*D1-D10*: 2% DMSO	Young > old: GST, CK18		[144]
Coculture									
LE/2, LE/6 oval cell line from CDE adult rat injury model		Subculture at 80–90% confluence	Irradiated 3T3 feeder layers on 3D collagen-gel sandwich	NS			−: CK19, HES6, H.4, DPPIV, GGT +: CK8, CK18, ALB Short-term culture		[104]

Table 3. (*Continued*).

Origin	Marker-based selection	Hepatic differentiation conditions: Cell density	Cell-matrix/cell-cell interaction	Serum	Growth factors-cytokines/nonepigenetic additives	Differentiation-inducing agents	Hepatic features: RNA + protein level	Functionality level	Ref.
LPC from 11 dpc mouse liver	cKit^{low}, CD45$^-$, TER119$^-$	1–5 × 10^3 11 dpc LPCs/cm^2	*D0-2*: Coculture of 11 dpc LPCs + cell-depleted 12 dpc liver fragments *D2-28*: transfer of fragments to filters				cKit^{low}, CD45$^-$, TER119$^-$, LPCs repopulated cell-depleted 12 dpc liver organoids → formation of ALB$^-$CK19$^+$ bile duct cells, ALB$^-$CK19$^-$, ALB$^+$CK19$^-$, ALB$^+$CK19$^-$ hepatocytes		[97]
LPCs from adult mouse liver	CD45$^-$, TER119$^-$, side-scatter^{low}	ND	Coculture of sorted cells with nonparenchymal cells	10% FBS	Insulin, dex, nicotinamide, ascorbic acid, 20 μg/l HGF		*FIC*: Integrin-α6$^+$, AFP$^+$, integrin-β1$^+$, cKit$^-$, Thy1.1$^-$ *Upon co-culture*: ALB, CK19		[101]
Oval cells from ethionine/PH injured rat liver		1042 cells/cm^2	On fibroblast feeder layers	NS	20 ng/ml SCF, 10 ng/ml HGF, 20 ng/ml HSS, 10 ng/ml EGF, 10 ng/ml LIF		*FIH*: OV6, CK19, ALB, CK7 *Cultured cells* (proliferation): ALB, CK7, CK19		[103]
Oval cells from E14 fetal rat liver	Thy1$^+$	4 × 10^3 cells/cm^2	On fibroblast feeder layers	/	BSA, dex, ITS, nicotinamide		*FIH*: AFP, ALB, cKit, CXCR4, HNF1α, HNF6, CK18, CK19 *Upon culture on feeder layers*: AFP, ALB, HNF1α, HNF6, CK18, CK19	Upon culture: glycogen storage	[198]
Aggregation									
BMEL from E14.5 mouse liver		6.4 × 10^4 cells/cm^2		/	/		*FIC + Basal media*: +: HNF1a, HNF4, GATA4, CK18, CK8, CK7, CD34, cKit, Cx43, CK19 −: apolipoprotein, ALB, Thy1, GGT IV *Upon aggregation*: AFP↑, aldolase B↑, ALB↑, apoB↑, apoA IV↑, ADH↑, TF, CK18, CK8, CK19		[107]
Failed transdifferentiation									
Growth factors/cytokines/matrices									
Oval cells from E14 fetal rat liver	Thy1$^+$	1–2 × 10^3 cells/cm^2	Collagen type I	10% FCS	(a) 50 μg/l EGF, insulin		(a) *Upon culture*: Fibroblastic morphology, no hepatic expression		[198]
					(b) insulin, dex, nicotinamide 50 μg/l HGF, 20 μg/l EGF		(b) *Upon culture*: Fibroblastic morphology, only CK18 expression		

(a), (b) and (1), (2), (3) indicate different protocols applied and order of serial steps, respectively. ↓, downregulation; ↑, upregulation; −, negative, +, positive).
[a]Simultaneous occurrence of differentiation and growth.
Abbreviations: AAF, 2-acetylaminofluorene; α1AT, α1-antitrypsin; AFP, α-fetoprotein; ALB, albumin; AP, alkaline phosphatase; 5-AzaC, 5-azacitidine; BDS, bile duct marker, BEC, biliary epithelial cell; bFGF, basic fibroblast growth factor; BMEL, bipotential mouse embryonic liver stem cell lines; BSA, bovine serum albumin; CD, cluster of differentiation; CDE, choline-deficient-DL-ethionine; C/EBP, CCAAT enhancer binding protein; CF-PH, colony forming parenchymal hepatocytes; CK, cytokeratin; CPS, carbamyl phosphate synthetase; Cx, connexin; CYP, cytochrome P450-dependent monooxygenases; 3D, three-dimensional; dex, dexamethasone; Dlk-1, delta-like 1 homolog Drosophila; DMSO, dimethylsulfoxide; dpc, days postcoitus; DPPIV, dipeptidylpeptidase IV; E, embryonic day; ECM, extracellular matrix; EGF, epidermal growth factor; EPCAM, epithelial cell adhesion molecule; FBS, fetal bovine serum; FCS, fetal calf serum; FEA, fetal antigen; FGF, fibroblast growth factor; FIC, freshly isolated cells; FLMPC, fetal liver multipotent progenitor cell; FN, fibronectin; FNRL, Fischer 344 neonatal rat liver; GF, growth factors; GGT, γ-glutamyltransferase; G6P, glucose-6-phosphatase; GST, glutathione S-transferase; HBC-3, hepatoblast cell line 3; HCV, hepatitis C virus; HEA, human epithelial antigen; HepPar1, hepatocyte paraffin 1; HGF, hepatocyte growth factor; HLSC, human liver stem cell; HNF, hepatocyte nuclear factor; HSS, hepatic stimulator substance; LA-BSA, linoleic acid-bovine serum albumin; LDH, lactate dehydrogenase; LE, liver epithelial; LEPC, liver epithelial progenitor cell; LIF, leukemia inhibitory factor; LPC, liver progenitor cell; MSC, mesenchymal stem cell; NPEC, nonparenchymal epithelial cells; OC/CDE, oval cell/choline-deficient-DL-ethionine; OSMR, oncostatin M receptor; PH, partial hepatectomy; Pref1 1+, preadipocyte factor 1; SB, sodium butyrate; Sca1, stem cell antigen; SCF, stem cell factor; SD, Sprague Dawley; SP, side population liver cells; SSEA, stage-specific mouse embryonic antigen; TAT, tyrosine aminotransferase; TF, transferrin; TGF, transforming growth factor; TTR, transthyretin; TO, tryptophan-2,3-dioxygenase.

Key signaling and molecular cross-talk events are thus patterned to occur in the right place at the right time [8]. Interactions between these various compartments accomplish homeostatic regulation of stem/progenitor cell functioning in vivo [2, 8]. Consequently, identification and simulation of these in vivo signaling patterns might comprise an approach to contribute to fate reprogrammation of stem/progenitor cells in vitro.

From Stem Cells to Hepatocytes: Current In Vitro Differentiation Strategies

Embryonic Stem Cells

ES cells spontaneously differentiate into cell types of the three germ layers, including hepatocytes, upon removal of leukemia inhibitory factor and feeder layers [34–37]. The processes of lineage establishment in developing embryoid bodies (EBs) appear to follow the events of embryogenesis, suggesting that ES cells can recognize and respond to the signals regulating embryonic development. The drawback is the yield of mixed cell types.

Addition of Soluble Medium Factors (Growth Factors/Cytokines/Corticosteroids/Hormones). The use of growth factors and cytokines is pivotal for hepatic growth of ES cells in vitro. Hormones and corticosteroids have a supporting role (Table 1).

Basically, activin A enriches ES cell cultures for endodermal populations [38, 39] and definite endoderm [40]. FGFs, but not BMP, are effective in mediating early hepatic differentiation, yet the choice of the most suitable FGF type depends on the species involved [41, 42]. HGF supports a midlate hepatic phenotype (e.g., ALB, dipeptidyl peptidase IV expression) [37, 43], but fails to induce functional maturation [34, 43]. Stepwise addition of FGF, HGF, and a mixture of insulin-transferrin-sodium selenite (ITS), dexamethasone, and OSM, on the other hand, seems successful [44–46]. Fine-tuning of the latter sequential strategy might even result in 70%–80% purity of ES cell-derived hepatocytes/hepatic progenitors within the culture system [47, 48]. Inherent to most differentiation protocols is the coexposure to serum. It contains hormones, growth factors, and other undefined substances that might contribute to stochastic differentiation of pluripotent ES cells [49, 50]. Nowadays, however, many efforts are being made to work under serum-free conditions. In particular, the use of serum-replacement factors has become a promising trend [47, 48, 51].

Reconstruction of In Vivo Cell-Matrix and Cell-Cell Interactions. Imitation of the ontogenic scaffold (particularly collagen) [42, 50, 52–58] and coculture with hepatic and nonhepatic cell types might provide an optimal in vitro environment to promote hepatogenic differentiation in ES cell and other stem cell cultures [49, 59]. However, intimate physical cell contact may result in cell fusion and requires highly sophisticated techniques to separate distinct cell populations. Hence, differentiation protocols currently use semipermeable membranes or filtered cell-conditioned media [60]. Fetal liver cells probably represent the most suitable cultivation partners [49, 59], because they, unlike cardiac mesoderm [61], contribute to hepatocellular functionality in ES cell cultures.

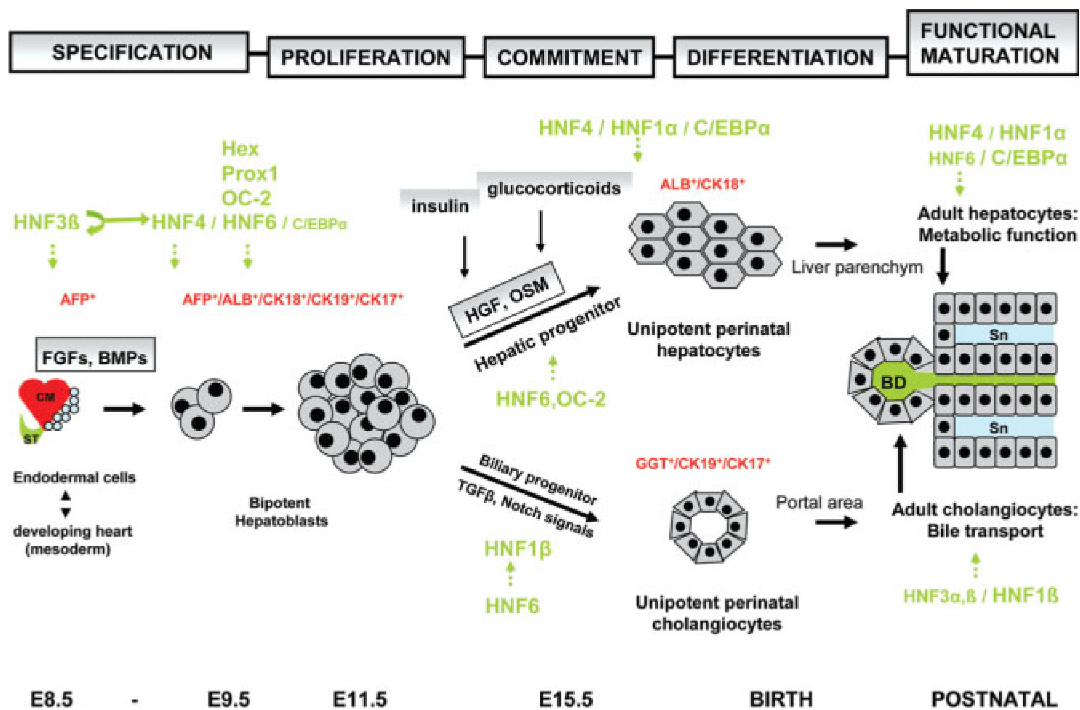

Figure 2. Schematic presentation of fetal liver development (modified from [2]). The establishment of a fully functional liver architecture is not accomplished before postnatal stages and follows upon a sequential array of tightly regulated intra- and extracellular signaling pathways, including liver-enriched transcription factors (LETFs) and growth factors, cytokines, glucocorticoids and hormones, respectively. To distinguish the level of expression and/or regulating role among diverse LETFs, different letter sizes are used. Abbreviations: ALB, albumin; AFP, α-fetoprotein, BMP, bone morphogenic proteins; C/EBP, CCAAT enhancer binding protein; CK, cytokeratin; CM, cardiogenic mesoderm; E, embryonic day in rodent liver development; FGF, fibroblast growth factors; GGT, γ-glutamyltransferase; HGF, hepatocyte growth factor; HNF, hepatocyte nuclear factor; OC-2, Onecut transcription factor; ST, septum transversum; TGF, transforming growth factor.

Determination of Cell Fate via Genetic Modification

(a) LETFS overexpression. HNF3β functions as a vital regulator of the initial intracellular signaling pathways in liver development/regeneration [28, 32] (Fig. 2). In addition, it may act as a driving force of ES cell differentiation along the hepatic lineage. In this context, HNF3β-transfected ES cells acquire a hepatic phenotype, more efficiently and far earlier than their untransfected counterparts [62–64]. Using this approach, ES cell differentiation in culture is in fact driven by the same transcriptional events as seen in early liver organogenesis in vivo. Hepatic gene expression and also hepatocellular functionality are found to be directly related to HNF3β levels [62]. A stated alternative is the recombinant expression of E-cadherin, most likely because adherens junction-mediated intercellular coupling has an integral role in hepatocyte functioning [52]. Major drawbacks of the constitutive overexpression of regulatory (transcription) factors are the risks of both unpredictable and nonintended gene upregulation in vitro.

(b) Epigenetic modification. The actual idea of changing cell fate via direct interference with the local chromatin structure of plastic cells was introduced only a few years ago. In 2003, ES exposure to 5 mM sodium butyrate led to 10%–15% enrichment with pure hepatic cells [65]. Lately, priming with alternating concentrations of sodium butyrate (0.5–1 mM) in the presence of activin A resulted in 10%-70% enrichment [51]. Basically, combined application of epigenetic modification and stepwise exposure to cytokine stimuli contributed considerably to homogeneity of the end population and acquirement of hepatic functionality [51]. Hitherto, both successful and failed differentiations were obtained using histone deacetylase inhibitors (HDACis), rendering their hepatotrophic effect ambiguous [43, 57]. Plausible explanations are described in From Stem Cells to Hepatocytes: Current Characterization Strategies and Their Limitations.

Multipotent Mesenchymal Stem Cells

Unidirectional/downstream differentiation into other mesenchymal cell types, such as adipocytes, chondrocytes, and osteoblasts readily occurs in the presence of a simple cocktail of growth factors and nutrients [66]. Successful bypassing of lineage borders depends mainly on multistep processes in a coordinated, synergistic signaling network (Table 2).

Addition of Soluble Medium Factors (Growth Factors/Cytokines/Corticosteroids/Hormones). Multipotent adult progenitor cells (MAPCs), discovered by Verfaillie and coworkers, were the first plastic cells found within adult bone marrow that gained the ability to undergo hepatic differentiation. Using combined exposure to FGF + HGF + ITS + dexamethasone, MAPCs transformed into cells with morphological, phenotypic, and functional characteristics of hepatocytes [67]. Yet, the resultant population was far from homogeneous. Significantly optimized differentiation was obtained via exposure of bone marrow stem cells to the same hepatogenic factors, but in a time-specific sequential manner, reflecting their secretion pattern during the hepatogenesis in vivo.

More than 85% of the thus sequentially cultured cells featured a highly differentiated hepatic phenotype and functionality, including inducible cytochrome P450 (CYP)-dependent activity [68]. Thus far, several research groups have revealed expression of distinct hepatocyte markers and functions, that is, ALB and urea secretion, glycogen storage, and low-density lipoprotein uptake upon stimulation of MSCs with hepatogenic factors exclusively, added either as a mixture (FGF + HGF [69–71]; FGF + HGF + OSM [72]) or separately (HGF [73]; HGF/OSM [74]; FGF/HGF/OSM [75]). Also combinations thereof (FGF + HGF followed by OSM [76, 77]) have been applied. In contrast, others emphasized the necessity of supplementary differentiation-inducing factors to enforce functional hepatic conversion of MSCs [78]. Basically, soluble medium factors such as dexamethasone, ITS, and nicotinamide synergistically affect the hepatic driving pathways [79]. In sharp contrast to the critical role of serum in MSC expansion and until recently in ES cell differentiation, serum-free conditions have been successfully applied on a routine basis for hepatic differentiation of MSCs [75, 77–79].

Reconstruction of In Vivo Cell-Matrix and Cell-Cell Interactions. Cocultures of stromal bone marrow cells with primary hepatocytes were at first designated to develop long-term functional hepatic in vitro models [80]. Jagged1 protein was considered responsible for the benign effects on hepatocyte differentiation by mediating differentiation events via the Notch signaling pathway [80, 81]. Later, Jagged1 and Notch were considered essential in driving bone marrow progenitors toward hepatocyte-lineage cells [81]. In a recent study by Lange et al. [78], coculture with liver cells was claimed to be the sole trigger able to shift MSC into cells with a hepatobiliary phenotype. The impaired differentiation capability of the chosen clonal MSCs or the high purity of high passaged MSCs (thus not contaminated with hematopoietic stem cells) [82, 83] was held responsible for failing growth factor-stimulated hepatic differentiation.

Another critical factor affecting cellular differentiation status is the spatial distribution between cells. Differentiation is usually initiated upon 60%–100% confluence (Table 1). Significantly promoted hepatic differentiation in areas of highest cellular density (maximal cell-cell contact) versus that in lower cellular density [67, 68, 74, 76, 82, 84] emphasized the relevance of intercellular communication during differentiation processes. Minor roles are ascribed to the type of coatings used. The natural scaffold collagen turns out to be most effective [68, 84].

Determination of Cell Fate via Genetic Modification

(a) LETFS overexpression. To the best of our knowledge, only one study thus far has investigated the putative inductive effect of LETFs on hepatic differentiating MSCs. More specifically, Talens-Visconti et al. [77] confirmed the contribution of C/EBPβ in driving adipose tissue-derived stem cells (ADSCs) and bone marrow-derived MSCs towards hepatic cells; yet, only trivial roles were ascribed to C/EBPα.

(b) Epigenetic modification. Epigenetic modification may contribute to overcome cell fate. determinism of MSCs. As such, we found previously that addition of 1 μM trichostatin A (TSA) to cultured human (h) MSCs, pretreated for 6 days with hepatogenic stimulating agents, triggers their "transdifferentiation" into cells with phenotypic and functional characteristics similar to those of primary hepatocytes [84]. In line with our results, Seo et al. [79] showed enhanced hepatic differentiation upon addition of 0.1% dimethylsulfoxide (DMSO) to hADSCs, prestimulated for 10 days with a mixture of hepatogenic cytokines. Recently, DNA methyl transferase inhibitors (DNMTis), either alone or combined with HDACis, also were introduced to alter cell fate [85–88]. Basically, DNMTis function as preconditioning agents before hepatic differentiation [87–89], whereas HDACis act as stimulants during or after differentiation [68, 79, 90, 91]. In general, chromatin remodeling seems, thus, to be a potential innovative strategy to overcome cell fate determinism and favor lineage-specific differentiation. This field is expected to emerge in the coming years.

Bipotent Liver Progenitor Cells

LPCs mainly comprise a bipotent progenitor cell population within the liver [92]. Their biliary/hepatic cell fate highly depends on cooperative cross-talks between extrinsic and intrinsic signaling pathways. Soluble factors, in particular, may execute pleiotropic effects (Table 3).

Addition of Soluble Medium Factors (Growth Factors/Cytokines/Corticosteroids/Hormones). Differentiation of LPCs into either the biliary or hepatic lineage greatly depends on the type of growth factor/cytokine used. (a) In midphase fetal liver, transforming growth factor (TGF) β promotes LPCs to undergo biliary differentiation [93], whereas HGF, FMS-like tyrosine kinase 3, stem cell factor (SCF), epidermal growth factor (EGF) [93–96], and members of the Gp130 receptor family, including OSM [97, 98], promote their initial hepatic differentiation and maturation, respectively. FGF propagates embryonic liver cultures toward hepatic progenitors. In this sense, FGF1 and FGF4 enrich for bipotential hepatic progenitors, whereas FGF8 further promotes the former enrichment for unipotential hepatocyte progenitors [99]. (b) In neonatal and adult rodent liver, HGF, FGF (FGF-1, FGF-2, and FGF-4), EGF, SCF, and TGFα,β might simultaneously play a central role in activation/proliferation, maintenance, and differentiation of LPCs such as liver epithelial cells and oval cells [100–103]. Some exceptions do occur, however [104].

Guidance of their cell fate by corticosteroids and hormones is less unidirectional. For example, dexamethasone upregulates the number of both hepatic- and bile duct-like cells in LPCs derived from midphase fetal mouse liver tissue [93]. Despite this scattered effect, when dexamethasone is accompanied by sodium butyrate, cultured oval cells shift solely toward the hepatocyte lineage [105]. Furthermore, growth-promoting effects have been ascribed to insulin, transferrin, α-tocopherol acetate, selenite, linoleic acid, nicotinamide, and hydrocortisone [95, 100–102, 106].

Reconstruction of In Vivo Cell-Matrix and Cell-Cell Interactions. The decisive factor of the microenvironment in directing the liver ontogeny underlines the importance of local cell and tissue paracrine signaling. In the context of this rationale, cocultivation of LPCs with stellate cells and mesenchymal feeder layers, including embryonic chick lung mesenchyme, growth-inhibiting embryonic STO fibroblast, or mesenchymal NIH3T3 fibroblast feeder layers, stimulate differentiation along the hepatic lineage [97, 101, 103, 104]. Cultivation in a three-dimensional collagen gel I matrix culture system provides further support [104]. In turn, removal of feeder layers and introduction of Matrigel leads to the formation of bile structures [104].

Besides signals secreted by the surrounding environment, cell density may also trigger differentiation. In essence, the

differentiation efficiency is linearly related to the level of confluence [107, 108].

Determination of Cell Fate via Epigenetic Modification. Long before their first introduction as cell fate modulators in ES and MSC cultures, epigenetic modification was found to actively contribute to the regulation of liver stem cell responses. The most commonly used HDACis in LPC cultures are DMSO and sodium butyrate [104–106, 108–111]. Depending on the cell type involved and the developmental/differentiation stage of the cells, these HDACis differentially direct cell fate determination. The following classes of HDACis can be distinguished on the basis of their potency to stimulate biliary and/or hepatic differentiation [112]: (a) hepatic-stimulatory sodium butyrate in FNRL cells [110] and WB-F344 cells [109]; (b) biliary- and hepatic-inducing DMSO in HepaRG [113]; and (c) hepatic-stimulatory DMSO and biliary-inducing sodium butyrate in explants of mouse E9.5 liver diverticulum [93] and in primary cultures of rat E12 liver cells [97]. Recently, priming of liver MSCs with the DNMTi 5-azacitidine was also found to trigger functional hepatic differentiation [114].

From Stem Cells to Hepatocytes: Current Characterization Strategies and Their Limitations

Stem cell-derived hepatocyte-like cells may be characterized in vitro at four levels: morphological, RNA, protein, and activity levels. Usually, the analytical work is limited to the elucidation of (a) endodermal/hepatogenic RNA transcripts via (quantitative) reverse transcriptase-polymerase chain reaction and (b) proteins by immunofluorescence. The most studied endodermal markers include LETFs (HNF1α,β, HNF3β, HNF4α, and C/EBPα,β), plasma proteins (AFP, ALB, transthyretin [TTR]), and cytoskeletal proteins (CK18, CK8) (Tables 1–3). A minority of studies have examined the expression of CYPs and other "late" enzymes such as tryptophan 2,3-dioxygenase (TO) and tyrosine amino transferase (TAT).

The following three features inherent to hepatic stem cell transitions need to be taken into account to perform accurate phenotyping. (a) The differentiation of stem/progenitor cells toward the hepatocyte lineage often involves uncontrolled processes, resulting in a heterogeneous cell population. Genes such as TAT [115], phosphoenolpyruvate carboxykinase [116], and LETFs [117–120] are also expressed in other somatic cells such as lung, intestine, pancreas, and kidney and thus cannot be considered as "true" hepatocyte markers. In addition, genes such as AFP and TTR are both expressed in liver tissue and in the extraembryonic yolk sac [121, 122]. Hence, exclusive analysis of one of the latter markers cannot count as proof for a genuine hepatic phenotype. The need thus arises to identify genes that are predominantly expressed in the liver and not in other tissues, enabling an accurate follow-up of the differentiation process and precise characterization of the end populations. In mouse, CYP7A1 is solely expressed in the liver and not in the yolk sac tissue, fulfilling its function as a reliable hepatocyte marker [36]. Alternatively, the synthesis of urea is a privileged function of hepatocytes [123, 124].

(b) The differentiation of hepatoblasts into hepatocytes is a steady process. It is known that embryonic, fetal, and adult hepatocytes differ in their molecular phenotype [1, 2]. Basically, hepatogenesis in vivo implies serial expression of early (HNF3β, AFP, and TTR), midlate (HNF1α, HNF4α, ALB, and CK18), and late (TO, TAT, C/EBPα, and CYPs) markers [28, 30–32, 125, 126]. Yet, some genes such as TTR and ALB are first expressed in early-midlate embryos and maintain expression in fetal and adult hepatocytes [126]. Positive expression of these genes may not enlighten the present differentiation state properly. AFP, on the other hand, is expressed very early in embryonic development and during the fetal stages. Its expression gradually levels off with increasing development and disappears entirely in adult life [125]. AFP thus represents a reliable marker to discriminate between distinct developmental stages. Alternatively, most, but not all, metabolic and detoxifying enzymes do not become functional before birth. Indeed, during the terminal step of liver organogenesis, the liver becomes a functional, metabolic organ: hepatocytes start to both control the levels of metabolites and serum proteins in the bloodstream and express numerous new genes and proteins related to specific functions of the adult liver [32, 123, 124]. Therefore, to state the differentiation stage of the resultant hepatocyte-like cells, functional assays for enzymes need to be carried out. At present, functional analysis is particularly focused on glycogen uptake, urea metabolism, and ALB secretion. Only a little attention has been paid to other metabolic functions, including CYP450-dependent activity and responsiveness to prototype inducers such as phenobarbital (human CYP2B6 and CYP3A4 and rat CYP2B1/2), rifampicin (CYP3A4), and 3-methylcholantrene (human and rat CYP1A1/2). If one bears in mind that inducible P450-dependent activity is considered to be a key determinant of the functional hepatic phenotype [123, 127], characterization must encompass the above-mentioned metabolic functionality assays as well.

(c) The ultimate proof of functional hepatic behavior is no doubt in vivo transplantation of ex vivo generated stem cells-based hepatic cells in (immunodeficient) animal models with liver injury [58, 70, 79, 82, 83, 87, 128]. Examples of recipients permissive for engraftment of both allogeneic and xenogeneic cells are partially hepatectomized *Pfp/Rag2*$^{-/-}$ [82]/nude [79] mice, carbon tetrachloride-injured severe combined immunodeficient (SCID) mice, and urokinase-type plasminogen activator$^{+/+}$/nonobese diabetic-SCID mice [79, 128, 129]. Positive homing, engraftment, repopulation, and functional maturation are basically explored by means of molecular imaging techniques, immunohistochemistry, in situ hybridization, and serology [58, 70, 79, 82, 83, 87, 128–130]. Despite seemingly irrefutable evidence that stem/progenitor cells could contribute to liver reconstitution, caution should be taken with production of false-positive results owing to application of inaccurate labeling techniques [131]. Also, one should keep in mind that, apart from generating fully functional stem cell-derived hepatocytes, other mechanisms including the bystander effect, fusion (cf. Introduction), partial transdifferentiation, and horizontal gene transfer [128, 129] might be responsible. For a more scrupulous insight in this complex matter, we refer the reader to Hengstler et al. [129].

In brief, accurate hepatic phenotyping in vitro should encompass the molecular analysis of a set of (non)specific hepatic markers in combination with ammonia formation and inducible CYP-dependent metabolism as functionality tests. Confirmation of the in vitro obtained results via rigorous in vivo tools might shed light on the therapeutic potential of stem/progenitor cells in various acute and chronic liver disorders.

Stem Cell Technologies: Current Shortcomings

Need for Standardization, Optimization, and Enrichment

ES cells harbor a unique pluripotent versatility compared with other fetal and adult multi- or bipotent stem/progenitor cell populations. They possess the unrestricted capacity to form embryonic and adult cell types, thereby reflecting the distinct developmental stages in vivo. Yet, the use of ES cell/EB technology encounters a complex differentiation environment, lack of organization, and inherent heterogeneity of the system [132–134]. In addition, although EBs may form functional and specialized cell types, including hepatocytes, the differentiation efficiency in number of lineage-specific cell types obtained is rather low [35, 135]. Culture of EBs in the presence of (a) differentiation inducers or (b) biologically derived signals (e.g., conditioned medium or purified growth factors) or other lineage-selective agents have been used to enrich for specific cell populations [133]. Thus, high-purity (70%–80%) ES cell-derived hepatocyte cultures have recently been produced on subculture and fine-tuning of the order/type of cytokine exposure [47, 48]. Yet, spontaneous differentiation is still predominant in many differentiation protocols. Differentiation is thus a default pathway of ES cells rather than replication. The opposite holds for adult stem cells [3]. For this reason, the use of adult stem/progenitor cells is often considered as a potential alternative. Basically, LPCs in culture differentiate either into hepatocytes, bile duct, or both (bipotency) [92, 97, 98, 136, 137]. The choice of matrices is the most important determinant for the direction taken. Lately, evidence has been provided that mesenchymal(-like) stem/progenitor cells from various sources (bone marrow, adipose tissue, skin, placenta, and umbilical cord) could occasionally overcome lineage borders and differentiate into endodermal (hepatocytes) and ectodermal (neural cells) cell types after specific in vitro induction [69, 74–76, 138–140]. It has now become clear that next to identification of hepatogenic cytokines or growth factors, their concentrations, mode of presentation, and order of application [8] also are crucial for hepatic differentiation and subsequent maturation into functional hepatocytes in vitro. As such, sequential exposure of bone marrow MSCs to hepatogenic factors reflecting their secretion pattern during liver embryogenesis in vivo results in a homogeneous population of functional hepatocytes. A downside of adult stem cell technology, however, is the level of reproducibility. Indeed, we found that only 25% of the bone marrow hMSC samples processed were “plastic” and consequently adopted a functional hepatic phenotype (intralaboratory variability). A number of unknown and consequently insufficiently controlled variables could be responsible. For example, the differentiation potential of MSC might depend on the following:

(a) The donor. Age, gender, lifestyle (e.g., smoking, alcohol consumption, or drug abuse, health condition (health/disease), intake of pharmaceutical agents, genetic differences, and others [141]. For example, the yield of MSCs within bone marrow is influenced by age, gender, the presence of osteoporosis, and prior exposure to high-dose chemotherapy or radiation [142]. In addition, both the differentiation and self-renewing capacity of bone marrow and liver MSCs was often, although not exclusively [143], found to level off with age [141, 142, 144]. In contrast, the adipogenic and myogenic differentiation ratios of ADSCs are not affected by the donor’s age [142]. To date, little is known about the relationship between disease (cancer) and stem cell behavior [142]. Yet, ADSCs derived from patients with gastric cancer were found to retain their endodermal differentiation potential [142, 145].

(b) The starting material. The harvest tissue varies, and the original characteristics of the starting material are often poorly defined (e.g., phenotypic profile, heterogeneity/conformity, and passage number). Phenotypic instability and plastic variability are inherent characteristics of MSCs [141, 146–148]. In this context, individual clones of cell lines derived from MSCs have different potentials for differentiation, indicating different stages of determination and levels of plasticity. Physiological alterations, resulting from exposure of clonal MSCs to a specific microenvironment during both proliferation and differentiation, may induce heritable and epigenetic cellular preconditioning, altering their original phenotype and manipulating their predestined cell fate [147]. In this regard, it was previously shown by DiGirolamo et al. [147] that some of the clonally derived MSCs from a single mother colony, expanded in separate cultures and subjected to identical osteogenic conditions, could efficiently differentiate into osteoblasts whereas others could not. This study clearly illustrates that clonal daughter cells, even when derived from a single mother cell, may have a different(ial) potential in response to soluble factors. The ambiguous definition of starting cell material remains a key obstacle for in vitro purposes and might even explain the global nonreproducibility or discrepancies in inter- and intralaboratory results reported thus far.

(c) The technology used. From an extensive review of the current literature, it appears that great variety exists among strategies to isolate, purify, expand, and differentiate postnatal stem cells. MSCs lack well-defined characterization and common surface markers that allow accurate isolation via fluorescence-activated cell sorting (FACS). For this reason, bone marrow MSCs are usually, but not exclusively, isolated via the plastic adherence technique. A major drawback of this strategy is its heterogeneous outcome, yielding a phenotypically mixed fibroblastoid cell population [141, 146, 148–150], often contaminated with hematopoietic cells at low passages [147, 151]. Basically, heterogeneity of initial populations hinders interpretation and reciprocal comparison of results among different research groups. Also, molecular cues necessary to enforce in vitro differentiation are complex and therefore are not easily identifiable or reproducible [152].

(d) Stress. Architectural and phenotypic diversification in response to stress might be misinterpreted as a true transdifferentiation phenomena. In fact, stem/progenitor cells removed from their natural niche and subsequently grown in a chemical ex vivo environment emit intrinsic (cellular) and chemical stress signals that in turn could lead to cytoskeletal collapse or pseudo-alchemical transitions [25, 26]. Unraveling the mechanisms underlying current successful and failed occurrences of adult stem cell plasticity and transdifferentiation is a complex and speculative undertaking that goes far beyond the scope of this review. Yet, we emphasize caution in interpreting data as spontaneous transitional processes.

It is conceivable that the factors enumerated above are only in part responsible for the variation in results observed in our studies and those of others. In this regard, it was postulated that phenotypic heterogeneity is intrinsic to stem cells because of their asymmetric self-renewal/differentiation potential.

Another critical factor for the commercial and clinical application (potential) of adult stem cells is the development of high-throughput scaling procedures. Today, most strategies to control and manipulate the cellular microenvironment of undifferentiated stem cells and their differentiated progeny are optimized on a laboratory scale. To be of pharmaceutical relevance, miniaturization and scaling up toward industrial needs are obligatory. In this context, bone marrow as source of hMSCs might not be ideal. Indeed, traditional bone marrow procurement procedures are risky for the patient and, in addition, bone marrow is also not readily available and yields only low numbers of multipotent stem cells upon processing [153]. A more easily accessible and readily available source of MSCs is human adipose tissue [75, 77, 78, 154] or human skin. These sources have the additional advantage that they may be obtained from healthy volunteers of diverse ages and gender. For these reasons, the latter alternative MSC sources are currently being explored.

Epigenetic Modification under Discussion?

Another point of interest is the role of epigenetic modifiers, particularly HDACis, in mediating hepatic-conditioned postnatal progenitor cells toward fully functional hepatocytes. In general, epigenetic modifiers affect a broad variety of cellular processes, including cell cycling, differentiation, and apoptosis [155–158]. For example, previous findings in our laboratory indicated that epigenetic alterations may represent a valuable approach to develop phenotypically stable primary hepatocyte cultures. It was revealed that addition of TSA to isolated primary hepatocytes impedes G_0/G_1 cell cycle transition and consequently favors the maintenance of hepatocellular functionality in vitro [155, 156, 158, 159]. Given this principle and the fact that covalent histone modification is central in processes determining lineage-specific gene expression and cell fate decisions [160, 161], we exposed postnatal bone marrow MSCs to TSA to obtain well-functioning mature hepatocytes. Critical factors in this process are onset of exposure, dose, and environmental conditions (cell-cell contact and cell densities) [155, 157, 158] as discussed in the following.

(a) Timing. Timing seems most essential in transdifferentiation processes. In this regard, addition of 1 μM TSA to undifferentiated bone marrow hMSCs and 0- to 5-day preconditioned bone marrow hMSCs resulted in massive cell death. On the other hand, hMSCs prestimulated with hepatogenic factors for at least 6 days before addition of 1 μM TSA underwent successful hepatic differentiation. Similar results were found by Seo et al. [79]. The importance of timing is also supported by the significant number of failed transdifferentiation experiments, producing nonhepatocyte-like cells [162–165]. In some cases, failure could be ascribed to inaccurately timing of exposure and determined concentrations.

(b) Dosage. Determination of the HDACi concentration that induces cell cycle arrest is another crucial factor, as the latter is generally a prerequisite for differentiation in vitro [166]. In primary hepatocyte cultures, differentiation and proliferation exclude each other [167]. Concentrations higher than this critical value may result in massive cell death. In preliminary experiments on bone marrow hMSCs, 5–25 μM TSA was found to be cytotoxic whereas 1 μM TSA, added from the 6th day of differentiation on, supported long-term culture and suppression of proliferation. Yet, at the molecular level a rather high apoptotic level was revealed. It was thought to be conceivable that TSA under hepatic-stimulating conditions selectively induced apoptosis of non(hepatic) differentiating cells, and simultaneously promoted the survival of hepatic differentiating cells. Although this is just a hypothesis and thus not based on stated evidence, it does stress the importance of timing and dose optimization of HDACis.

(c) Biotransformation. TSA is metabolically instable and undergoes intensive phase I biotransformation in primary rat hepatocytes [168]. With a 30-min incubation time, virtually all TSA is metabolized into inactive metabolites. It might thus be optional to use HDACi compounds that are more metabolically stable than TSA [169]; however, the latter only becomes an issue when stem cell-derived hepatocytes acquire metabolic activity.

Another point that can be raised here is the fact that HDACis, being modulators of chromatin, are by nature considered to be genotoxic. To date, data available in the literature are scarce. However, the genotoxic factor may have important consequences once one aims to use HDACi/DNMTi-treated hepatocytes in cell therapy or transplantation.

Perspectives

From the discussion in From Stem Cells to Hepatocytes: Current in Vitro Differentiation Strategies and From Stem Cells to Hepatocytes: Current Characterization Strategies and Their Limitations, it becomes clear that standardization of the production of functional hepatocytes out of postnatal progenitors and improvement of the hepatic potency of the initial progenitor population are tasks for the future. Here we state some ideas that may help to guide future stem cell research.

(a) A precise characterization of the undifferentiated initial cell populations is of utmost importance for future exploitation of stem cell technology. Phenotyping based on surface markers has thus far been insufficient. Instead, characterization should be performed at morphological, molecular, and functional levels. However, if the hypothesis that heterogeneity is inherent to stem cells is true, efforts hereto may be futile. With microarray analysis of gene expression pattern(s) and proteomics we will learn more. It also remains to be clarified whether physiological markers of MSCs and LPCs become lost or undergo changes during isolation and expansion/subculture procedures. Aging and stress during growth and subculture might also affect the phenotype of progenitors [141, 170–173]. Selection of reliable cell surface markers is therefore desirable to accurately isolate, select, and purify well-defined populations of plastic progenitors via FACS. Public accessibility of phenotypic profiling via databases and the Web may facilitate standardization and comparative inter- and intralaboratory studies.

(b) Stem cells differ significantly in their surface receptor expression profiles for cytokines/growth factors at successive developmental stages [8,27–29,31–33]. Dosage, timing, and combinations of cytokines/growth factors should thus be fine-tuned according to the differentiated state and type of stem cell involved. The suitability of epigenetics to promote hepatic (trans)differentiation requires a delicate balance between biological activity, pharmacokinetic, and toxicological characteristics; proliferation/ differentiation; and finally apoptosis/cell survival. Successful improvement of the hepatocellular phenotype and functionality of stem cell cultures relies, as is the case for primary hepatocyte cultures, on appropriate selection of type of epigenetic modifier applied and optimal fine-tuning of its dose and timing of exposure [163].

(c) Another major consideration is the dichotomy between hepatocyte proliferation and expression of differentiated functions (overview in [166]). In contrast to the in vivo situation, in which cellular proliferation and differentiation go hand in hand, in vitro differentiation is mostly associated with cell cycle arrest (with the exception of some in vitro cultured LPCs) [102, 103]. Most commonly, cells exit from the cell cycle and then undergo differentiation, resulting in either a terminal, irreversible cell specialization or a particular developmental step in the life cycle [166]. Hence, the dosage and combination of soluble medium additives should be fine-tuned, according to this dichotomy between proliferation and differentiation of the cells.
(d) Finally, in addition to variability at the in vitro level, it should be clarified whether or not the multipotency of stem/progenitor cells significantly depends on the donor's profile [141, 171, 174]. Simple questions on the effect of age (young or elderly donors), lifestyle (e.g., smokers or nonsmokers), health condition, and other factors should be answered before practical application is considered.

In conclusion, a more scrupulous understanding of the instructive signals emanating from the stem cell niche, together with a deeper analysis of cell-intrinsic mechanisms governing replication versus differentiation-inducing signals, is needed to reliably expand and differentiate stem/progenitor cells. Identification of reliable surface markers, useful for accurate and efficient isolation of plastic progenitor cells may upregulate the current hepatic potential of MSC and eventually serve to construct efficient and standardized devices that enable specific direction of MSCs and other progenitors towards the hepatocyte lineage. Standardization is, in any case, a sine qua non for prospective preclinical and clinical purposes of stem cells and their differentiated progeny.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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