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REVIEW ARTICLE

Cholesterol: recapitulation of its active role during liver regeneration

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Keywords
cholesterol – hepatic stellate cells – hepatocytes – Kupffer cells – liver regeneration

Abbreviations
aP2, adipocyte fatty acid-binding protein; *cav1*$^{-/-}$ KO, caveolin-1 knockout mice; c-Met, mesenchymal epithelial transition factor receptor; Cyp7a, cholesterol 7α-hydroxylase; Cyp8B1, sterol 12á-hydroxylase; DAG, diacylglycerol; DHCR7, 7-dehydrocholesterol reductase; DNA, deoxyribonucleic acid; ECM, extracellular matrix; ERK1/2, extracellular signal-regulated kinase; FSP-27, adipocyte marker; FXR, farnesoid X receptors; HDL, high-density lipoproteins; HGF, hepatocyte growth factor; Hmgc1, 3-OH-3-methylglutaryl-CoA lyase; HMG-CoAR, 3-OH-3-methylglutaryl-CoA reductase; HMGCS, 3-OH-3-methyl-glutaryl-CoA synthase; Hmgcs1, 3-OH-metyl-glutaryl-CoA synthase 1; Hmgcs2, 3-OH-metyl-glutaryl-CoA synthase; HSC, hepatic stellate cells; IL6, interleukin 6; INSIG, insulin-induced gene proteins; IP3, inositol 1,4,5-trisphosphate; JNK, c-Jun N-terminal kinase; LXR, liver X receptors; mRNA, messenger ribonucleic acid; NMR, nuclear magnetic resonance; PI-PLC, phosphoinositide phospholipase C; PKC, protein kinase C; PMCA, plasma membrane Ca^{2+}-ATPase; PPAR, peroxisome-proliferator-activated receptors; S3-12, (member of PAT proteins, target for PPARγ); SCAP, SREBP-cleavage activating protein; SRE, sterol regulatory element; SREBPs, sterol regulatory element-binding proteins; TGFβ, transforming growth factor-β; TNFR1, tumour necrosis factor receptor Type I; UTRs, untranslated regions; VLDL, very-low-density lipoproteins.

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Abstract
Liver regeneration is a compensatory hyperplasia produced by several stimuli that promotes proliferation in order to provide recovery of the liver mass and architecture. This process involves complex signalling cascades that receive feedback from autocrine and paracrine pathways, recognized by parenchymal as well as non-parenchymal cells. Nowadays the dynamic role of lipids in biological processes is widely recognized; however, a systematic analysis of their importance during liver regeneration is still missing. Therefore, in this review we address the role of lipids including the bioactive ones such as sphingolipids, but with special emphasis on cholesterol. Cholesterol is not only considered as a structural component but also as a relevant lipid involved in the control of the intermediate metabolism of different liver cell types such as hepatocytes, hepatic stellate cells and Kupffer cells. Cholesterol plays a significant role at the level of specific membrane domains, as well as modulating the expression of sterol-dependent proteins. Moreover, several enzymes related to the catabolism of cholesterol and whose activity is down regulated are related to the protection of liver tissue from toxicity during the process of regeneration. This review puts in perspective the necessity to study and understand the basic mechanisms involving lipids during the process of liver regeneration. On the other hand, the knowledge acquired in this area in the past years, can be considered invaluable in order to provide further insights into processes such as general organogenesis and several liver-related pathologies, including steatosis and fibrosis.

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Liver cells are normally arrested in the G_0 phase of the cell cycle. However, a diverse range of stimuli are capable of inducing approximately 95% of the hepatic cells to enter a replicative state (G_1/S) through a process known as liver regeneration. This process involves the coordinated intervention of growth-promoting cytokines to prepare liver cells for mitosis, growth factors to initiate proliferation and inhibitory cytokines to return liver cells to a quiescent state (1). Currently, comprehensive reviews of this process have accounted for the myriad of molecular mechanisms involved in the switch from a quiescent state to an actively dividing state. However, fewer studies have addressed how hepatocytes, as well as non-parenchymal cells, function after undergoing a significant metabolic process such as tissue regeneration, while still maintaining vital functions for the body. Although, most metabolic pathways are involved in the regeneration process, in this review we focus on pathways that involve structural as well as bioactive lipids. For liver cells, lipids are not only a significant source of energy but they also represent an important structural component of the eukaryotic plasma membrane. In particular, the role of cholesterol during liver regeneration will be discussed as it constitutes $>30\%$ of the lipids present in the cell plasma membrane, modulates the permeability and the fluidity of the cell membrane, controls the activity of membrane proteins and contributes to the regulation of protein trafficking and transmembrane signalling (2). The capacity for lipids within the nuclear matrix to serve as active molecules (i.e. secondary messengers) in the control of metabolism, and as regulators of chromatin structure, will also be discussed.

Lipid storage during liver regeneration

Initial reports have documented that the accumulation of fat in the liver, mainly in the form of triglycerides (TG) and cholesterol esters (CE), after a 70% partial hepatectomy affects the metabolism of lipids and their corresponding enzymes (Table 1) (3–5). In hepatocytes, this accumulation is apparent as lipid droplets and reaches maximum levels at 12 h after a partial hepatectomy in rats, and between 12 and 24 h in mice (6, 7). In both cases, the levels of cholesterol and TG in the serum were found to decrease (Fig. 1). In an analysis of rat serum following partial hepatectomy, an increase in the fatty acid levels was detected after 8 h, with the highest levels detected 20 h after the surgery (6). In addition, levels of glycerol 3-phosphate acyltransferase located in the microsomal fraction were found to increase, and a seven-fold increase in TG levels were detected 24 h after the surgery with respect to sham-operated rats (8). Therefore, it is clear that steatosis comprises a temporal event during the liver regeneration, although the mechanisms involved are not completely understood (Table 1). Furthermore, early cytokine signalling appears to have a role in the release of fatty acids from adipose tissues into the circulatory system and in their subsequent esterification and storage as TG (9).

Before the accumulation of fat needed for liver regeneration, a well-organized gene expression pattern is orchestrated that shares similarities with the gene expression pattern for adipocyte differentiation. This similarity suggests that a strict level of gene regulation is associated with liver regeneration, and this has been shown to include the induction in mice of adipogenic genes such as *adipsin aP2* (adipocyte fatty acid-binding protein),

Table 1. Correlation of main events occurring during rat liver regeneration and changes in lipids levels

Phase	Main events	TG (5, 8) mg/g	Cholesterol (18, 19) μg/mg	PL (18) μg/mg	CER (89) nmoles FA/mg	SM (89) nmoles FA/mg
Normal liver (G_0)	Quiescent tissue	2–3.0 LP	98.0 MP 0.15 LP	500.0 MP	0.25 LP	1.10 LP
Priming ($G_0 \rightarrow G_1$)	↑GP-cytokines					
0–4 h	↑Early genes		120.0 MP (3 h)	470.0 MP (3 h)	0.31 LP (4 h)	1.19 LP
4–8 h	↑Delayed genes ↑Growth factors	8.0 LP (10 h)	75.0 MP (6 h)	500.0 MP (6 h)		
Proliferative ($G_1 \rightarrow S$) 12 h	↑DNA synthesis*	12.6 LP (18 h) 14.0 LP (24 h)	80.0 MP (12 h) 80.0 MP (24 h) 0.15 LP (24 h)	550.0 MP (12 h) 460.0 MP (24 h)	0.33 LP (12h) 0.35 LP (24 h)	1.20 LP 1.19 LP
30 h	Mitosis†					
Inhibitory ($S \rightarrow G_0$)	↑I-cytokines					
48 h	↓DNA synthesis		87.0 MP 0.24 LP	450.0 MP		
72 h	80% restored		88.0 MP	400.0 MP		
168 h	Restored liver		95.0 PM 0.30 LP	470.0 MP		

*Maximum deoxyribonucleic acid (DNA) synthesis at 24 h in hepatocytes; at 48 h in KC and HSC; at 96 h in endothelial cells.

†Maximum mitosis at 48 h in hepatocytes.

CER, ceramides; FA, fatty acids; GP, growth-promoting or proinflammatory cytokines; HSC, hepatic stellate cells; KC, Kupffer cells; I-cytokines, inhibitory cytokines; LP, liver protein; MP, membrane protein; PL, phospholipids; SM, sphingomyelin; TG, triglycerides.

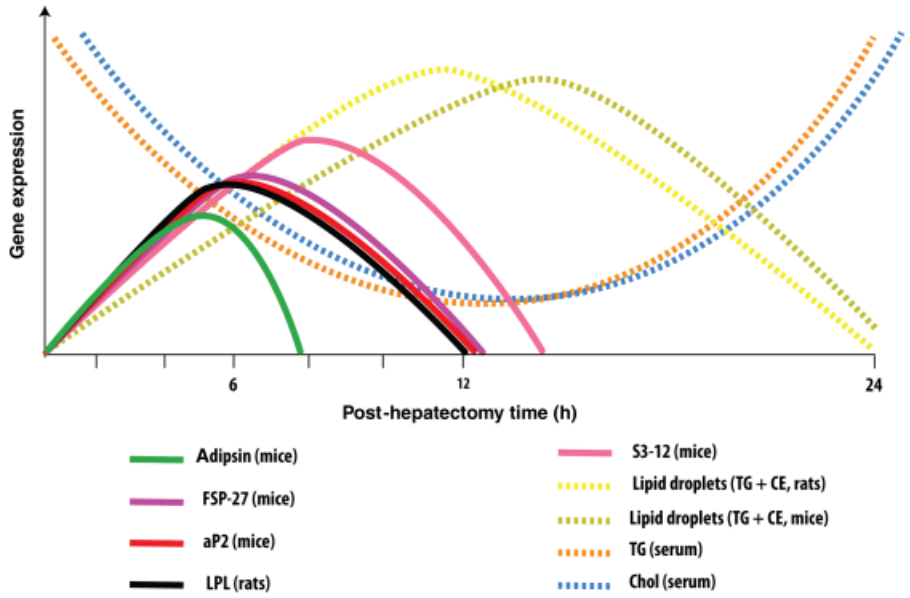

Fig. 1. Expression pattern of gene transcripts related to lipid accumulation after partial hepatectomy. Lipid droplets containing triglycerides (TG) and cholesterol esters (CE) are observed in hepatocytes, with maximum expression levels detected at 12 and 12–24 h post-partial hepatectomy in rats and mice respectively. A concomitant decrease in serum cholesterol and TG was also observed in both models (6, 7). Before lipid accumulation, several adipogenic genes are induced, including lipoprotein lipase in rats, the adipocyte marker, FSP-27 and the adipocyte fatty acid-binding protein, aP2, in mice. All of these show a peak induction occurring 6 h post-partial hepatectomy (11), although adipsin reaches maximal levels as early as 4 h post-partial hepatectomy, while at 8 h S3-12, a member of the Perilipin/adipophilin/TIP47 protein family and target for peroxisome-proliferator-activated receptors-γ, reaches its maximum levels in mice (7). In combination with the induction of cytokines (i.e. tumour necrosis factor-α, interleukin-6) detected at earlier time points of post-partial hepatectomy, these changes in gene expression may correlate with the release of fatty acids into the circulatory system, which are later stored as TG (9).

S3-12 [member of Perilipin/adipophilin/TIP47 protein family that targets peroxisome-proliferator-activated receptors (PPAR)γ] and the adipocyte marker, *FSP-27* (Fig. 1) (7). Interestingly, if the adipogenic process is blocked by administration of recombinant leptin, or by inactivation of a specific receptor involved in the regulation of adipocyte expression (i.e. glucocorticoid receptors), liver regeneration is impaired because of the delay in fat accumulation (7). Recently, it has been shown in rats that the resemblance between light lipid droplets (density > 1.02 g/ml) observed in regenerating liver and adipocytes is not limited not only to their gene expression but also to their composition, basically abundant in TG and devoid of CE (10). In addition, dense droplets (density > 1.06 g/ml) identified during the liver regeneration were poor in TG and rich in phosphatidylethanolamine (10).

After partial hepatectomy, several metabolic precursors are imported into the liver from other tissues, or their expression is up-regulated in the liver cells. For example, lipoprotein lipase (LPL) that hydrolyzes TG present in serum delivers fatty acids to the liver for re-esterification and to provide an energy source. LPL is normally expressed in early development; however, during liver regeneration, hepatocytes experience a dedifferentiation process that initiates fetal gene expression patterns. As a result, in rats LPL activity has been shown to increase 2.5-fold over control levels 6 h after partial hepatectomy, and to return to normal levels at 24 h (Fig. 1). Correspondingly, messenger ribonucleic acid (mRNA) levels of LPL have also been shown to increase between 6 and 24 h post-partial hepatectomy (11).

Cholesterol as a structural lipid during liver regeneration

Changes in membrane fluidity

Cholesterol is a structural component of biological membranes that influences properties such as permeability and fluidity, while also contributing to the modulation of the membrane proteins, protein trafficking, and transmembrane signalling. Importantly, cholesterol is also a precursor for the generation of steroids and bile acids, which contribute to the solubilization of other lipids and function as signal transducers (2).

Studies of lipid metabolism during liver regeneration have focused on an analysis of changes in the content and ratios of structural lipids, and therefore, on membrane properties. Membrane fluidity is a multifactorial parameter that depends on the presence of cholesterol, proteins, phospholipids and the extent of saturation and chain length for fatty acid chains. Micro-environmental conditions such as temperature and pressure are also factors to be considered. Experimentally, lipid bilayers

with a known composition have been investigated using paramagnetic resonance to quantitatively evaluate differences between two or more physical phases (12). On the basis of this approach, interactions between phosphatidylcholine and cholesterol can be explained in terms of a liquid-ordered phase that exhibits highly ordered, rapid translational movement. Moreover, this liquid-ordered phase can co-exist with a liquid-disordered phase of phosphatidylcholine and cholesterol (13).

Post-hepatectomy changes in fluidity are not limited to the plasma membrane, because important modifications have also been detected in microsomal (14) and nuclear membranes (15). For example, early studies of these changes performed in rats detected a decrease in the cholesterol/phospholipid ratio of these membranes, and an increase in plasma membrane fluidity between 15 and 24 h post-hepatectomy (14, 16). However, a similar increase in the phospholipid and cholesterol levels 32 h after partial hepatectomy was also observed, although these increases did not affect cholesterol/phospholipid ratios (17). Moreover in rat, up to 168 h post-hepatectomy, the liver plasma membrane composition was not found to change significantly, while membrane fluidity, measured as the cholesterol/phospholipid ratio, increased as early as 3 h following partial hepatectomy (18). Increased levels of cholesterol and phosphatidylethanolamine content in membranes were also detected, consistent with the increase in fluidity and the apparent cholesterol/phospholipid ratio detected between 2 and 7 days after partial hepatectomy (Table 1) (19).

Relevance of cholesterol-rich domains: caveolae and lipid rafts

By itself, cholesterol modulates plasma membrane-associated proteins to affect the physical state of membranes (20, 21). For example, when levels of cholesterol were increased in cardiac sarcolemma, a decrease in the enzyme activity of plasma membrane Ca^{2+}-ATPase (PMCA) was observed, as well as an increase in its thermal stability (21–24). However, the opposite results have been observed when the cholesterol content in sarcolemmal membranes was reduced (25, 26). Correspondingly, the evolutionary relevance of cholesterol and its ability to improve protein stability in membranes have been discussed (21). Furthermore, we propose that in addition to the presence of cholesterol, specific changes in the structural conformation of the membrane proteins increase their stability and also enhance the ability of cells to withstand a thermal challenge (21).

To date, it is known that cholesterol within the plasma membrane of eukaryotic cells is organized into different microdomains, including caveolae, rafts and non-raft-domains (27–29). Caveolae are sphingomyelin/cholesterol-rich domains forming part of invaginations that contain associated proteins or caveolins (types 1–3). In liver cells, there are two types of caveolin-rich domains, one which is soluble in Triton X-100 and is closely related to the cytoskeleton, while the other which is insoluble in Triton X-100 and is associated with the smaller molecular weight fractions of a gradient (i.e. detergent-resistant membranes/rafts) (30). The functions of caveolae are related to the regulation of cholesterol homeostasis, as well as with signal transduction pathways that mediate lipid metabolism (31). Moreover, the regulation of cellular proliferation and lipogenesis by caveolae and caveolins has been shown to be important (32). For caveolin-1, its role as a fatty acid transporter in the plasma membrane has been demonstrated (33). Furthermore, in white adipose tissue of caveolin-1 knockout ($cav1^{-/-}$ KO) mice, the absence of caveolin-1 reduces the ability of adipocytes to store or use TG for the synthesis of fatty acids (34). Accordingly, $cav1^{-/-}$ mice do not accumulate the same levels of TG in their hepatocytes as wild-type mice (35). At 72 h post-partial hepatectomy, the survival of $cav1^{-/-}$ mice was ~28%, compared with 100% for wild-type mice. However, when glucose was administered to $cav1^{-/-}$ mice, survival rates increased to match those of wild-type mice (35). Therefore, this last study points to the fact that impairment in regeneration 24 h after partial hepatectomy is mainly because of the unavailability of a source of energy and/or lipid molecules needed to reconstruct membranes and to participate in signalling and proliferation processes. On the other hand, it was not evident increased apoptosis that otherwise might have indicated some degree of injury (35). Experiments performed in $cav1^{-/-}$ mice with different genetic backgrounds, yet with wild-type levels of fat and TG accumulation, identified a faster rate of proliferation for hepatocytes after partial hepatectomy. However, no effects on survival were observed (36). An increase in cav-1 levels, as well as a redistribution of cav-1 proteins, was also associated with wild-type mice following partial hepatectomy (36). Concerning the possible mechanisms involved in the accelerated process of liver regeneration of *cav1*-deficient mice, the delocalization of receptors for transforming growth factor (TGF)-β seems to alter its signalling, producing an induction of proto-oncogenes [(Ski-related novel protein N (SnoN)] and an imbalance between actions promoted by TGF-β and hepatocyte growth factor (HGF) (37). It has been proposed that SnoN and Ski are important inhibitors of TGF-β antiproliferative signals during liver regeneration (38). Despite these insights, additional studies are still needed to determine whether caveolins provide a true 'metabolic push' for the regeneration of liver tissue to recover its initial mass (33).

On the other hand, lipid rafts are sphingomyelin/cholesterol-rich membrane domains associated with flotillins (39). Raft domains are larger than caveolae and are similar to platforms that become aggregated to mediate signal transduction in the cell (27). Fewer studies have been performed to address changes in lipids rafts that may occur following partial hepatectomy. However, it is likely that these domains modulate several liver tissue proteins during liver regeneration. Plasma membranes of

hepatocytes also contain higher levels of cholesterol compared with other cell types. Accordingly, preliminary observations in our laboratory have demonstrated that a partial depletion of cholesterol from hepatocyte-isolated plasma membranes decreases the level of PMCA activity as a function of a decrease in cholesterol concentration (B. Delgado-Coello *et al.*, unpublished results).

To our knowledge, a few groups have reported experiments with cholesterol-depleting reagents (for instance, statins) using only short-term primary hepatocyte cultures. One such study reports a dose-dependent effect of pravastatin on deoxyribonucleic acid (DNA) synthesis when hepatocytes are induced to proliferation with epidermal growth factor (40). In our experience, the treatment of primary rat hepatocytes with concentrations between 1.5 and 2.0 mM of methyl-β cyclodextrin in the absence of growth factors, compromises the cells' viability (B. Delgado-Coello *et al.*, unpublished results). Therefore, in order to study the effect of cholesterol on hepatocyte proliferation, experiments should be carried out under moderate depleting conditions when using growth factors. Studies are in progress to analyse the influence of cholesterol content on PMCA activity of proliferating hepatocytes, which may represent a parallel model for liver regeneration.

Concerning other functions of cholesterol, the extracellular signals that hepatocytes receive after partial hepatectomy are mediated by receptors located in the plasma membrane. For example, the scavenger receptor B1 obtains cholesterol from high-density lipoproteins (HDLs), while cholesterol is also obtained from the sinusoidal domain via transcytosis and transferred to the bile canalicular domain for secretion into bile (2). Additional studies have indicated that during liver regeneration, the trafficking of cholesterol drastically changes as indicated by the low recovery of late endosomes, which leads to an accumulation of cargo proteins in recycling/transcytotic compartments (41).

Changes in enzymes that are involved in cholesterol biosynthesis during liver regeneration

Regulation of cholesterol synthesis

Lipid-activated receptors, such as PPAR (types α, β or δ, and γ), liver X receptors (LXR, types α and β) and farnesoid X receptors (FXR, types α1–α4) regulate cell processes of development, reproduction, inflammmation and metabolism, among others (42). While PPAR-α is abundantly expressed in the liver and regulates enzymes catalysing fatty acid oxidation, PPAR-γ is also expressed in adipose tissue where it intervenes in the control of adipocyte differentiation. Alternatively, LXR receptors function as cholesterol sensors that modulate cholesterol homeostasis both at the cellular and systemic levels. In mice, the LXR system is considered the main catabolic pathway mediated by oxysterol nuclear receptors (43). In wild-type mice while cholesterol levels increase in the liver after partial hepatectomy, the transcriptional pathway involving LXR is down-regulated representing a necessary condition to an adequate proliferation of hepatocytes (43). LXR-null mice ($LXR\alpha\beta^{-/-}$), although showing a similar regenerative capacity, do not show apparent differences in the expression pattern of cytokines and growth factors, when compared with $LXR\alpha\beta^{+/+}$ (43). In a complementary fashion, the FXR in the liver are activated by endogenous bile acids, inducing a transcriptional feedback repression that becomes relevant for the appropriate progress of liver regeneration. Moreover, recently a new direct FXR target (Foxm1b) has proven to be involved in cell cycle regulation and ageing regenerating livers (44). FXR function results in the repression of cholesterol 7α-hydroxylase (Cyp7a)1 transcription that in turn can be stimulated by diverse cholesterol metabolites via their association to LXR (45, 46) Together, these receptors participate avoiding the catabolic modification of cholesterol, leading to bile synthesis and secretion during liver regeneration.

On the other hand, both cholesterol synthesis and its exogenous recovery from low-density lipoproteins in the plasma via receptor-mediated endocytosis are rigorously regulated in mammals through a well-known feedback mechanism. For example, promoters of sterol-regulated genes include a sterol regulatory element (SRE), which is bound by sterol regulatory element-binding protein (SREBP) transcription factors in response to cholesterol. SREBPs control more than 30 genes encoding proteins that mediate the synthesis and uptake of cholesterol, as well as unsaturated fatty acids, TG and phospholipids (47, 48). In their inactive form, SREBPs form a complex with SREBP-cleavage activating proteins (SCAPs) and insulin-induced gene proteins (INSIG) to be maintained as membrane-associated proteins in the endoplasmic reticulum or nucleus, thereby preventing their binding of SREs. However, when SCAPs sense low levels of sterol, SREBPs are released to activate gene transcription. In addition, SREBP–SCAP complexes can relocate from the endoplasmic reticulum to the Golgi apparatus to mediate cholesterol transport (47).

Differential expression of genes encoding enzymes for cholesterol synthesis during liver regeneration

In addition to its structural function as a component of cell membranes, cholesterol can also modulate cell-cycle progression. Moreover, this function cannot be substituted with other sterols and achieve the same efficiency (49). The majority of studies on cholesterol biosynthesis have investigated key enzymes in this pathway, including 3-OH-3-methylglutaryl-CoA reductase (HMG-CoAR), Cyp7a and sterol 12α-hydroxylase, up to the point where sterols are eliminated as bile acids (Fig. 2). Mevalonate pathway is necessary for the regulation of cholesterol levels in the liver and plays an important role in controlling cell proliferation by producing prenyl intermediates that are needed to anchor important signalling proteins to the cell membrane, including small G proteins.

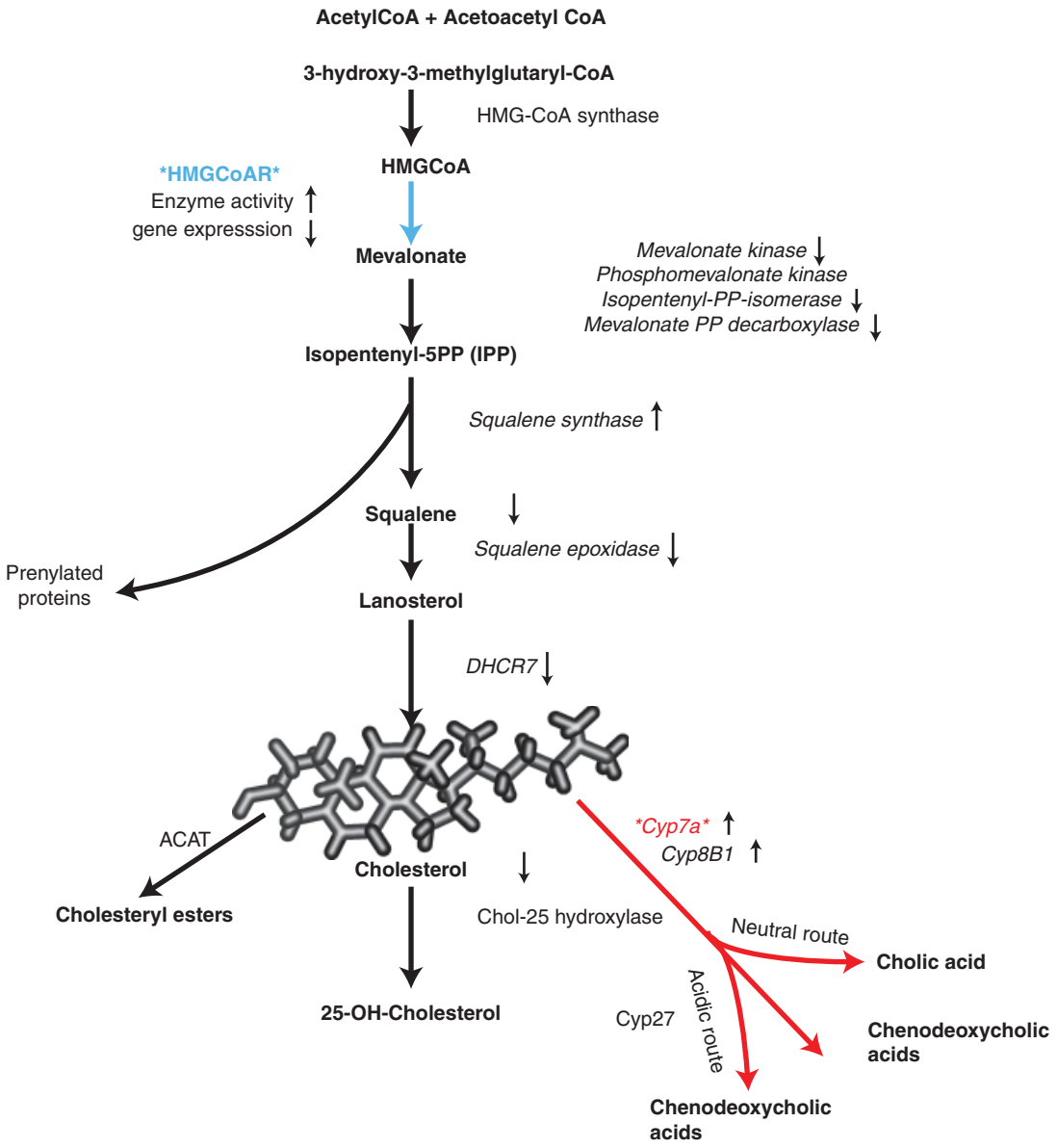

Fig. 2. An abbreviated mevalonate pathway and potential changes that may occur during liver regeneration. Hydroxy-methylglutaryl CoA reductase (HMG-CoAR) is a rate-limiting enzyme, whose kinetics and gene expression profile have been extensively studied during liver regeneration (51, 52, 55, 76). Synthesis of cholesterol from lanosterol involves 19 intermediate reactions; however, only the step involving 7-dehydro-cholesterol-reductase (DHCR7) is shown in this figure. Cholesterol is important in the liver as a precursor in the production of cholesteryl esters, or for sterol elimination via bile acids through the rate-limiting enzyme, cholesterol 7α-hydroxylase (Cyp7a). Cyp7a and Cyp8B1 mRNAs are down-regulated in mice at early times after partial hepatectomy, correlating with a diminished enzyme activity that may protect the liver avoiding toxicity because of an overproduction of bile acids (76–78); these mRNAS are up-regulated in the long-term in rats (71). The possible deviation of the mevalonate pathway to generate isoprenylated proteins from intermediates of squalene (i.e. geranyl-PP and farnesyl-PP) synthesis as a result of increased HMG-CoAR activity during liver regeneration is indicated (54).

A differential distribution of important enzymes has been reported in the liver acinus, where HMG-CoAR and 3-OH-3-methylglutaryl-CoA synthase (HMGCS) predominate in periportally located hepatocytes (50). During liver regeneration, the activity of HMG-CoAR increases, which represents a rate-limiting enzyme in the mevalonate pathway. However, reduced production of cholesterol is also observed, suggesting that a deviation of the mevalonate pathway occurs so that the synthesis of non-steroid products and isoprenylated proteins are more dominant (Fig. 2) (51, 52).

Recently, gene expression patterns during liver regeneration, or in immortalized hepatocytes, have been

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Table 2. Changes at transcript level of genes encoding enzymes of biosynthesis of cholesterol during liver regeneration and steatotic models

↑Gen (fold change)	↓Gen (fold change)	Technique	Organism/model	References
	Mevalonate kinase (− 5) *DHCR7* (− 7)	SAGE library	Rats/PH	(72)
CYP7A1 (1 day/+1; 7 days/+3.5) *CYP8B1* (1 day/+1)	*CYP27* (1 day/ − 1)	qPCR	Rats/PH (1–14 days)	(71)
	Hmgcs1 *Hmgcs2* *Hmgc1*	Microarrays	Rats+CCl_4/(6 h–2 weeks)	(53)
	HMG-CoAR *IDI1* *SQLE*	Microarrays	Rats/PH (0.5–168 h)	(55)
	CYP8B1 (− 5)	Microarrays	Mice/PH (12 h)	(7)
Squalene synthase		Microarrays/NB	Mice/PH (6 h)	(54)
	CYP7A1	qPCR/WB	Mice/PH (1–6 h)	(78)
	HMGS1 soluble (− 6.17) *DHCR7* (− 2.11) *HMG-CoAR* (− 2.11) *CYP51A1* (− 1.55) *NADP-dependent steroid dehydrogenase* (− 1.68) *SQLE* (− 2.58) *IDI1* (− 2.62)	Microarrays/qPCR	Steatotic HH	(56)
	CYP7A1 (− 0.95)	qPCR	HH+HGF	(77)

CYP27, cholesterol 27 ∝-hydroxylase; *CYP51A1*, cytochrome P450, family 51, subfamily A, polypeptide 1; *CYP8B1*, sterol 12 ∝-hydroxylase; *DHCR7,* 7-dehydrocholesterol reductase; HGF, hepatocyte growth factor; HH, human hepatocytes; *Hmgc1,* 3-OH-3-methylglutaryl-CoA lyase; *HMG-CoAR,* 3-OH-3-methylglutaryl-CoA reductase; *HMGCS1*, 3-OH-3-methylglutaryl-CoA synthase (soluble); *Idi1,* isopentenyl-PP-isomerase; *IDI1*, isopentenyl-diphosphate delta isomerase1; qPCR, quantitative polymerase chain reaction; NB, northern blot; PH, partial hepatectomy; SAGE, serial analysis of gene expression; *SQLE,* squalene epoxidase; WB, western blot.

analysed. A comparison of these findings has shown that a high variability is associated with these results depending on the model and the sensitivity of the techniques used (Table 2). Although models of liver regeneration using induction by CCl_4, or by partial hepatectomy, do not involve the same mechanisms, some of the effects on lipid homeostasis in these models are similar. For example, gene expression following treatment with CCl_4 correlates with previous findings that HDL and very-low-density lipoproteins production is reduced, along with the synthesis of apolipoproteins A-IV and C-III and transcription of the corresponding genes (53). Differential gene expression has also been detected for enzymes that synthesize cholesterol, 3-OH-metyl-glutaryl-CoA synthases 1 and 2 (*Hmgcs1* and *Hmgcs2*) and 3-OH-3-methylglutaryl-CoA lyase (*Hmgc1*) (53). Similarly, down-regulation of genes encoding enzymes that participate in the β-oxidation pathway and the catabolism of fatty acids has been observed (53). In contrast, up-regulation of the squalene synthase gene and down-regulation of HMG-CoAR and squalene epoxidase have been detected post-partial hepatectomy (54, 55). Results from *in vitro* studies to induce steatosis in human hepatocytes show the down-regulation of cholesterol metabolism-related genes, including enzymes, *Hmgcs1*, *Cyp51a1*, *7-dehydrocholesterol reductase* and *HMG-CoAR* (Table 2) (56).

For cholesterol homeostasis, different levels of regulation are involved. For example, an increase in levels of TG in human hepatocytes induces steatosis by post-transcriptional mechanisms that up-regulate enzyme activity, rather than affecting transcription of the enzymes involved (51). HMG-CoAR is another enzyme whose expression is tightly regulated at the transcriptional, post-transcriptional and post-translational levels. The down-regulation of genes encoding the HMG-CoAR observed in rat hepatocytes after partial hepatectomy (55) or in human hepatocytes induced to steatosis (56) corresponds well with the strict regulation of sterol synthesis in mammals (47, 57, 58). Therefore, the increase of cholesterol concentration in the hepatocytes occurs apparently at the expense of using cholesterol from plasma and blocking its elimination as bile acids. High cholesterol levels function as a biochemical signal to keep the complex SREBP–SCAP–INSIG in an inactive status, i.e. attached to the endoplasmic reticulum and avoiding the transcription of HMG-CoAR genes. Accordingly, an early *INSIG* mRNA (before known as CL-6) overexpression after partial hepatectomy has been reported (59, 60). The overexpression of INSIG inhibits the SREBP cleavage from the complex, i.e. it remains inactive. The opposite is observed in KO mice ($INSIG1^{-/-}$/$INSIG2^{-/-}$) (61, 62) (Table 3), where the lack of both *INSIG* isoforms produces a higher expression of the enzyme than was

Table 3. Sterol regulatory element-binding proteins –SREBP-cleavage activating protein–insulin-induced gene proteins complex from the proteolytic activation of nuclear sterol regulatory element-binding proteins to the transcription of 3-OH-3-methylglutaryl-CoA reductase genes in the liver of different experimental models

Experimental model	Liver cholesterol	Proteolytic activation of SREBP	Reference
WT mice (↓*INSIG*)	Normal	On demand	(61)
WT mice+PH → ↑ *INSIG*	↑	No	(60)
TG mice ↑↑ *INSIG*	↑	No	(61)
L-SCAP^{-} mice	↓	↓	(65)
SCAP mutants: D443N/Y298/L316F	↑	Yes	(64)
Knockout mice *INSIG 1-2*$^{(-/-)}$	↑	Yes	(62)

HMG-CoAR, 3-OH-3-methylglutaryl-CoA reductase; *L-SCAP*$^{-}$ mice, conditional deficiency of SCAP in liver; INSIG, insulin-induced gene proteins; PH, partial hepatectomy; SCAP, SREBP-cleavage-activating protein; SREBPs, sterol regulatory element-binding proteins; TG, transgenic mice; WT, wild-type mice.

observed for the corresponding mRNA HMG-CoAR (63). In a similar manner, in mutant Chinese hamster ovary cells where a single substitution in SCAP (D443N, Y298, L316F) prevents its binding to INSIG (64), SREBP is permanently activated and therefore promotes the transcription of HMG-CoAR genes (58) (Table 3). Conditional L-SCAP^{-} mice show a diminished proteolysis of SREBP because of the compensatory action from adipocytes (65, 66).

Experiments using cells overexpressing SREBP-1a through deprivation of lipids reproduce observations obtained with transgenic mice overexpressing the nuclear SREBP-1a after being partially hepatectomized (67). The phenotype of these transgenic mice shows a fatty liver as a result of the constitutive activation of the biosynthesis of cholesterol and fatty acids (67, 68). In general, the overexpression of SREBP-1a inhibits cell proliferation because this isoform is involved in the regulation of cell cycle and growth. Therefore, when it is abundantly expressed, an induction of cyclin-dependent kinase inhibitors is produced and cells are arrested in the G_1 phase (67).

Therefore, the cell is provided with several mechanisms by which cholesterol synthesis can be regulated, and each of these mechanisms have the potential to affect different enzymes to control levels of protein in the cell. However, in some cases, there are enzymes, such as the squalene epoxidase, that appear to be regulated only at the transcriptional level (69, 70). Moreover, RNA availability in the early stages of post-partial hepatectomy has been found to depend on transcriptional regulation, whereas changes in RNA levels during the proliferation phase post-partial hepatectomy have been found to be dependent on post-transcriptional mechanisms of regulation (71, 72). Alternatively, chromatin structure may also play an important role in controlling the transcriptional rate of cholesterol synthesis-associated enzymes. For example, histone deacetylase-3 has been shown to down-regulate gene expression of lanosterol synthase, consistent with the classical model of chromatin regulation where deacetylation and methylation promote transcriptional repression of a gene (73).

The RNA stability can also represent a post-transcriptional mechanism for the control of cholesterol synthesis enzymes as demonstrated in studies targeting the 3′ untranslated regions of *cyp7a* mRNA (74). In addition, protein stability of transcription factors can be modified to affect protein levels, without affecting basal transcription of a gene. This occurs for the regulation of SREBP-1 degradation during post-natal development (75). Studies of human hepatocytes treated with HGF also support these findings based on the decrease in *cyp7a1* transcription and subsequent reduction in bile synthesis after partial hepatectomy that has been observed in several species (76–78). As down-regulation of *cyp7a1* mRNA can be reversed by inhibiting c-Jun N-terminal kinase, extracellular signal-regulated kinase and protein kinase C (PKC), or by knocking down the expression of the receptor recognizing HGF (i.e. mesenchymal epithelial transition factor receptor), it has been suggested that HGF directly modulates the expression of *cyp7a1* via different signalling pathways (76). Moreover, early down-regulation of *cyp7a1* mediated by HGF after partial hepatectomy may be necessary to avoid liver toxicity because of overproduction of bile acids (77, 78).

Knockout mice deficient in growth-promoting cytokines that become activated after partial hepatectomy have provided valuable insight into the role of specific genes in the process of liver regeneration (79). For example, KO mouse models of interleukin 6 (IL6), or tumour necrosis factor receptor type I, experience high post-partial hepatectomy mortality rates, as well as a significant delay in their recovery of liver mass (80, 81). In both cases, these abnormalities are reversible if IL6 is exogenously provided.

Role of signalling lipids during liver regeneration

Although lipids have been well-characterized as structural components of cell membranes, they have also been shown to have an active role in signalling pathways that regulate diverse cellular processes such as cell proliferation, apoptosis, motility and cell–cell recognition (82). During liver regeneration, several lipid molecules function as secondary messengers and as precursors for new messengers that diffuse from the plasma membrane into

the nucleus to affect the transcription of genes that induce changes in the homeostasis of vital cell functions. Correspondingly, inositol lipids, including sphingomyelin, ceramides, lysophosphatidic acid and plasmalogens, among others, are recognized as important signalling lipids (83). Sphingomyelin illustrates the versatility of lipids as signalling molecules as it is not only the main sphingolipid component of eukaryotic cells but it is also a precursor of secondary signalling messengers such as ceramide, sphingosine and sphingosine-1-phosphate (84, 85). These messengers are synthesized by ceramidases, sphingomyelinases and sphingosine-kinases respectively (84, 85). Sphingomyelin and derived messengers also undergo important changes while regulating cell cycle progression, apoptosis and differentiation (84). Sphingomyelin and ceramide are present in the liver where they regulate cell survival and apoptosis during homeostasis and regeneration (86–88). In addition, a synergistic increase in sphingomyelinase and ceramidase activities has been reported to be associated with survival and proliferation signals that result from a decrease in ceramide levels and an increase in levels of sphingosine (86, 87). Although non-significant changes in total sphingomyelin content have been observed between 4 and 24 h after partial hepatectomy in rat liver, a sustained increase in neutral sphingomyelinase activity and sphingosine content was observed (89). An increase in acidic ceramidase activity was observed only 4 h following partial hepatectomy (89). These results suggest that the simultaneous increase in levels of sphingosine and decrease in levels of ceramide mediated by ceramidase are synergistic, and they also correlate with the temporal window of cell proliferation observed after hepatectomy.

It has been shown that ceramides inhibit the PI3K/Akt pathway through the activation of PP2A (90). This observation correlates with the enhanced survival of wild-type hepatocytes observed following inhibition of sphingomyelinases by imipramine, and with the low rate of apoptosis in primary hepatocytes observed in sphingomyelinase-deficient mice (87). However, as there is evidence to indicate that ceramide does not induce cell death via apoptosis in primary hepatocyte cultures, but rather via necrosis through an increase in the permeability of the inner membrane in mitochondria, controversy regarding the apoptotic mechanism of ceramide action is ongoing (91). The results obtained using primary cultures of hepatic cells vs. hepatic cell lines indicate that the effects of ceramide are cell type dependent, thereby explaining the suppression of cell proliferation that has also been associated with ceramide (85, 92–94).

The role of sphingosine-1-phosphate should also be considered, because of its capacity to mobilize intracellular calcium and induce cell proliferation, migration, differentiation and apoptosis (85, 95). Sphingosine-1-phosphate is an intracellular secondary messenger that mediates signalling downstream of five types of G protein-coupled receptors associated with sphingosine-1-phosphate named $S1P_{1-5}$ (96). Following a partial hepatectomy, an increase in sphingosine-1-phosphate content has been observed, which is hypothesized to be an important characteristic of liver tissue proliferation (87, 89). However, in cultured hepatocytes, sphingosine-1-phosphate is associated with an antiproliferative effect that is mediated by the activation of Rho through the sphingosine-1-phosphate receptor 1, or $S1P_1$ (a G-protein-coupled receptor also known as Edg-1) (97). In addition, the receptor $S1P_2$ has been shown to have a primary role in the proliferation of hepatic myofibroblasts and in the induction of wound-healing markers such as TIMP-1, TGF-β and platelet-derived growth factor B. These effects have also been associated with reduced rates of cell proliferation rate in $S1P_2^{-/-}$ mice (96).

Phosphatidylinositol (PI) is phosphorylated or dephosphorylated to form a variety of lipids that function as secondary messengers. These include PI, PI(4)P and $PI(4,5)P_2$ (98, 99). The action of the specific phosphoinositide phospholipase C (PI-PLC) on PI(4,5)P2 produces inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (100, 101). Furthermore, there is evidence to suggest that mitotic activity and accumulation of [^{32}P] PI/phosphatidylserine increase following partial hepatectomy (102). During liver regeneration, an increase in PI phosphorylation is also observed immediately following partial hepatectomy, and this is suppressed when high levels of DNA synthesis are induced and IP3 synthesis is reduced (103).

Interestingly, during liver regeneration, a correlation between changes in Ca^{2+} concentration, PKC activity and cell proliferation has been suggested. A decrease in IP3 concentration and an increase in DAG concentration 20 h after partial hepatectomy correlate with the translocation of PKC to the nucleus (104). In rat liver, isoforms β1 and γ1 of PI-PLC have been detected inside the nucleus, and an increase in their activity has been detected during liver regeneration (105–108). Specifically, reduced expression of the isoform PI-PLC-γ1 in the nucleus has been detected up to 16 h post-partial hepatectomy, while levels returned to baseline by 22 h. Accordingly, PI(4,5)P2 synthesis from PI(4)P post-partial hepatectomy was found to decrease after 3 h, increase by 16 h and then decrease again after 22 h. In combination, these observations suggest a relevant role for PI-PLC-γ1 as a modulator of the phosphoinositides cycle in the nucleus (109).

Changes in lipid metabolism in non-parenchymal cells during liver regeneration

The close relationship between parenchymal and non-parenchymal cells is fundamental to understanding liver physiology. However, detailed information concerning changes in lipid metabolism during liver regeneration in non-parenchymal cells remains scarce. In the case of hepatic stellate cells (HSC), these cells represent the main vitamin A reservoir in the body, and are also where the biosynthesis of extracellular matrix (ECM) occurs.

Lipogenesis has been shown to play a fundamental role in the metabolism of HSCs, and during the development of hepatic fibrosis and cirrhosis, HSCs undergo dramatic changes in their phenotype. For example, during liver regeneration, or after being activated, HSCs have a myofibroblast-like morphology and are depleted of retinoic acid. Activated HSCs also exhibit contractile activity and an increased production of ECM (110). During activation of HSCs in culture, a decrease in expression of adipogenic transcription factors has been detected (111), an aspect that has been shown to be important on the basis of the ability of an adipocyte differentiation mixture (isobutylmethylxanthine/dexamethasone/insulin) to induce activated HSCs to become quiescent (111). Furthermore, *in vivo* studies have shown that activation of HSCs because of bile duct ligation, or treatment with CCl_4, express higher levels of cholesterol 25-hydroxylase, which intervenes in the production of the oxysterol, 25-OH-cholesterol, a potent inhibitor of sterol synthesis that is able to induce an adipocyte-like phenotype (112). Nevertheless, these results should be cautiously interpreted because non-parenchymal cells have an established communication system between themselves. This communication is evident as differences in gene expression have been observed for HSC cultures, or when HSCs are activated *in vivo*. Likewise, cholesterol 25-hydroxylase is up-regulated in HSCs when they are cocultured with Kupffer cells (112), which play a fundamental role in liver regeneration based on their production of cytokines that trigger proliferation of liver tissue. Nevertheless, specific changes in the metabolism of lipids in Kupffer cells during liver regeneration have been less studied and therefore need to be investigated.

Although a large body of evidence points to the key role of non-parenchymal cells in the progress of chronic pathologies as steatosis of alcoholic and non-alcoholic types, it has been proposed that the primary accumulation of lipids in hepatocytes represents the first step that triggers the process. Importantly, the accumulation of cholesterol in mitochondria of hepatocytes leads to mitochondrial glutathione depletion, which in turn increases the sensitivity to tumour necrosis factor-α and Fas-mediated pathways (113, 114). After treatment with atorvastatin, an HMG-CoAR inhibitor, the mitochondrial free cholesterol decreases, and therefore the signs of steatohepatitis improve. Further work searching for similar mechanisms in HSC and the cross-talk between different hepatic cells will be of paramount relevance in the understanding of liver pathology.

Future directions

Recent studies clearly indicate the importance of systemic fat stores for a successful liver regeneration, because using *fld* mice that have diminished peripheral adipose stores, impaired liver regeneration in comparison with normal mice has been shown (115). Therefore, although it is clear that lipids are important structural molecules in membranes as well as regulators of hepatic tissue metabolism, additional research is needed to better understand the dynamic function of lipids in the nuclear matrix during liver regeneration (116, 117). To facilitate these studies, a complete metabonomic analysis of urine, liver and serum using sensitive techniques, such as nuclear magnetic resonance spectroscopy, might provide valuable insight into the mechanisms involved (118). Currently, there is a strong evidence to indicate that intranuclear lipids and several important enzymes mediate the induction of a lipid-specific metabolic programme in the nucleus, which affects both the phosphoinositides cycle and the metabolism of sphingomyelin (119–121). In addition, given the importance of chromatin structure in the control of gene expression by eukaryotic cells, further studies are needed to identify the manner by which lipids contribute to the condensation of chromatin, and the establishment of epigenetic marks that regulate specific genes in the hepatic cells during liver regeneration.

Another aspect to consider is the interactions that occur between proteins, such as PMCA, and their lipid environment in the plasma membrane of hepatocytes vs. non-parenchymal cells. Integral proteins of the membrane are distributed according to their intrinsic preference for a specific physical phase present in the lipid bilayer. Accordingly, proteins are recruited to the membrane on the basis of their association with specific domains containing high concentrations of cholesterol. However, it remains unclear whether proteins induce the formation of such domains, or whether these domains are established in the membrane (12). It is also unclear whether differences in the membrane domains explain the kinetic behaviours associated with cholesterol-enriched vs. cholesterol-depleted preparations of plasma membranes from different cell types. For example, in neurons, PMCA is associated with both lipid raft and non-raft domains, and exhibits different sensitivities to the changes in the cholesterol content of the plasma membrane (122). Recent studies in our laboratory indicate that this might also be the case for PMCAs expressed in the liver tissue (Delgado-Coello *et al.*, unpublished results). Moreover, hepatocytes expressing multiple PMCA isoforms exhibit an increased variability in their affinities for Ca^{2+} and calmodulin, and may further exhibit changes in function when cholesterol is present in their immediate environment.

Considering the important knowledge obtained from hepatocyte primary cultures (56, 77, 123–125), the hepatocyte transplantation possibility in humans has emerged as a promissory alternative in the improvement of liver regeneration (126, 127).

On the other hand, although the relevance of studying the basic molecular mechanisms in humans related to liver regeneration and several liver diseases that lead to the eventual possibility of liver transplantation is clear, the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) was created in 2002 to investigate

the potential risks of this procedure (128–130). Together with the Organ Procurement and Transplantation Network in coordination with the Scientific Registry of Transplant Recipients, the A2ALL study has compared data that eventually will dictate policy development, performance evaluation and research in the USA. Countries like Mexico are still working towards establishing their own coordination units in direct relationship between an adequate registry database and the important scientific issues to be addressed.

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