Hepatoma Research

Review

Liver regeneration: metabolic and epigenetic regulation

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Abstract

The liver is bestowed with an extraordinary regenerative capability, which is accomplished by a well-coordinated cellular and molecular response at different phases of regeneration. Metabolism, as the primary function of liver, displays various alterations as a consequence of hepatic insufficiency from an injury. These metabolic perturbations are physiologically relevant for promoting hepatocellular proliferation and regeneration. On the other hand, proliferation of otherwise quiescent hepatocytes and accompanied regeneration are regulated by transient, but precisely regulated transcriptional reprogramming. This phase- cell- and time-specific gene expression is controlled by epigenetic mechanisms. Hence, both metabolic and epigenetic changes regulate liver regeneration events. But the cross-talk between metabolic and epigenetic changes for a successful liver regeneration needs to be explored. Since most of the enzymatic players of epigenetic mechanisms rely upon metabolites for their substrates and co-factors, we expect a highly coordinated inter-dependence between metabolism and epigenetics during liver regeneration too. In the present review, we discuss various metabolic and epigenetic regulatory mechanisms for liver regeneration, and put forward the possible metabolic-epigenetic-liver regeneration link for a better understanding of the process and identification of novel targets for liver-related diseases in clinical settings.

Keywords: Liver regeneration, Metabolic regulation, Epigenetic regulation

INTRODUCTION

The vital functions of digestion, absorption, detoxification and synthesis of plasma proteins make the liver an indispensable organ of our body. While performing these essential functions, the liver gets exposed to toxin-rich blood from the gut, and thus, to the associated damage to its cells. This might be the reason why liver possesses a unique and extraordinary regenerative power. Though liver regeneration is an extensively studied phenomenon for decades because of its clinical importance, yet, in liver associated diseases that are the leading cause of mortality worldwide[1,2], regeneration failure remains the primary cause. Success of liver transplants, the only cure for end stage liver treatment, relies on the liver's regenerative ability of both the living-donor and the recipient. Similarly, patients undergoing surgical resection for the treatment of hepatocellular carcinoma[3] also depend on the regenerative success of the remnant liver. In the light of these clinical examples, it is imperative to understand the mechanism underlying successful liver regeneration. A liver regeneration event can be mediated by either hepatocytes or progenitor cells, depending on the severity of injury[4]. The sequence of events is broadly categorized into the phases of priming, proliferation, and termination[5,6]. Each phase is executed by a synchronous and well-coordinated interplay of several cellular and molecular players[7,8]. Over the past few decades, a large number of studies have revealed these players and their roles in the kinetics of liver regeneration. However, the inter-connection/dependence of these players need to be elaborated in order to understand the regeneration process better and develop specific therapeutics. One such provocative, yet unproven link, is between metabolism and epigenetics during liver regeneration[9]. Metabolic perturbations during liver regeneration and phase-specific gene expression via epigenetic mechanisms are two important branches. Recently, many studies have shown that metabolic alterations and epigenetic mechanisms, particularly the histone modifications and DNA methylation are inter-connected during liver regeneration. In the present review, we have summarized recent novel insights in the metabolic and epigenetic regulation of liver regeneration and their interplay thereof.

LIVER REGENERATION: MODELS AND MECHANISMS

The liver is composed of various specialized cell types, of which hepatocytes are the major ones. Hepatocytes remain in proliferative quiescence, but can start dividing if stimulated. Upon injury, hepatocytes can enter the cell cycle and restore the structural and functional hepatic loss by compensatory hyperplasia. But, if the injury is severe, liver specific stem/progenitor cells play the major role in regeneration. Hence, depending on the severity of damage and cell types involved, liver regeneration is of two types, hepatocyte mediated or progenitor/stem cell mediated[4]. The progenitor cell mediated liver regeneration is executed when regeneration by mature hepatocytes is impaired. Compared to stem/progenitor cell mediated regeneration, hepatocyte mediated compensatory hyperplasia is well characterized[4]. In order to dissect the underlying mechanism of liver regeneration, several model systems have been developed over the past few decades.

Experimental paradigms to study liver regeneration

A wide variety of model systems are available to study different aspects of liver regeneration. While *in vitro* studies using hepatocyte cell cultures are used to investigate liver regeneration specific signalling pathways, *in vivo* studies are used to get insights into the complex interactions between various hepatic cell types. Recently, long-term three dimensional organoid culture systems for hepatocytes and cholangiocytes from mice and humans have also been established, which recapitulate morphological, functional and transcriptional features of liver regeneration[10]. These organoid systems have opened up experimental avenues for regenerative medicine, disease modeling, gene therapy, and toxicology studies related to the liver[11]. In animal model systems, the first step to provoke regeneration response in otherwise rarely proliferating healthy liver is to induce an injury. Thus, depending on the type of injury, two broadly classified animal models of liver regeneration are: surgical resection and toxin-induced injury[12]. Both of

them can be used in animals with a wide range of size, from the zebra fish to pigs[12,13]. The small-sized animals provide financial, logistical, and ethical advantages, but are less suitable for clinical studies due to their differences in size, anatomy and liver metabolism from humans. On the other hand, the larger animals are anatomically and physiologically more similar to humans and provide clinically relevant results, but suffer from logistical, financial, and ethical disadvantages. Thus, both small and large sized animals have their own advantages and disadvantages, and thus, the ultimate choice depends upon the research question being addressed.

Liver regeneration models can also be grouped according to the cell type that is induced to proliferate: liver progenitor cell independent and liver progenitor cell dependent[14]. The liver progenitor cells - hepatic oval cells in rodents and intermediate hepatobiliary cells in humans - mediate liver regeneration only in cases of severe injuries and hepatocyte replication failure[15]. Otherwise, self-replication of hepatocytes contributes to liver regeneration with very little or no contribution of progenitor cells[16].

The commonly used surgical methods of liver regeneration are partial hepatectomy and portal ligation. In partial hepatectomy, upon surgical resection of two-thirds of the liver, the volume of the remaining liver segments increases and gets restored within seven days. This regeneration is more functional than the complete anatomical restoration[4]. The method of two-third partial hepatectomy in rats was first devised by Higgins *et al.*[17] in 1931; but because the surgery is easy to perform, is reproducible, and well tolerated[6], it has been employed in mice[18], dogs[19], pigs[20,21] and monkeys[22]. Partial devascularization that involves ligating portal vein branches to specific lobes, has been found to cause atrophy of the ligated or portal-deprived lobes, and concomitant compensatory growth of non-occluded residual liver[23]. The portal branch ligation model is simple and reproducible, if adequate operative care is given. In contrast to partial hepatectomy, it is reversible[12] and has been used in mice[24], dogs[25], pigs[26] and monkeys[27]. Though surgical methods are widely used for inducing liver regeneration, their outcome is influenced by a number of factors, including the age of animals[28], fasting before surgery[29], time of surgery[30], and anaesthetics used[29]. Hence, all these experimental variables must be duly considered while using surgery-based liver regeneration models.

The toxin-based, pharmacogical or hepatotoxic models, are relatively easy to perform and are clinically more relevant. However, these models also have various drawbacks such as the lack of reproducibility; variation in regenerative response with respect to dose and mode of administration of the drug; species; age; and the nutritional status of animals[12,31]. Some of these toxin-induced models are also the models for intrinsic drug-induced liver injury (DILI) studies; in fact, acetaminophen (APAP) overdose is one of the most common models for DILI[32]. Some of the commonly used hepatotoxins for liver injury and subsequent regeneration are summarized in Table 1.

Besides these, there are different dietary models to induce liver injury and regeneration in mice. 1,4-dihydro-2,4,6-trimethyl-pyridine-3,5-dicarboxylate (DDC)[50] is one such diet, which causes biliary injury and fibrosis[51]. Mice on DDC diet respond poorly to partial hepatectomy[50]. Another commonly used diet is choline-deficient, ethionine-supplemented (CDE) diet, first used in mice by Passman *et al.*[52], which causes hepatocellular injury with steatosis[53,54]. Though rodents have been the model of choice for liver regeneration studies, the zebrafish has recently been developed as a liver regeneration model that uses surgical partial hepatectomy[55], drug-induced injury[55] and nitroreductase-mediated hepatocyte ablation[56]. The advantages of using zebrafish for liver regeneration studies are low cost, rapid analysis and easy *in vivo* chemical screening due to the animal's small size and translucent body, respectively.

Mechanisms of liver regeneration
The restoration of structural and functional hepatic loss due to internal or external injuries can be accomplished by either compensatory hyperplasia of hepatocytes or progenitor/stem cell mediated

Table 1. Commonly used drugs for hepatotoxic/pharmacological liver regeneration models

S. No.	Drug/ Hepatotoxin	Principal Cell Affected	Effect
01	Carbon tetrachloride	Hepatocytes	Cell membrane damage, dependent on lipid peroxidation by trichloromethyl radicals produced due to metabolism of carbon tetrachloride; acute liver injury that manifests itself in the form of centrilobular necrosis, followed by hepatocyte regeneration[33-35]. Gastric administration can lead to hepatic cirrhosis[36].
02	Thioacetamide	Hepatocytes	Acute liver damage by biotransformation into thioacetamide sulfoxide and sulfone causing centrilobular necrosis, followed by hepatocyte regeneration[37,38]. Continued exposure induces hepatic cirrhosis[36].
03	Acetaminophen (or paracetamol)	Hepatocytes/ Oval cells	Acute liver failure, due to formation of N-acetyl-benzoquinoneimine, resulting in centrilobular apoptosis and necrosis, activation of Kupffer cells, followed by hepatocyte regeneration[39,40]. Exerts its effect only when present in excess and normal detoxifying pathways are saturated[41]. Higher dose leads to oval cell activation in the smallest portal tracts[42].
04	D-Galactosamine	Hepatocytes/ Oval cells	Acute liver injury by causing intracellular depletion of uridine nucleotides and consequent pan-lobular necrosis, followed by hepatocyte regeneration[43,44]. Increased/repeated dose leads to activation of progenitor or oval cells that proliferate and differentiate into hepatocytes and biliary cells[45,46].
05	Ethanol	Hepatocytes/ Oval cells	Damage due to hepatocyte steatosis and necrosis, but large variations seen among different studies due to mode and duration of administration[12,47,48]. Causes weakened and delayed liver regeneration after partial hepatectomy[49]

regeneration[4]. Compensatory hyperplasia takes place in the absence of significant hepatocyte senescence. In such cases, hepatocytes majorly contribute to liver regeneration with very little or no involvement of progenitor/stem cells[16]. On the other hand, if there is a failure of hepatocyte replication or there is severe injury, then hepatic stem/progenitor cells play a major role[15].

Hepatic progenitor cells (HPCs) play a dual role in liver injury, i.e., regeneration and fibrosis[57-59]. HPCs are activated in the periportal area after substantial liver damage. The activated HPCs are then directed towards biliary and hepatic regeneration via up-regulation of the Notch and Wnt-β-catenin pathways, respectively. In addition to regeneration, proliferation of HPCs also leads to fibrosis during liver injury. Different mechanisms are involved in HPC mediated fibrosis and inflammation: Activation of hepatic stellate cells (HSCs) via transforming growth factor (TGF-β) and sonic hedgehog (SHh) signals to produce abnormal extracellular matrix (ECM); recruitment of activated macrophages via chemokine ligand (CCL-2), Tweak, chemokine ligand 5 (CCL5)/RANTES, and intercellular adhesion molecules (ICAMs); stimulation of abnormal angiogenesis by liver endothelial cells[57]. Hence, HPCs participate both in regeneration and fibrogenesis. Also, the presence of progenitor cells and the HPC response have been associated with liver tumor formation[60]. The cellular source for all these functions is provided by the heterogenous cell population in the HPC niche[58,61].

Though various studies have revealed the genes, cytokines, growth factors, and signalling pathways involved in progenitor/oval cell based liver regeneration[62-65], the cellular and molecular mechanisms involved in progenitor/stem cell mediated regeneration are relatively less characterized[4]. Thus, in order to design HPC-based, pro-regenerative and anti-fibrotic therapies, a deeper understanding of the origin of HPCs, their niche components consisting of heterogeneous cell populations, signalling molecules involved in activation, proliferation, migration and differentiation of HPCs, signalling pathways and their relative relevance is required[14,57,58,66-69].

Although the possibility of hepatocyte differentiation from HPCs is not completely excluded, another school of thought also suggests that new hepatocytes and cholangiocytes are derived only from pre-existing hepatocytes and not from HPCs in liver injuries[16,70-72].

Besides these two mechanisms, Nagy *et al.*[73] (2001) have also shown that if both hepatocyte proliferation and stem cell activation are prevented by dexamethasone or 5-fluorouracil, liver restoration is achieved by hypertrophy/enlargement of periportal hepatocytes.

The whole process of regeneration is completed in three phases, i.e., priming (increased capacity of hepatocytes to replicate), proliferation (attainment of required functional cell mass) and termination (end of proliferation)[5,6]. Each phase is executed by a well-coordinated network of various parenchymal and non-parenchymal cells[74-76], hormones from various glands[7], growth factors, and signalling networks[7,8]. Both intra-hepatic and extra-hepatic cells are involved in liver regeneration, i.e., hepatocytes, sinusoidal endothelial cells (SECs), Kupffer cells, hepatic stellate cells (HSCs), hepatic stem cells, biliary epithelial cells, platelets, eosinophils, platelets, and natural killer T (NKT) cells. Hormones (insulin, glucagon, serotonin, somatostatin, norepinephrine, T3, *etc.*) from various glands (thyroid, adrenal, pancreas, duodenum), cytokines [tumor necrosis factor (TNF)-α, interleukin [IL]-6, interferon α and γ], transcription factors [NF-κB, STAT3, CCAAT-enhancer binding protein (C/EBP) b, farnesoid X receptor (FXR), cAMP regulatory element-binding protein, activator protein 1] and different growth factors [epidermal growth factor (EGF), transforming growth factor (TGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) *etc.*] also affect the process of regeneration. Optimum liver regeneration results from various signalling mechanisms that are turned on and off at specific times. Some of the prominent pathways involved are: IL-6/Jak/STAT3 pathway in hepatocyte proliferation, PI3-K/PDK1/Akt pathway in hepatocyte growth, HGF/PI3-K/Akt and FXR-p62/SQSTM1 pathways in liver protection *etc*. An exhaustive list and roles of all these major players in the execution and regulation of liver regeneration has been extensively reviewed elsewhere[7-9,13,77-82]. Besides these cellular and molecular mechanisms, stress, hypoxia, and gut microbiome have also been directly or indirectly linked with liver regeneration, as reviewed by Preziosi *et al.*[8].

Despite of the tremendous knowledge about these players, we are yet to understand the essence of hepatostat, i.e., the adjustment of liver size to 100% for required homeostasis[83]. Metabolic alterations are one of the early events in hepatic insufficiency, and play an important role in the execution and regulation of liver regeneration[9]. Equally important are the recently reviewed[84-86] epigenetic mechanisms that tightly regulate the expression of genes, specific for each phase of regeneration. Here, we review the potential relationship between metabolism, epigenetics, and liver regeneration.

METABOLIC REGULATION OF LIVER REGENERATION

Since metabolism is the major function of liver, metabolic alterations are bound to occur in an injured liver. Many studies have shown that both hepatic and systemic metabolism are altered in injury induced liver regeneration. Some of these changes, as reviewed by Huang *et al.*[9] include: the suppression of liver glycolysis and induction of gluconeogenesis, depletion of glucagon in remnant liver, decline in lean and adipose tissue mass, depletion of systemic fat, steatosis, increase in lipolysis and serum free amino acids, decline in hepatic ATP content and increase in AMP in remnant liver, and β-oxidation of fatty acids as a primary source of ATP production in regenerating liver. These metabolic changes occur before the initiation of surgery or toxin-based hepato-cellular proliferation that is subsequently promoted by cyclin-CDK complexes. The metabolic perturbations get resolved with advancement of regeneration events[87-89].

Various studies have implicated that these changes are not only metabolic perturbations in response to hepatic insufficiency, but physiological determinants of liver regeneration too. For example, the physiological importance of hypoglycaemia is revealed by the fact that glucose supplementation has been shown to impair liver regeneration[87,90]. Similarly, inhibiting liver fat accumulation by using drugs[89,91,92] or knockout mice[88,89] has been shown to suppress liver regeneration. Several other studies have demonstrated that alteration in amino acid uptake and metabolism, which is another metabolic response to hepatic insufficiency, also has physiological relevance in regenerative hepatocellular proliferation[93-97]. Thus, there exists a well-coordinated balance between hepatic insufficiency associated metabolic changes and ensuing regeneration events.

Huang *et al.*[9] have also suggested various candidate molecular mediators that link metabolism with liver regeneration, though they require definite identification. These molecules probably serve as substrates for energy and synthesis of macromolecules during regeneration. One such class of mediators is xenobiotics, which have been shown to induce hepatocellular hyperplasia and hypertrophy in the absence of liver injury in rodents[98]. Such xenobiotic-induced hepatocellular proliferation is executed by various nuclear receptor transcription factors. Peroxisome proliferator-activated receptor (PPAR)-α is one such nuclear receptor transcription factor, whose expression is required for clofibrate and Wy-14,636 xebobiotic-induced hepatomegaly in rodents[99]. But recently, endogenous lipid metabolites have been reported as ligand activators of PPAR-α[100], which indicates that these naturally occurring ligands might connect the post-hepatectomy lipid accumulation with subsequent hepatocyte proliferation during regeneration. Other such xenobiotic-induced transcription factors, which might link hepatic insufficiency induced altered metabolites with liver regeneration, include constitutive androstane receptor (CAR), famesoid X receptor (FXR) and liver X receptor (LXR)[9].

Metabolism also regulates the signalling molecules and pathways essential for regeneration. For example, epidermal growth factor receptor (EGF-R) ligands regulate hepatocyte proliferation in experimental liver regeneration models[78-80]. EGF-R also plays an important role in lipid and fatty acid metabolism in quiescent and regenerating liver, and also in steatosis in a murine model of non-alcoholic fatty liver diseases (NAFLS)[101-103]. EGF-R expression and activity is inhibited by hyperglycemia in other models[104,105]. Thus, hypoglycaemia induced by partial hepatectomy probably promotes EGF-R signalling and hepatocyte proliferation in liver regeneration. Similar possibility exists for glycogen synthase kinase (GSK)-3 to link hepatic insufficiency induced hypoglycaemia with liver regeneration, as reviewed by Huang *et al.*[9].

Taken together, these examples suggest that altered metabolism as a result of hepatic insufficiency in an injured liver is physiologically relevant for regulating liver regeneration.

EPIGENETIC REGULATION OF LIVER REGENERATION

Transient, but precisely regulated gene expression is the hallmark of different phases of a liver regeneration event[83]. The transcriptional reprogramming involves early activation of otherwise latent genes of a quiescent liver by transcription factors[106,107], and repression of genes associated with hormone biosynthesis and lipid/ steroid metabolism[108,109]. These transcriptional events are time, cell, and context dependent. Such a tightly controlled gene expression can be accomplished by epigenetic mechanisms, and hence, epigenetic mechanisms must be involved in regulating liver regeneration.

The epigenetic means of transcriptional regulation include DNA methylation, post-translational modifications of histones, microRNA, and chromatin remodelling. So far, only limited reports are available on epigenetic regulation of liver regeneration. Within those, microRNA mediated regulation of liver regeneration has been studied more, which has been reviewed recently[110-112]. Chen *et al.*[112] have reviewed that miR-16, miR-22, miR-23, miR-24, miR-26a, miR-29, miR-30, miR-31, miR-33, miR-122a, miR-126, miR-127, miR-145 and miR-150, miR-378 are down-regulated; while miR- 34a, miR-122, miR-203, and miR-221 are up-regulated during liver regeneration. miR-21, miR-26b, miR-192 and miR-194 are up-regulated in the first day, and down-regulated in the subsequent two days. miR-21, miR-23b, miR-122, miR-203 and miR-221 are promoters for entry of quiescent hepatocyte into the cell cycle during liver regeneration, whereas miR-26a, miR-33, miR-34a, miR-127, miR-150, and miR-378 are inhibitors for the same.

Among chromatin remodelers, Arid1a, a SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodelling complex component, suppresses hepatocyte proliferation and regeneration[113]. Proliferation of hepatocytes is enhanced in murine liver regeneration models with hepeatocyte-specific deletion of Arid1a.

Arid1a limits the access of hepatocyte transcription factors, C/EBPα, hepatocyte nuclear factor α (Hnf4α) and the E2 factor (E2F), to their target genes[113]. Our group has previously reported the switching of Brahma-related gene (BRG-1) and Brahma (BRM) containing SWI/SNF complexes during different phases of thioacetamide induced liver regeneration in mice[114]. This differential expression of SWI/SNF complexes, correlated with histone modification marks, probably regulates the expression of different sets of genes during injury and proliferation phases of liver regeneration.

A cross-talk between DNA methylation and histone modification was recently revealed by Wang *et al.*[115] in liver regeneration. A dynamic expression of UHRF1, an epigenetic regulator for DNA methylation, was observed during liver regeneration. Also, they found an early and sustained activation of pro-regenerative genes and thus enhanced liver regeneration in partially hepatectomized UHRF1 deleted livers. In these organisms, H3K27me3, a marker for transcriptional repression, was redistributed from promoters to transposones, thus allowing the expression of liver regeneration specific genes.

Bromodomains of bromodomain and extraterminal (BET) proteins, which regulate transcription by binding to acetylated lysine residues on histone tails, also regulate hepatocellular proliferation in hepatocyte-driven liver regeneration[116]. Russell *et al.*[116] have shown that the drug JQ1, a specific inhibitor of Brd4 (one of BET proteins), impairs hepatocyte proliferation in partial hepatectomy and acetaminophen-induced liver regeneration with significant reduction in E2f2 genes and cyclin-D1.

A couple of studies have shown that p300 histone acetyltransferase (HAT) along with C/EBP transcription factor also regulates hepatocyte proliferation[117-119]. Hepatocellular proliferation gets suppressed when a broad inhibitor of histone deacetyalses (HDACs), suberoylanilide hydroxamic acid (SAHA), is administered in rodents subjected to partial hepatectomy[120]. An absence of enhancer of zeste homologs (EZH) 1 and 2, the methyltransferases, causes loss of regeneration capacity in CCl_4 and partial hepatectomy induced liver regeneration. Partly, this is attributed to a decrease in H3K27me3 levels on the promoters of genes that encode G1/S phase cell cycle inhibitors like CDKN2A and CDKN2B[121].

Recently, the highly conserved Hippo pathway and its downstream effectors, the transcriptional co-activators, yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ or WWTR1), have been shown to mediate liver regeneration[122-125] by activating hepatocyte proliferation and trans-differentiation into stem cell like progenitor cells[126]. Interestingly, Hippo signalling is, in turn, regulated by lysine demethylase 3A (KDM3A), the enzyme responsible for removing H3K9me2 and recruiting p300[127]. Also, the EZH2- H3K27me3-DNMT1 complex regulates the *wwc1* gene, a key upstream factor of the Hippo pathway[128]. Thus, Hippo signalling, which plays an important role in regeneration, is also regulated by epigenetic mechanisms. Aloia *et al.*[129] have also shown that epigenetic remodelling through Ten-eleven translocation (TET1)-mediated hydroxymethylation, licences adult cholangiocytes to initiate organoids and activate the liver regeneration through transcriptional regulation of stem-cell genes and regenerative pathways, including YAP-Hippo signalling.

This information, summarized in Figure 1, highlights the regulatory role that epigenetic mechanisms exert during liver regeneration. However, phase and cell-specific studies will help unravel the regulatory role of epigenetic mechanisms in liver regeneration via orchestration of transcriptional reprogramming.

METABOLITES AS EPIGENETIC MODIFIERS: AN INTERPLAY

Hepatic insufficiency due to liver injury causes metabolic perturbations, which are physiologically relevant for liver regeneration. The regeneration event is executed by tightly regulated and phase-specific gene expression that is under epigenetic control. Hence, we hypothesize the existence of a metabolic-epigenetic-liver regeneration link.

A

| Epigenetic marks/players	Quiescent liver	Regenerating liver
Epigenetic marks on histones	Site-specific histone modifications to maintain cellular homeostasis	H3K9Ac ↓ H3K4me3 ↑ (at transcription start sites) H3K36me3 ↑ (at gene bodies) H3K27me3 ↑ (occupancy to transposons, with concomitant ↓ occupancy to promoters)
DNA methylation (overall change)	Maintained to obtain cellular homeostasis	Transient, genome-wide increase in TET1-mediated hydroxymethylation for organoid formation from adult cholangiocyte. Also, gene specific demethylation of few genes during regeneration is reported
Histone methyltransferase and demethylase activity	Basal level	EZH1 and EZH2 expression are crucial. KDMD4 recruited to specific promoter to erase H3K9me3 marks. SIRT1 also recruited to specific gene promoters
DNA methyltransferase and demethylase activity	DNMT1 ↓ DNMT3a ↓ DNMT3b ↓	DNMT1 ↑ DNMT3b ↑
Histone acetyltransferase activity	Basal level	No reports on gross changes in acetylation of histones. Few gene-specific acetylation reported. p300 has been shown to negatively modulate proliferation, during liver regeneration
Histone deacetylase activity	Basal level	Increased activity at chromatin level HDAC1 ↑ HDAC9 ↓ HDAC4 ↑ HDAC11 ↓ HDAC8 ↑ HDAC5 (translocated to the nucleus) SIRT1 ↑
miRNA levels	Basal level regulation for normal cell survival.	miR-16, miR-22, miR-23, miR-24, miR-26a, miR-29, miR-30, miR-31, miR-33, miR-122a, miR-126, miR-127, miR-145, miR-150, miR-378 ↓ miR-34a, miR-122, miR-203, miR-221 ↑

B
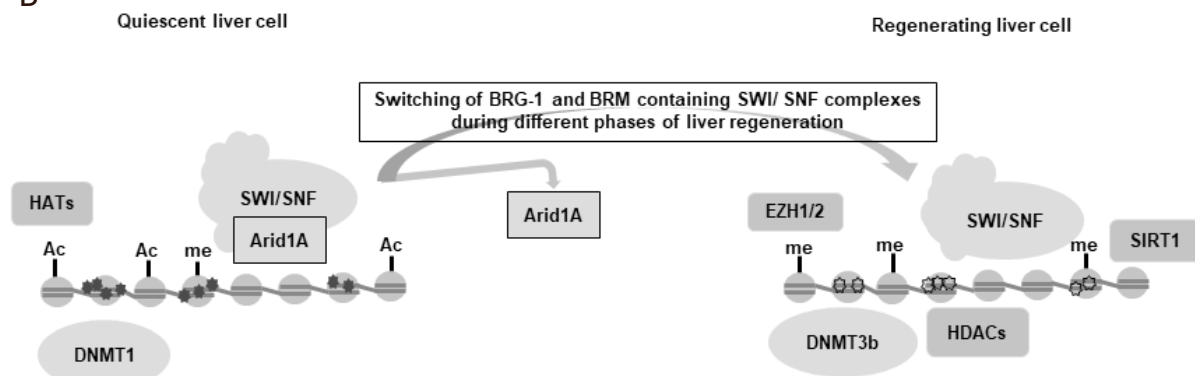
Figure 1. Summary of epigenetic modifications associated with liver regeneration[84,86,112,126-129]. A: Table representing the comparison of a quiescent and regenerating liver. "↑" represents an increase in expression and "↓" represents a decrease in expression; B: A schematic representation of the changes associated with the transition of a quiescent liver to a regeneration phase. During regeneration, a reorganization of histone modifications (primarily, acetylation, and methylation) and DNA methylation occurs. However, a detailed epigenetic code for liver regeneration is yet to be decoded

Epigenetic mechanisms control transcription with the help of various enzymes like DNA methyltransferases (DNMT), Histone acetyltransferases (HAT), Histone deacetylases (HDAC), Histone methyltransferases (HMT), *etc*. The activity of these enzymes in turn depends on the availability of their substrates or cofactors, which are nothing but cellular intermediary metabolites. For example, acetyl-CoA, a metabolite, is required for histone acetylation by HAT enzymes, NAD^+ is required by sirtuins (class III HDACs) for histone deacetylation, S-adenosylmethionine (SAM) is required for histone/DNA methyltransferases for methylation, FAD^+ is needed for demethylases, and so on[130]. Thus, a coupling exists between the metabolic state of a cell and the chromatin-dependent gene regulation[131-134].

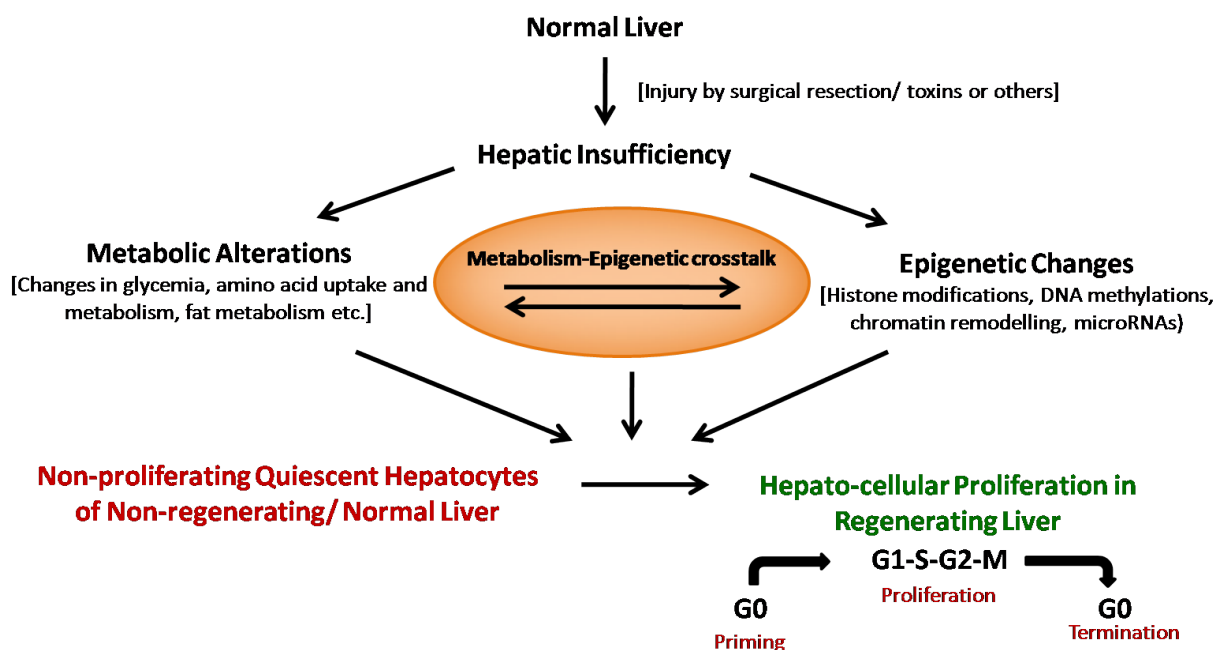Here, we cite certain examples which support the idea of existence of a metabolic-epigenetic-liver regeneration link.

Figure 2. Hepatic insufficiency caused by surgical or toxin-induced injury induces metabolic alterations and epigenetic changes in the liver. Besides the isolated metabolic and epigenetic regulation of hepatocyte proliferation and regeneration, a well-controlled interplay between metabolism and epigenetics controls liver regeneration events

Metabolic alteration regulates DNA methylation during liver regeneration

Mato and Lu (2007)[135] have shown that the synthesis of S-adenosyl-methionine (SAMe) by methionine-adenosyl transferase 1A (MAT1A) from methionine remains tightly regulated during liver regeneration, and gets disrupted in liver diseases including cancer. An altered expression of MAT1A disrupts methionine metabolism, which has been shown to inhibit liver regeneration in mice[93]. Since SAMe is required for methyltransferases' activity, DNA methylation might be altered in such cases. DNA methylation, in turn, regulates liver regeneration, similar to what has been demonstrated in another study, wherein, azacytidine, the methyltransferase inhibitor, suppresses liver regeneration[136].

Another example linking metabolism and DNA methylation during liver regeneration comes from α-ketoglutarate (α-KG). α-KG is an amino group acceptor in an alanine aminotransferase catalyzed reaction for the production of a gluconeogenic precursor, pyruvate, from alanine. The metabolic alteration in levels of α-KG is likely to occur along with glycolytic and gluconeogenic changes in a regenerating liver. α-KG is also the co-factor for demethylation reactions[137]. Also, the TET-1 catalytic activity required for cholangiocyte organoid initiation and maintenance depends on α-KG[129,138]. Hence, metabolic changes in α-KG might alter DNA methylation and ensuing gene expression patterns in a regenerating liver[9].

Metabolic alteration regulates histone acetylation during liver regeneration

Parallel to hypoglycaemia, zinc-dependent HDAC activity increases and global liver histone acetylation decreases in liver regeneration. SAHA, the HDAC inhibitor, is reported to suppress liver regeneration[139]. Thus, glycemia seems to affect liver regeneration by regulating protein acetylation level. Also, the sub-cellular localization of HDAC5, a class-IIa zinc-dependent HDAC, is also regulated by glycemia. In partial hepatectomy, the glycemic alteration brings about this localization in the nucleus[140].

Shimazu *et al.*[141] have reported that β-hydroxybutyrate, a ketone body, is a specific and endogenous inhibitor of class-I HDACs, supporting the idea of coupling between epigenetic transcriptional regulation and metabolic status of a cell. There is a possibility that other metabolites generated during hepatic

insufficiency in liver regeneration might also activate/inhibit the epigenetic players. A possible link between glucose homeostasis and fat metabolism with the SIRT1, a class-III HDAC, has been reported during liver regeneration[142].

These examples indicate that a metabolic-epigenetic-liver regeneration link exists that helps in the well-regulated execution of molecular events during liver regeneration as depicted below in Figure 2.

SUMMARY AND CONCLUSIONS

Hepatic insufficiency caused by liver injury generates altered metabolites. These metabolites are physiologically relevant for hepatocellular proliferation and regeneration. These metabolites also supply the substrates/cofactors for various enzymes involved in epigenetic-mediated transcriptional regulation. Though an increasing number of isolated reports are available for metabolic and epigenetic regulation of liver regeneration, studies focusing on the inter-dependence between metabolism and epigenetics during liver regeneration need to be investigated. Investigation of the metabolic-epigenetic interplay and its molecular characterization during liver regeneration can provide better insights in identifying potential targets for clinical treatments in liver related diseases.

DECLARATIONS

Authors' contributions
Wrote the major part of the manuscript: Verma S
Helped in writing the manuscript and made the epigenetic model: Purohit JS
Helped in material collection and reference arrangement: Arora A, Sinha S
Envisaged the concept, designed an outlines, and corrected the manuscript: Chaturvedi MM

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
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