Minireview

Role of AMP-activated protein kinase in the control of hepatocyte priming and proliferation during liver regeneration

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Abstract

The enzyme AMP-activated protein kinase (AMPK) is the main energy sensor in cells and is responsible for controlling the balance of anabolic/catabolic processes under metabolic stress conditions. This metabolic control exerted by AMPK is critical for energy-demanding situations, such as liver regeneration. Immediately after partial hepatectomy (PH), the liver undergoes the priming phase, mediated by the proinflammatory cytokines tumor necrosis factor (TNF) and interleukin-6, which promote responsiveness of hepatocytes to growth factors, such as hepatocyte growth factor (HGF) and epidermal growth factor, which lead to proliferation. In addition to its metabolic function, AMPK is likely to be a key mediator in both hepatocyte priming and the proliferative phases, induced by TNF-α and HGF, respectively. TNF-α-induced AMPK activation has been shown to be necessary for nuclear factor κappa B (NF-κB)-induced inducible nitric oxide synthase expression and for blocking TNF-α-induced apoptosis. On the other hand, HGF-induced LKB1/AMPK activation has been found to play a critical role in controlling Hu antigen R cytosolic localization and endothelial nitric oxide synthase activation, and consequently *Cyclin D1* and *Cyclin A* expressions, and nitric oxide generation, respectively. During PH, levels of *S*-adenosylmethionine (SAMe), the principal methyl donor in the liver, have to decrease to allow liver proliferation. Our studies also show that SAMe inhibits hepatocyte proliferation by controlling the hepatocyte's responsiveness to mitogenic signals such as HGF through the inhibition of AMPK activity. In summary, these data highlight the essential role of AMPK in controlling the balance between hepatocyte metabolic adaptations, cell cycle progression and apoptosis during liver regeneration.

Keywords: AMPK, TNF, HGF, liver regeneration, *S*-adenosylmethionine

Experimental Biology and Medicine 2011; **236**: 402–408. DOI: 10.1258/ebm.2011.010352

Introduction

The liver is a unique organ that can restore its mass and function after injury. In healthy liver, hepatocytes remain quiescent and have a low turnover. However, liver resection, 'small for size' transplantation, viral infections and toxicant-induced tissue necrosis promote hepatocyte proliferation that restores the original liver size in mammals.[1,2]

In rodents, the most commonly used experimental method to study liver regeneration is partial hepatectomy (PH) of two-thirds (70%) of the liver, which unlike other methods, such as CCl_4 treatment, is not associated with necrosis or acute inflammation.[3,4] Immediately after PH, the liver undergoes the priming phase, which is dependent on the proinflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 derived from non-parenchymal cells (mainly Kupffer cells)[5–7] and the right amount of nitric oxide (NO) and reactive oxygen species (ROS).[8,9] This priming phase promotes the transition of hepatocytes from the quiescence (G0) to the G1 phase and increases their responsiveness to growth factors (such as hepatocyte growth factor [HGF] and epidermal growth factor [EGF]), which lead to proliferation.[10] This compensatory hyperplasic response leads to the restoration of the liver mass and function.

Under normal conditions, liver size is restored by proliferation of the mature cellular populations of the liver, which undergo one to three rounds of replication but are not dependent on resident progenitor or stem cells. Hepatocytes are the first to proliferate with a peak of DNA synthesis in periportal and pericentral hepatocytes

at 24 and 36–48 h, respectively,[11] whereas the other liver cells have a peak of DNA synthesis at 48 h or later.[12,13] However, in situations where proliferation of either hepatocytes or biliary epithelial cells is inhibited, each cell can function as a stem cell for the other, undergoing a process of transdifferentiation.[14]

Rat liver can regenerate each time even after 12 sequential hepatectomies,[15] and surprisingly they can simultaneously proliferate and perform all of the essential homeostatic functions, such as glucose and protein synthesis, detoxification, and bile secretion. In 2006, Fausto *et al.*[16] proposed that the essential circuitry required for liver regeneration is encompassed by three types of pathway comprising cytokine, growth factor and metabolic networks that link liver function with cell growth and proliferation. The regenerative response has been widely investigated, although so far, blockade of a single pathway cannot completely abrogate liver regeneration. This highlights the complex network of signaling pathways that are active during the regenerative response in the liver. Identification of critical hubs in this complex network that controls inflammation, metabolism and growth of the liver is critical to understand the process. The present review focuses on the critical role of the metabolic sensor, AMP-activated protein kinase (AMPK), during liver regeneration as a controller of the inflammatory and proliferative response after PH.

AMPK and TNF-α-induced inducible nitric oxide synthase expression in hepatocytes

The priming phase during liver regeneration is dependent on the proinflammatory cytokines TNF-α and IL-6. Both cytokines promote the activation of different signaling pathways, such as nuclear factor κappa B (NF-κB), signal transducer and activator of transcription 3 (STAT-3), activating protein-1 and CCAAT/enhancer-binding protein β that lead to activation of target genes in hepatocytes, promoting DNA synthesis and cell proliferation.[17–20] NF-κB is a nuclear transcription factor that remains inactive in the cytoplasm when bound to the inhibitory protein I kappa B alpha (IκBα). Upon receptor-mediated stimulation (as TNF-α/TNF-R1/2), the Ikappa B kinase (IKK) complex (IKK1/α, IKK2/β and the regulatory subunit NEMO/IKKγ) gets activated and phosphorylates IκBα, leading to its ubiquitination and degradation by the proteasome system. This allows the translocation of NF-κB into the nucleus where it promotes the transcription of target genes.

Several studies have shown that NF-κB is important during the regenerative response in the liver, although its concrete role remains controversial.[21–24] After PH, Kupffer cells rapidly release TNF-α, which activates NF-κB signaling in neighboring hepatocytes in an autocrine manner, leading to IL-6 secretion that further activates the STAT-3 signaling cascade. Blocking NF-κB signaling using either an adenoviral IκB super-repressor infection of whole liver or transgenic mice carrying an inducible IκB mutant exclusively in hepatocytes, induces apoptosis and impairs liver regeneration respectively.[22,23] More recent studies using conditional knockout (KO) mice have shown that inactivation of IKK2 in hepatocytes leads to a rapid activation of NF-κB in non-parenchymal cells, while showing delayed hepatocyte p65 activation, leading to an earlier acute phase response and faster cell cycle progression.[24] Taken together, these studies underline the major relevance of NF-κB activation in the different cell compartments in protecting hepatocytes from cell death and regulating the proper cell proliferation during liver regeneration.

TNF-α is also crucial for liver regeneration since TNFR1-KO (tumor necrosis factor receptor 1-knockout) mice have impaired DNA synthesis after PH[25] and *in vivo* injection of antibodies against this cytokine impairs liver regeneration.[5] In regenerating liver, TNF-α generates an excess of ROS, which although normally acting to promote apoptosis, are also necessary for liver regeneration. After PH, the excess in ROS production after TNF-α stimulation is blocked for a few hours, preventing apoptosis. This neutralization of TNF-α-induced ROS in normal hepatocytes is mainly dependent on the action of GSH (reduced glutathione) and other thiols. In addition, an increase in the uncoupling protein-2 plays a role in decreasing the TNF-α-induced ROS.[26,27] On the other hand, several studies have shown that TNF/IL-6-induced iNOS (inducible nitric oxide synthase) expression is an important hepatoprotective factor in regenerating liver, and that NO prevents TNF-α-mediated hepatoxicity after PH. Deficiency of iNOS leads to impaired hepatocyte DNA synthesis and liver regeneration despite normal TNF-α, IL-6, STAT-3 and NF-κB expression.[8,28] This impairment in the mitogenic responses is reverted by the administration of a NO donor.[29]

Hepatocytes are resistant to TNF-α-induced apoptosis unless they are treated with inhibitors of mRNA or protein synthesis such as cycloheximide or actinomycin D.[30] Also, blocking NF-κB activation significantly enhances TNF-α-mediated apoptosis in hepatocytes.[31,32] Thus, complete ablation of hepatocyte/NF-κB signaling in conditional NEMO-KO mice promotes excessive oxidative stress, massive apoptosis, low cell proliferation and death after PH (Malato *et al.*, unpublished).

Finally, we have recently observed that in cultured hepatocytes AMPK is phosphorylated after TNF-α stimulation, and that inhibition of AMPK induces TNF-α mediated apoptosis.[33] TNF-α-induced *iNOS* transcription is dependent on NF-κB activity,[34] and our data indicate that the inhibition of AMPK in TNF-α-treated hepatocytes induces abnormal p65 accumulation in the nuclear compartment, together with an inhibition of TNF-α-induced *iNOS* mRNA expression. Taken together, these data suggest that AMPK activation is necessary for *iNOS* mRNA expression and antiapoptotic function after TNF-α stimulation in hepatocytes.

AMPK and HGF-induced hepatocyte proliferation

HGF is the classical mitogenic factor that promotes hepatocyte proliferation and thus plays an essential role in liver regeneration.[1,3] In the rat, plasma concentrations of HGF rise more than 20-fold within one hour after PH,[35] although

the mechanisms involved in this rise are still unclear. HGF concentrations decrease slowly during the next 24 h although they remain elevated for more than three days. HGF remains in its inactive form (single-chain HGF) when bound to the intracellular matrix. Shortly after PH, matrix breakdown may cause a rapid release and activation of HGF.[36–39] Active HGF (two-chain HGF) rapidly induces activation of the c-met receptor-signaling cascade, which includes the activation of the Ras (RAt Sarcoma)/ERK/ mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase/Akt, Rac (Ras-related C3 botulinum toxin substrate)/p21-activated kinase (Pak) and Crk/ Ras-related protein 1 (Rap1) pathways.[39] Several studies have demonstrated that blocking the HGF receptor c-met leads to impaired cell cycle progression.[40,41]

Recently, we have found that a non-canonical signaling pathway for HGF driven by LKB1 (serine/threonine protein kinase 11)/AMPK is essential for hepatocyte proliferation. Although AMPK and LKB1 have been considered traditionally as a metabolic tumor suppressor, we have observed that in hepatocytes, LKB1/AMPK promote hepatocyte proliferation after HGF stimulation.[42] We found that HGF-mediated AMPK phosphorylation in cultured hepatocytes induces cytosolic localization of the RNA-binding protein Hu antigen R (HuR), which increases the half-life of mRNAs for targets such as cyclin D1 and A2.[43] We also showed that the LKB1/AMPK/HuR cascade was activated during liver regeneration after PH.[33,42]

As mentioned above, NO production plays an important role in liver regeneration. In hepatocytes, *iNOS* expression and NO production sensitize hepatocytes to respond to HGF.[29] AMPK has been shown to phosphorylate and activate eNOS (endothelial nitric oxide synthase) in response to HGF both after PH and in HGF-treated hepatocytes in culture. In addition, DNA synthesis after PH was impaired in mice lacking eNOS.[42] The finding that AMPK is the kinase responsible for regulating eNOS (Thr1172) expression in response to the major mitogenic stimuli highlights the importance and the wide range of action of this protein as a hub in the complex network of signaling and metabolic pathways that converge during the liver regeneration process.

This proliferative effect of AMPK activation seems to be exclusive of hepatocytes.[44] Activation of AMPK by adiponectin, AICAR (5-aminoimidazole-4-carboxamide-riboside) or metformin inhibits proliferation of human immortalized hepatic stellate cell (HSC) lines and myofibroblasts derived from primary rat and human HSCs in response to platelet-derived growth factor.[45,46] The antiproliferative effect of AMPK on HSCs appears to be mediated via suppression of ROS production and subsequent inhibition of the Akt pathway. In addition, an increase in the expression of the cyclin-dependent kinase inhibitors, cyclin-dependent kinase inhibitor p27 and cyclin-dependent kinase inhibitor p21, has been detected. Further experiments in this context should be performed in order to establish the physiological environment that determines the differential response of AMPK in the cellular subpopulations of the liver.

S-adenosylmethionine-regulated and AMPK-mediated hepatocyte proliferation

It is well established that *S*-adenosylmethionine (SAMe), the major methyl donor group in the cell, is inhibitory to cell cycle progression and hepatocyte proliferation.[47] Thus, a

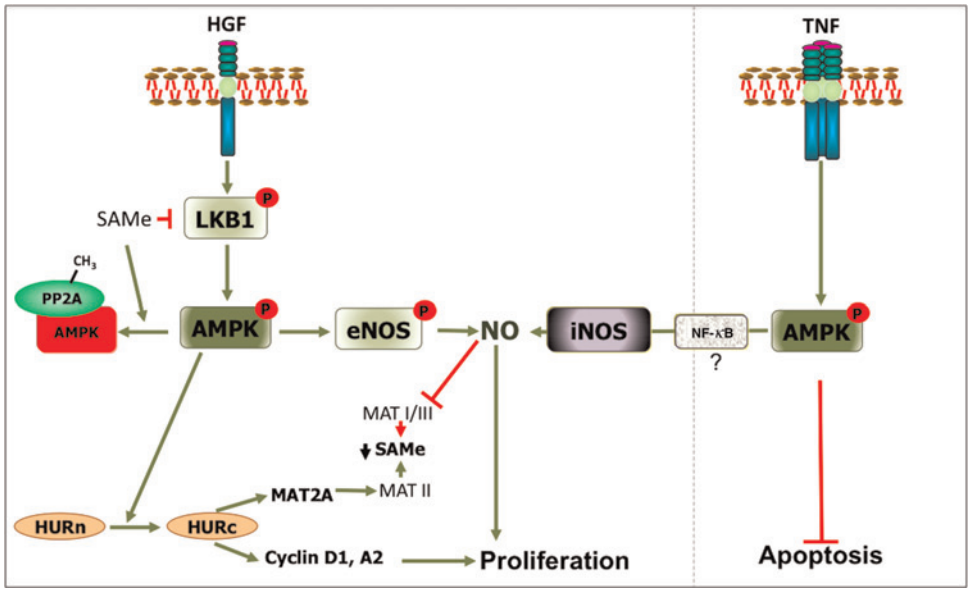

Figure 1 Schematic representation of AMP-activated protein kinase (AMPK)-mediated hepatocyte proliferation. Tumor necrosis factor alpha (TNF-α)-induced priming of hepatocytes (via nuclear factor kappa B [NF-κB] activation and increased inducible nitric oxide synthase [*iNOS*] expression) is probably mediated by AMPK activation. Both TNF-α-induced iNOS and hepatocyte growth factor (HGF)-induced endothelial nitric oxide synthase (eNOS) (via LKB1/AMPK) contribute to an increase in nitric oxide (NO) levels, which leads to proliferation. HGF-induced LKB1/AMPK activation also induces nucleo-cytoplasmic shuttling of HuR (Hu antigen R) translocation leading to increased stability of *cyclin D1* and *A2* mRNA. HuR also stabilizes *MAT2A* mRNA, thereby increasing MATII expression. This together with the inhibitory effect of NO on MATI/III activity contribute to a decrease in *S*-adenosylmethionine (SAMe) level, which is necessary for allowing proliferation to progress, since SAMe is an inhibitor of LKB1 and AMPK activity

decrease in SAMe levels has been observed in regenerating liver after PH.[48]

In hepatocytes, SAMe blocks HGF-induced proliferation by inhibiting AMPK activation through the induction of AMPK interaction with the protein phosphatase 2A. This inhibits AMPK phosphorylation at the threonine residue 172 (Thr 172) and therefore blocks its activation. SAMe also inhibits HGF-induced HuR nucleo-cytoplasmic shuttling mediated by AMPK activation, reducing the levels of HuR in the cytosolic compartment, and decreasing the stabilization of the mRNA stabilized by HuR, as cyclin D1 and A2.[43]

Methionine adenosyltransferases (MAT) are the main enzymes responsible for SAMe synthesis.[49,50] MATI/III, encoded by the *MAT1A* gene, and MATII, encoded by the *MAT2A* gene, are the main enzymes responsible for SAMe synthesis in adult hepatocytes, and fetal and proliferative liver, respectively. A decrease in SAMe levels, induced by a switch from *MAT1A* to *MAT2A* gene expression, has been observed in regenerating liver after PH[51] and in proliferating hepatocytes after HGF stimulation.[42,52] This is due to the fact that MATII, unlike MATI/III, can be inhibited by its own product SAMe. We recently showed that this switch from *MAT1A to MAT2A* gene expression is regulated by the mRNA binding proteins HuR and AUF1 (A + U-rich RNA-binding factor) and that both AICAR (an AMPK activator) and HGF increased HUR–MAT2A mRNA interaction in cultured hepatocytes.[53] In summary, HGF-induced hepatocyte proliferation, mediated by AMPK activation, leads to an increase in HuR cytosolic localization that promotes stabilization of *MAT2A* mRNA and increased MATII protein expression. This results in a decrease in SAMe levels (Figure 1). Also, since it has been shown that NO has an inhibitory effect on MATI activity, increased NO production after HGF-induced activation of LKB1/AMPK/eNOS pathway contributes to the decrease in SAMe levels (Figure 1).

The observation that SAMe levels have to decrease to allow liver proliferation has been confirmed by studies done in mice lacking the GNMT (glycine *N*-methyltransferase) enzyme (mice lacking GNMT [GNMT-KO]). GNMT is the main enzyme responsible for SAMe catabolism in the liver. GNMT-KO mice have a chronic elevation in SAMe and levels of serum transaminases, and develop steatosis and steatohepatitis (non-alcoholic steatohepatitis [NASH]) at three months, and fibrosis and hepatocellular carcinoma (HCC) at eight months. We found that lack of GNMT in these mice induces aberrant methylation of DNA and histones, resulting in epigenetic modulation of critical carcinogenic pathways (Janus Kinase/STAT).[54]

GNMT-KO mice also have impaired liver regeneration and increased mortality after PH. Even though DNA synthesis is induced in these mice after PH, SAMe levels fail to decrease after PH and this excess of SAMe exerts an inhibitory effect on the HGF-induced LKB1/AMPK/eNOS

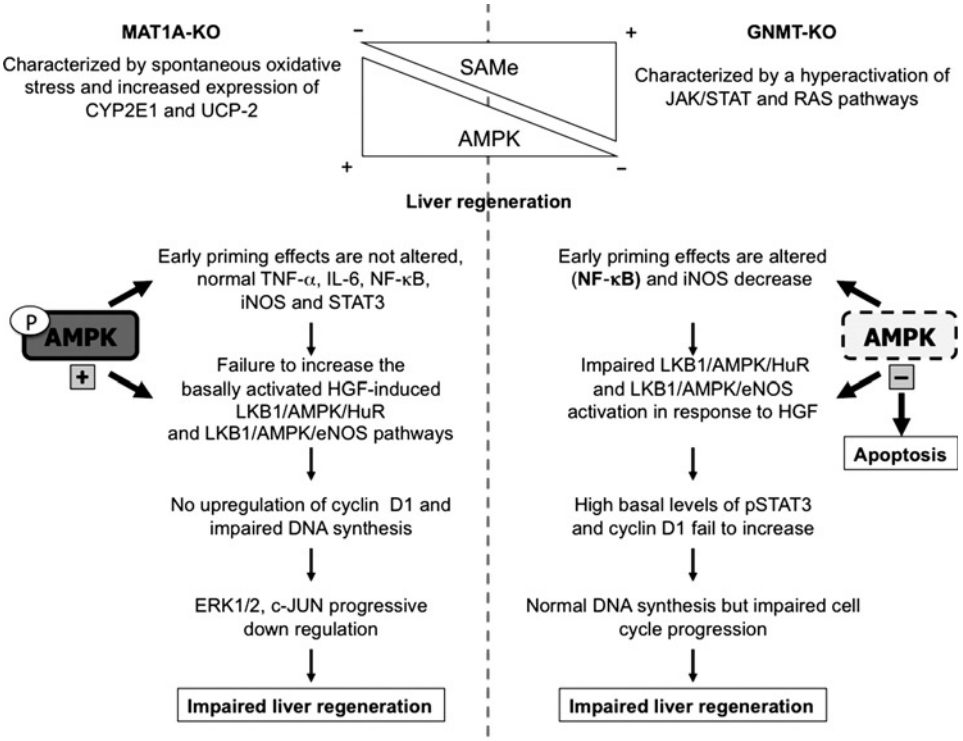

Figure 2 Schematic representation of the mechanisms involved in impaired liver regeneration in MATIA and GNMT knockout mice. *S*-adenosylmethionine (SAMe) levels have to decrease to allow liver proliferation as shown by studies done in mice lacking MATI/III enzyme (MAT1A-KO) and mice lacking GNMT enzyme (GNMT-KO), the main enzymes involved in SAMe synthesis and catabolism, respectively. This figure summarizes the main signalling pathways affected after partial hepatectomy in both mice. UCP-2, uncoupling protein-2; AMPK, AMP-activated protein kinase; NF-κB, nuclear factor kappa B; eNOS, endothelial nitric oxide synthase; HGF, hepatocyte growth factor; STAT3, signal transducer and activator of transcription 3; iNOS, inducible nitric oxide synthase; IL-6, interleukin-6

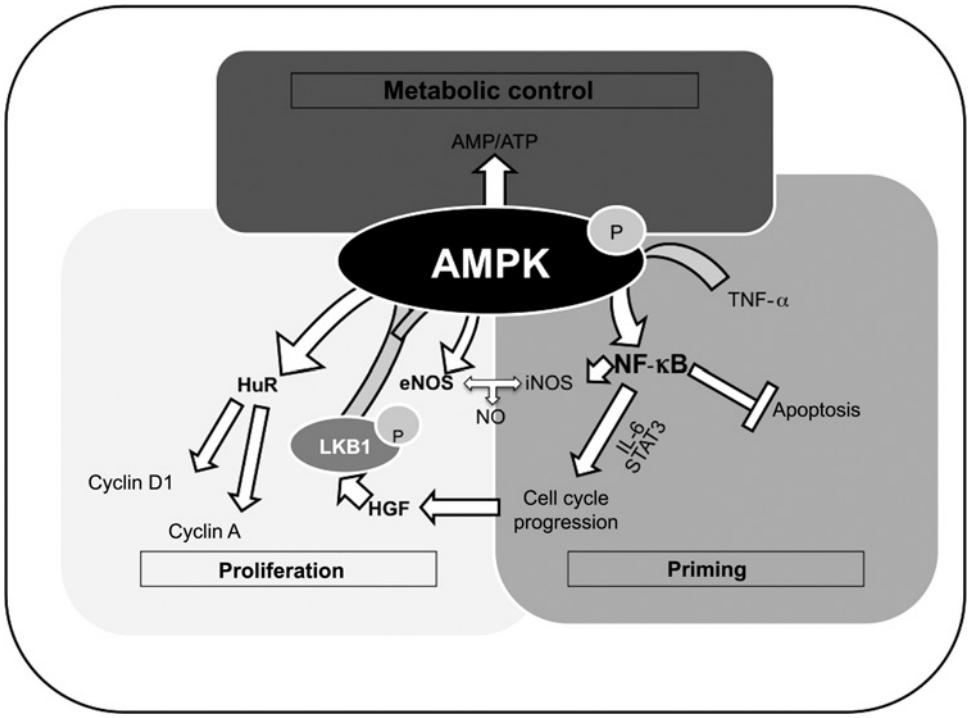

Figure 3 AMP-activated protein kinase (AMPK) activation in hepatocytes in response to metabolic signals, tumor necrosis factor alpha (TNF-α) and hepatocyte growth factor (HGF). TNF-α-induced AMPK possibly controls nuclear factor kappa B (NF-κB)-induced inducible nitric oxide synathase (*iNOS*) expression and blocks TNF-α-induced apoptosis during the priming response after partial hepatectomy (PH) whereas HGF-induced LKB1/AMPK controls HuR (Hu antigen R) cytosolic localization and endothelial nitric oxide synthase (eNOS) activation, and therefore Cyclin D1 and cyclin A expressions, and nitric oxide (NO) generation, respectively. Modulation of liver growth after PH by *S*-adenosylmethionine is exerted by its inhibitory effect on AMPK, which impacts on ATP metabolism, TNF-α signalling and HGF proliferative response

and AMPK/HuR/CyclinD1 pathways, blocking cell cycle progression.[33] This lack of HGF-induced LKB1/AMPK activation was also observed in mice treated with SAMe before PH[53] and in hepatocytes isolated from GNMT-KO mice and stimulated with HGF[33] (Figure 2).

Our data also show that the inhibition of AMPK activity mediated by the chronic elevated SAMe level in the GNMT-KO mice could also play an important role in the increased mortality observed in these mice. As mentioned above, NF-κB activation in response to TNF-α leads to increased *iNOS* expression in normal mice after PH, providing a hepato-protective function. In hepatocytes with impaired activation of AMPK (after treatment with the AMPK inhibitor compound C), TNF-α-induced NF-κB activation is not able to increase *iNOS* mRNA expression.[33] Similarly in the GNMT-KO mice, there is an abnormal basal accumulation of p65 in the nuclear compartment that fails to increase after PH, which results in impaired *iNOS* transcription.[33] Also, recently we have observed that hepatocytes isolated from GNMT-KO mice show increased nuclear p65 compared with wild-type hepatocytes, and TNF-α failed to induce AMPK phosphorylation and *iNOS* mRNA expression. In addition, TNF-α also induces apoptosis in GNMT-KO hepatocytes after 16 h of stimulation (unpublished data). Together, these data suggest that in hepatocytes, AMPK phosphorylation after TNF-α stimulation is necessary for blocking TNF-α-induced apoptosis.

Although our studies above show that the SAMe level has to decrease to allow successful liver regeneration, we have also found that a chronic decrease in SAMe level also can affect liver regeneration. Mice lacking MAT1A (MAT1A-KO mice), characterized by a chronic deficiency in SAMe level develop steatosis, NASH and HCC,[55] and show impaired liver regeneration after PH.[48] In these mice, the early priming events after PH, such as TNF-α and IL-6 secretion, and NF-κB, c-jun or STAT3 activation, appear to be intact, whereas the progression into the G_1 phase and the response to growth factors are impaired. We observed reduced DNA synthesis and progressive down-regulation of Jun-N-terminal kinase following PH.[48] In addition, HGF-induced pathways such as ERK, LKB1/AMPK/HuR/CyclinD1 and LKB1/AMPK/eNOS were more activated at baseline as compared with WT but failed to increase following PH (Figure 2). The fact that the SAMe level in the MAT1A-KO did not fall following PH is likely to be a key contributor to the lack of response to mitogenic signals in these mice.

Summary

The liver is the main organ responsible for storage and release of carbohydrates and synthesis of fatty acids, playing a critical role in the control of the whole-body energy status. Activation of AMPK in response to low ATP levels increases the ATP-producing catabolic pathways and decreases the ATP-consuming anabolic pathways.[56] The metabolic control exerted by AMPK is critical for

energy-demanding situations such as liver regeneration. In addition to its energy controlling function, AMPK is potentially a critical mediator in the priming response and in the proliferative phase after PH (Figure 3).

TNF-α and HGF are critical mediators of the priming and proliferative response after PH, respectively. In hepatocytes, AMPK is activated in response to both TNF-α and HGF. TNF-α-induced AMPK activation has been shown to be necessary for NF-κB-induced *iNOS* expression and for blocking TNF-α-induced apoptosis. HGF-induced LKB1/AMPK activation, on the other hand, has been found to play a critical role in controlling Cyclin D1 and cyclin A expressions through HuR cytosolic localization and NO generation through eNOS activation.

Our results also suggest that modulation of liver growth after PH by SAMe is exerted by its inhibitory effect on AMPK, which impacts on ATP metabolism, TNF-α signaling and HGF proliferative response. Finally, SAMe levels have to be under tight control during hepatocyte proliferation after PH, since a deficiency or a chronic excess of hepatic SAMe levels leads to impaired liver regeneration.

Author contributions: MV-R, NB and MLM-C were involved in manuscript preparation. MV-R was involved in figure preparation. SCL and JMM did critical revision of the manuscript.

ACKNOWLEDGEMENTS

This work is supported by grants from NIH AT-1576 (to SCL, MLM-C and JMM) and DK51719 (SCL), SAF 2008-04800, HEPADIP-EULSHM-CT-205 and ETORTEK-2008 (to MLM-C) and Sanidad Gobierno Vasco 2008 (to MLM-C), the Instituto de Salud Carlos III, FIS PS09/02010 (to NB) and 'Ramón y Cajal' Program of the Ministry of Science and Innovation, Spain (to NB). Ciberehd is funded by the Instituto de Salud Carlos III.

REFERENCES

1 Fausto N. Liver regeneration. *J Hepatol* 2000;**32**(Suppl.):19–31
2 Taub R. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol* 2004;**5**:836–47
3 Michalopoulos GK, DeFrances MC. Liver regeneration. *Science* 1997;**276**:60–6
4 Michalopoulos GK. Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. *Am J Pathol* 2010;**176**:2–13
5 Akerman P, Cote P, Yang SQ, McClain C, Nelson S, Bagby GJ, Diehl AM. Antibodies to tumor necrosis factor-alpha inhibit liver regeneration after partial hepatectomy. *Am J Physiol* 1992;**263**(Part 1):G579–85
6 Trautwein C, Rakemann T, Niehof M, Rose-John S, Manns MP. Acute-phase response factor, increased binding, and target gene transcription during liver regeneration. *Gastroenterology* 1996;**110**:1854–62
7 Iwai M, Cui TX, Kitamura H, Saito M, Shimazu T. Increased secretion of tumour necrosis factor and interleukin 6 from isolated, perfused liver of rats after partial hepatectomy. *Cytokine* 2001;**13**:60–64
8 Rai RM, Lee FY, Rosen A, Yang SQ, Lin HZ, Koteish A, Liew FY, Zaragoza C, Lowenstein C, Diehl AM. Impaired liver regeneration in inducible nitric oxide synthasedeficient mice. *Proc Natl Acad Sci USA* 1998;**95**:13829–34
9 Lee FY, Li Y, Zhu H, Yang S, Lin HZ, Trush M, Diehl AM. Tumor necrosis factor increases mitochondrial oxidant production and induces expression of uncoupling protein-2 in the regenerating mice [correction of rat] liver. *Hepatology* 1999;**29**:677–87
10 Webber EM, Bruix J, Pierce RH, Fausto N. Tumor necrosis factor primes hepatocytes for DNA replication in the rat. *Hepatology* 1998;**28**:1226–34
11 Rabes HM, Wirsching R, Tuczek HV, Iseler G. Analysis of cell cycle compartments of hepatocytes after partial hepatecomy. *Cell Tissue Kinet* 1976;**9**:517–32
12 Grisham JW. A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver; autoradiography with thymidine-H3. *Cancer Res* 1962;**22**:842–9
13 Widmann JJ, Fahimi HD. Proliferation of mononuclear phagocytes (Kupffer cells) and endothelial cells in regenerating rat liver. A light and electron microscopic cytochemical study. *Am J Pathol* 1975;**80**:349–66
14 Michalopoulos GK. Liver regeneration: alternative epithelial pathways. *Int J Biochem Cell Biol* 2009;**43**:173–9
15 Stöcker E, Wullstein HK, Bräu G. Capacity of regeneration in liver epithelia of juvenile, repeated partially hepatectomized rats. Autoradiographic studies after continous infusion of 3H-thymidine (author's transl). *Virchows Arch B Cell Pathol* 1973;**14**:93–103
16 Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology* 2006;**43**(Suppl. 1):S45–53
17 FitzGerald MJ, Webber EM, Donovan JR, Fausto N. Rapid DNA binding by nuclear factor kappa B in hepatocytes at the start of liver regeneration. *Cell Growth Differ* 1995;**6**:417–27
18 Greenbaum LE, Cressman DE, Haber BA, Taub R. Coexistence of C/EBP alpha, beta, growth-induced proteins and DNA synthesis in hepatocytes during liver regeneration. Implications for maintenance of the differentiated state during liver growth. *J Clin Invest* 1995;**96**:1351–65
19 Cressman DE, Diamond RH, Taub R. Rapid activation of the Stat3 transcription complex in liver regeneration. *Hepatology* 1995;**21**:1443–9
20 Taub R. Liver regeneration 4: transcriptional control of liver regeneration. *FASEB J* 1996;**10**:413–27
21 Cressman DE, Greenbaum LE, Haber BA, Taub R. Rapid activation of post-hepatectomy factor/nuclear factor kappa B in hepatocytes, a primary response in the regenerating liver. *J Biol Chem* 1994;**269**:30429–35
22 Chaisson ML, Brooling JT, Ladiges W, Tsai S, Fausto N. Hepatocyte-specific inhibition of NF-kappaB leads to apoptosis after TNF treatment, but not after partial hepatectomy. *J Clin Invest* 2002;**110**:193–202
23 Iimuro Y, Nishiura T, Hellerbrand C, Behrns KE, Schoonhoven R, Grisham JW, Brenner DA. NFkappaB prevents apoptosis and liver dysfunction during liver regeneration. *J Clin Invest* 1998;**101**:802–11
24 Malato Y, Sander LE, Liedtke C, Al-Masaoudi M, Tacke F, Trautwein C. Beraza hepatocyte-specific inhibitor-of-kappaB-kinase deletion triggers the innate immune response and promotes earlier cell proliferation during liver regeneration. *Hepatology* 2008;**47**:2036–50
25 Yamada Y, Kirillova I, Peschon JJ, Fausto N. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. *Proc Natl Acad Sci USA* 1997;**94**:1441–6
26 Yuan L, Kaplowitz N. Glutathione in liver diseases and hepatotoxicity. *Mol Aspects Med* 2009;**30**:29–41
27 Diehl AM. Cytokine regulation of liver injury and repair. *Immunol Rev* 2000;**174**:160–71
28 Hortelano S, Dewez B, Genaro AM, Díaz-Guerra MJ, Boscá L. Nitric oxide is released in regenerating liver after partial hepatectomy. *Hepatology* 1995;**21**:776–86
29 García-Trevijano ER, Martínez-Chantar ML, Latasa MU, Mato JM, Avila MA. NO sensitizes rat hepatocytes to proliferation by modifying *S*-adenosylmethionine levels. *Gastroenterology* 2002;**122**:1355–63
30 Leist M, Gantner F, Bohlinger I, Germann PG, Tiegs G, Wendel A. Murine hepatocyte apoptosis induced *in vitro* and *in vivo* by TNF-αlpha requires transcriptional arrest. *J Immunol* 1994;**153**:1778–88
31 Xu Y, Bialik S, Jones BE, Iimuro Y, Kitsis RN, Srinivasan A, Brenner DA, Czaja MJ. NF-kappaB inactivation converts a hepatocyte cell line TNF-αlpha response from proliferation to apoptosis. *Am J Physiol* 1998;**275**:C1058–66
32 Plümpe J, Malek NP, Bock CT, Rakemann T, Manns MP, Trautwein C. NF-kappaB determines between apoptosis and proliferation in hepatocytes during liver regeneration. *Am J Physiol Gastrointest Liver Physiol* 2000;**278**:G173–83
33 Varela-Rey M, Fernández-Ramos D, Martínez-López N, Embade N, Gómez-Santos L, Beraza N, Vázquez-Chantada M, Rodríguez J, Luka Z,

Wagner C, Lu SC, Martínez-Chantar ML, Mato JM. Impaired liver regeneration in mice lacking glycine *N*-methyltransferase. *Hepatology* 2009;**50**:443–52
34 Guo Z, Shao L, Du Q, Park KS, Geller DA. Identification of a classic cytokine-induced enhancer upstream in the human iNOS promoter. *FASEB J* 2007;**21**:535–42
35 Lindroos P, Tsai WH, Zarnegar R, Michalopoulos GK. Plasma levels of HGF in rats treated with tumor promoters. *Carcinogenesis* 1992;**13**:139–41
36 Masumoto A, Yamamoto N. Sequestration of a hepatocyte growth factor in extracellular matrix in normal adult rat liver. *Biochem Biophys Res Commun* 1991;**174**:90–5
37 Kim TH, Mars WM, Stolz DB, Petersen BE, Michalopoulos GK. Extracellular matrix remodeling at the early stages of liver regeneration in the rat. *Hepatology* 1997;**26**:896–904
38 Naldini L, Vigna E, Bardelli A, Follenzi A, Galimi F, Comoglio PM. Biological activation of pro-HGF (hepatocyte growth factor) by urokinase is controlled by a stoichiometric reaction. *J Biol Chem* 1995;**270**:603–11
39 Birchmeier C, Birchmeier W, Gherardi E, Van de Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003;**4**:915–25
40 Borowiak M, Garratt AN, Wüstefeld T, Strehle M, Trautwein C, Birchmeier C. Met provides essential signals for liver regeneration. *Proc Natl Acad Sci USA* 2004;**101**:10608–13
41 Huh CG, Factor VM, Sánchez A, Uchida K, Conner EA, Thorgeirsson SS. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. *Proc Natl Acad Sci USA* 2004;**101**:4477–82
42 Vázquez-Chantada M, Ariz U, Varela-Rey M, Embade N, Martínez-Lopez N, Fernández-Ramos D, Gómez-Santos L, Lamas S, Lu SC, Martínez-Chantar ML, Mato JM. Evidence for LKB1/AMP-activated protein kinase/endothelial nitric oxide synthase cascade regulated by hepatocyte growth factor, *S*-adenosylmethionine, and nitric oxide in hepatocyte proliferation. *Hepatology* 2009;**49**:608–17
43 Martínez-Chantar ML, Vázquez-Chantada M, Garnacho M, Latasa MU, Varela-Rey M, Dotor J, Santamaria M, Martínez-Cruz LA, Parada LA, Lu SC, Mato JM. *S*-adenosylmethionine regulates cytoplasmic HuR via AMP-activated kinase. *Gastroenterology* 2006;**131**:223–32
44 Wang W, Fan J, Yang X, Fürer-Galban S, Lopez de Silanes I, von Kobbe C, Guo J, Georas SN, Foufelle F, Hardie DG, Carling D, Gorospe M. AMP-activated kinase regulates cytoplasmic HuR. *Mol Cell Biol* 2002;**22**:3425–36
45 Caligiuri A, Bertolani C, Guerra CT, Aleffi S, Galastri S, Trappoliere M, Vizzutti F, Gelmini S, Laffi G, Pinzani M, Marra F. Adenosine monophosphate-activated protein kinase modulates the activated phenotype of hepatic stellate cells. *Hepatology* 2008;**47**:668–76
46 Adachi M, Brenner DA. High molecular weight adiponectin inhibits proliferation of hepatic stellate cells via activation of adenosine monophosphate-activated protein kinase. *Hepatology* 2008;**47**:677–85
47 Lu SC, Mato JM. *S*-adenosylmethionine in cell growth, apoptosis and liver cancer. *J Gastroenterol Hepatol* 2008;**23** (Suppl. 1):S73–7
48 Chen L, Zeng Y, Yang H, Lee TD, French SW, Corrales FJ, García-Trevijano ER, Avila MA, Mato JM, Lu SC. Impaired liver regeneration in mice lacking methionine adenosyltransferase 1A. *FASEB J* 2004;**18**:914–6
49 Finkelstein JD. Methionine metabolism in mammals. *J Nutr Biochem* 1990;**1**:228–37
50 Mato JM, Alvarez L, Ortiz P, Pajares MA. *S*-adenosylmethionine synthesis: molecular mechanisms and clinical implications. *Pharmacol Ther* 1997;**73**:265–80
51 Huang ZZ, Mao Z, Cai J, Lu SC. Changes in methionine adenosyltransferase during liver regeneration in the rat. *Am J Physiol* 1998;**275**:G14–21
52 Latasa MU, Boukaba A, García-Trevijano ER, Torres L, Rodríguez JL, Caballería J, Lu SC, López-Rodas G, Franco L, Mato JM, Avila MA. Hepatocyte growth factor induces MAT2A expression and histone acetylation in rat hepatocytes: role in liver regeneration. *FASEB J* 2001;**15**:1248–50
53 Vázquez-Chantada M, Fernández-Ramos D, Embade N, Martínez-Lopez N, Varela-Rey M, Woodhoo A, Luka Z, Wagner C, Anglim PP, Finnell RH, Caballería J, Laird-Offringa IA, Gorospe M, Lu SC, Mato JM, Martínez-Chantar ML. HuR/methyl-HuR and AUF1 regulate the MAT expressed during liver proliferation, differentiation, and carcinogenesis. *Gastroenterology* 2010;**138**:1943–53
54 Martínez-Chantar ML, Vázquez-Chantada M, Ariz U, Martínez N, Varela M, Luka Z, Capdevila A, Rodríguez J, Aransay AM, Matthiesen R, Yang H, Calvisi DF, Esteller M, Fraga M, Lu SC, Wagner C, Mato JM. Loss of the glycine *N*-methyltransferase gene leads to steatosis and hepatocellular carcinoma in mice. *Hepatology* 2008;**47**:1191–9
55 Martinez-Chantar ML, Corrales FJ, Martinez-Cruz LA, Garcia-Trevijano ER, Huang ZZ, Chen L, Kanel G, Avila MA, Mato JM, Lu SC. Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A. *FASEB J* 2002;**16**:1292–4
56 Winder WW, Thomson DM. Cellular energy sensing and signaling by AMP-activated protein kinase. *Cell Biochem Biophys* 2007;**47**:332–47