S-adenosylmethionine and proliferation: new pathways, new targets

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Abstract

SAMe (*S*-adenosylmethionine) is the main methyl donor group in the cell. MAT (methionine adenosyltransferase) is the unique enzyme responsible for the synthesis of SAMe from methionine and ATP, and SAMe is the common point between the three principal metabolic pathways: polyamines, transmethylation and transsulfuration that converge into the methionine cycle. SAMe is now also considered a key regulator of metabolism, proliferation, differentiation, apoptosis and cell death. Recent results show a new signalling pathway implicated in the proliferation of the hepatocyte, where AMPK (AMP-activated protein kinase) and HuR, modulated by SAMe, take place in HGF (hepatocyte growth factor)-mediated cell growth. Abnormalities in methionine metabolism occur in several animal models of alcoholic liver injury, and it is also altered in patients with liver disease. Both high and low levels of SAMe predispose to liver injury. In this regard, knockout mouse models have been developed for the enzymes responsible for SAMe synthesis and catabolism, MAT1A and GNMT (glycine *N*-methyltransferase) respectively. These knockout mice develop steatosis and HCC (hepatocellular carcinoma), and both models closely replicate the pathologies of human disease, which makes them extremely useful to elucidate the mechanism underlying liver disease. These new findings open a wide range of possibilities to discover novel targets for clinical applications.

SAMe metabolism

Since its discovery, SAMe (*S*-adenosylmethionine; also known as AdoMet and SAM) has emerged as an important molecule that controls numerous cellular functions. Liver plays a central role in the homoeostasis of SAMe as the major site of its synthesis and degradation [1]. The liver is where up to half of the daily intake of methionine is converted into SAMe and up to 85 % of all methylation reactions take place [2]. MAT (methionine adenosyltransferase) is the only enzyme responsible for biosynthesis of SAMe from methionine and ATP [1], making it indispensable for the survival of an organism. In mammals, of the two genes (*MAT1A*, *MAT2A*) that encode MAT, *MAT1A* is mainly expressed in adult liver, whereas *MAT2A* is expressed in all extrahepatic tissues. *MAT2A* and its gene product also predominate in the fetal liver and are progressively replaced by *MAT1A* during development [3,4]. In the liver, *MAT1A* expression is associated with a differentiated phenotype, whereas *MAT2A* expression is associated with rapid growth and de-differentiation.

SAMe is the link to three key metabolic pathways: polyamine synthesis, transmethylation and transsulfuration (Figure 1). In polyamine synthesis, SAMe is decarboxylated and the remaining propylamino moiety is donated to putrescine to form spermidine and MTA (methylthioadenosine) and to spermidine to form spermine and a second molecule of MTA. In transmethylation, SAMe donates its methyl group to a large variety of acceptor molecules in reactions catalysed by methyltransferases. SAMe is largely regulated by GNMT (glycine *N*-methyltransferase) [5], the major methyltransferase that accounts for 1 % of the soluble protein in rat liver [6,7]. The transsulfuration pathway is particularly active in the liver, making SAMe an important precursor of glutathione (GSH) [8]. All mammalian tissues express MAT and methionine synthase, whereas BHMT (betaine homocysteine methyltransferase) is limited to the liver and kidney. In the liver, SAMe inhibits MTHFR (methylenetetrahydrofolate reductase) and methionine synthase, and activates CBS (cystathionine β-synthase) [9,10]. Thus, when SAMe is depleted, homocysteine is channelled to remethylation to regenerate SAMe, whereas when SAMe level is high, homocysteine is channelled to the transsulfuration pathway.

SAMe and proliferation

In hepatocytes, SAMe levels control the differentiation status of the hepatocyte. Regarding this point, quiescent and proliferating hepatocytes display different SAMe contents, being lower in the growing cells [11]. In addition, after partial hepatectomy the levels of SAMe are decreased dramatically, coinciding with the onset of DNA synthesis and the induction

Key words: glycine *N*-methyltransferase (GNMT), HuR, liver, methionine adenosyltransferase (MAT), non-alcoholic steatohepatitis (NASH), *S*-adenosylmethionine (SAMe).

Abbreviations used: AMPK, AMP-activated protein kinase; BHMT, betaine homocysteine methyltransferase; CBS, cystathionine β-synthase; GNMT, glycine *N*-methyltransferase; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; MAT, methionine adenosyltransferase; MTA, methylthioadenosine; MTHFR, methylenetetrahydrofolate reductase; NASH, non-alcoholic steatohepatitis; OA, okadaic acid; PP, protein phosphatase; SAMe, *S*-adenosylmethionine.

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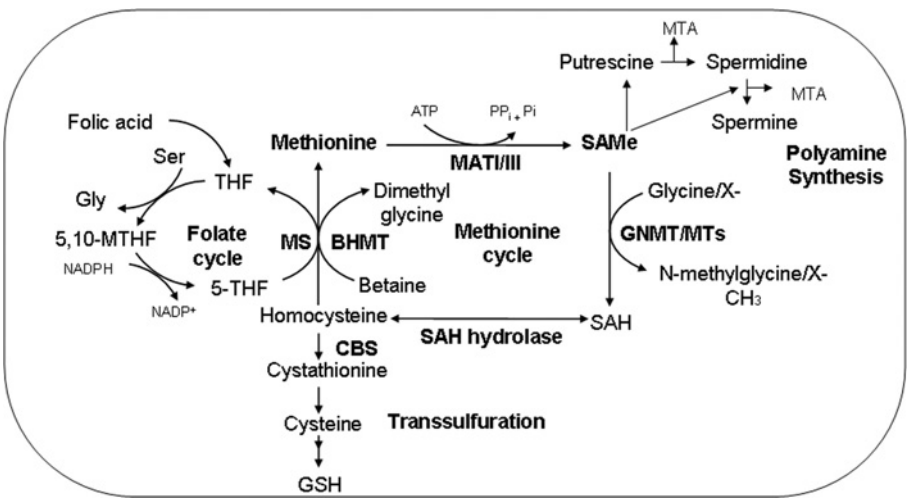
Figure 1 | **Methionine metabolism**

SAMe is synthesized by MAT enzymes and SAH (*S*-adenosylhomocysteine) is generated as a product of transmethylation and is hydrolysed to form homocysteine (Hcy) and adenosine through a reversible reaction catalysed by SAH hydrolase. SAH is a potent competitive inhibitor of methylation reactions and prompt removal of adenosine and homocysteine is required to prevent accumulation of SAH. Homocysteine can be remethylated to form methionine by two enzymes: methionine synthase ('MS'), which requires normal levels of folate and vitamin B_{12}; and BHMT, which requires betaine, a metabolite of choline. Remethylation of homocysteine via methionine synthase requires 5-MTHF (5-methyltetrahydrofolate), which is derived from 5,10-MTHF (5,10-methylenetetrahydrofolate) in a reaction catalysed by MTHFR. 5-MTHF is then converted into THF (tetrahydrofolate) as it donates its methyl group and THF is converted into 5,10-MTHF to complete the folate cycle. Homocysteine can also undergo the transsulfuration pathway to form cysteine [the rate-limiting precursor for glutathione (GSH)] via a two-step enzymatic process catalysed by CBS and cystathionase, both requiring vitamin B_6.

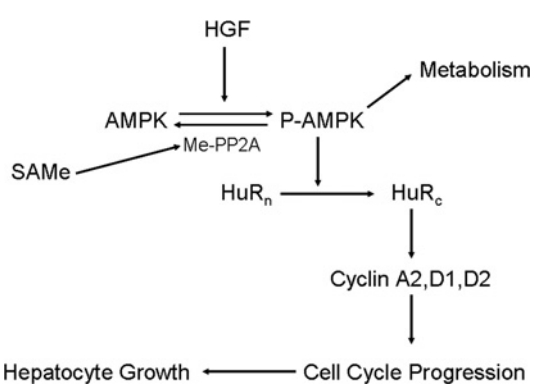


of early-response genes [12]. When this decrease in SAMe is blocked by exogenous SAMe administration, hepatocyte DNA synthesis is inhibited [13]. Moreover, the treatment with SAMe inhibits the proliferation of the hepatoma cells [11], prevents the development of HCC (hepatocellular carcinoma) in rats previously treated with a hepatocarcinogen [14] and blocks the mitogenic effect of HGF (hepatocyte growth factor) in hepatocytes [15]. The question open at this point is to identify the mechanism by which SAMe exerts its effect on growth modulation in hepatocytes.

In primary hepatocytes, SAMe is able to prevent the response induced by HGF, including the normal up-regulation of cyclins D1 and D2 obtained after the mitogenic stimulus. This effect is independent of ERK, suggesting that MAPK (mitogen-activated protein kinase) pathway is not the target of SAMe. In agreement with this, HGF induces the phosphorylation of AMPK (AMP-activated protein kinase) in hepatocytes, and SAMe prevents this process [16]. There is clear evidence that AMPK regulates the localization of HuR (RNA-binding protein) between cellular compartments [17]. In hepatocytes, the activation of AMPK by HGF induces the transport of HuR from the nucleus to the cytoplasm. This process involves the stabilization and increase in the half-life of several cell-cycle genes regulated by HuR binding [18]. Between these target genes are cyclin D1 and cyclin A2, both of them up-regulated after HGF treatment. SAMe inhibits the activation of AMPK through a mechanism that involves the activation of PPs (protein phosphatases), probably PP2A, and in relation to this effect it blocks the transport of HuR and the stabilization of cell-cycle genes [16]. A hypothetical scheme for the involvement of AMPK and HuR in HGF-mediated cell growth and modulation by SAMe is shown in Figure 2.

Figure 2 | **AMPK and HuR in hepatocyte cell growth**

HGF activates AMPK, which contributes to the translocation of HuR from the nucleus to the cytosol and subsequently cell-cycle progression. Increased SAMe level and level of methyl-PP2A (Me-PP2A) inhibit AMPK activation and hepatocyte proliferation.



AMPK is the metabolic sensor of the cell regulating the AMP/ATP ratio [19]. When AMPK is activated, there is a switch from anabolic pathways that consume ATP to catabolic pathways that generate ATP [20]. In this context, the regulation of the activation of AMPK by SAMe modulates liver lipid, carbohydrate homoeostasis and hepatocyte growth. In summary, SAMe is the central molecule in which metabolism and proliferation converge.

SAMe regulates apoptosis in transformed hepatocytes

As mentioned above, SAMe exerts hepato-protective and chemo-preventive effects and prevents the development of liver tumours. This effect of SAMe is accompanied by an increase in apoptotic bodies [14,21,22]. *In vitro* studies show that SAMe exerts opposing apoptotic effects in normal versus transformed hepatocytes. It has a pro-apoptotic effect on HepG2 and HuH-7 cells (transformed hepatocytes) but not on primary hepatocytes. Normal hepatocytes undergo apoptosis after treatment with OA (okadaic acid). OA is an inhibitor of PPs that leads to the release of cytochrome *c* from the mitochondria into the cytosol and activation of downstream effector pro-apoptotic caspases, which cleave a number of cellular proteins facilitating DNA fragmentation and cell death [23]. SAMe treatment in these hepatocytes, however, can prevent OA-mediated apoptosis by preventing the release of cytochrome *c*. In addition, SAMe treatment also prevents caspase 3 activation and PARP [poly(ADP-ribose) polymerase] cleavage induced by OA. However, in transformed hepatocytes, SAMe induces the release of cytochrome *c* from the mitochondria into the cytosol. Thus, in transformed hepatocytes treated with OA, there is a net amplification of cytochrome *c* release after SAMe treatment rather than a suppression of its release, as in normal hepatocytes.

This effect of SAMe on cytochrome *c* release in transformed hepatocytes has been shown to be mediated by regulation of Bcl-X_S levels. Bcl-2 family members (such as Bcl-X_L, Bcl-X_S and Bax) are known to modulate apoptosis at the mitochondrial level [24] by controlling the release of cytochrome *c* into the cytoplasm. Bcl-X is alternatively spliced, producing two major distinct mRNAs and variant proteins: the anti-apoptotic larger Bcl-X_L and the pro-apoptotic shorter Bcl-X_S. In transformed hepatocytes, SAMe selectively up-regulates Bcl-X_S by an alternative splicing mechanism, involving promoters 2 and 3 of the Bcl-X gene, and this process is independent of methylation [25]. Furthermore, SAMe regulates the SR protein (serine/arginine-rich protein) dephosphorylation, required for the progression of the splicing reaction [26] by the activation of PP1 activity. This is mediated by an increase in PP1 catalytic subunit mRNA transcription. In normal hepatocytes, however, SAMe treatment has no effect on Bcl-X_S transcription or PP1 activity [25]. In addition, these anti-apoptotic effects of SAMe in primary hepatocytes are due, at least in part, to their capacity for inhibiting the JNK (c-Jun N-terminal kinase)/AP-1 (activator protein-1) pathway [27]. These results support the idea that SAMe exerts its anti-apoptotic action in hepatocytes and its pro-apoptotic action in hepatoma cells by different mechanisms.

SAMe and liver pathology

Abnormalities in methionine metabolism occur in several animal models of alcoholic liver injury in mammals [1,28–31]. In rats fed intragastrically with ethanol, hepatic levels of methionine, SAMe and DNA methylation fell by ~40 % [31]. Additionally, when a diet deficient in choline is administered to rats and mice, the liver develops steatosis in a few weeks, and subsequently steatohepatitis and sometimes HCC [13].

Methionine metabolism is also altered in patients with liver disease. Low levels of SAMe have been described in several pathologies. Patients with liver injury showed impairment in methionine clearance after methionine load [32] and patients with cirrhosis have diminished *MAT1A* expression, lower MAT activity and thus decreased SAMe biosynthesis, which can lead to reduced transmethylation and GSH synthesis [1]. Moreover, patients hospitalized for alcoholic hepatitis (some of them just with fibrosis) presented decreased *MAT1A* expression and reduced hepatic SAMe levels [33].

Abnormally elevated plasma methionine and SAMe levels have also been described in patients with GNMT mutations [34], and a GNMT polymorphism (1289C>T) has been associated with HCC [35]. However, although these individuals show evidence of mild liver disease, they appear to be quite normal.

The relationship between low levels of SAMe with the development of liver disease, and the important role of SAMe in normal hepatic physiology, led to testing the effect of SAMe supplementation in a variety of models of liver disease. For many years, SAMe has been used in the treatment of cholestasis (given orally or parenterally). When SAMe was administered to alcohol-fed animals, GSH levels were increased and liver damage diminished [28]. Administration of SAMe improved survival in animals with liver injury caused by different toxics (galactosamine, acetaminophen and thioacetamide) [9] just as it reduced neoplastic nodules in animals with HCC [36] and liver fibrosis in rats treated with carbon tetrachloride (CCl_4) [9]. In humans, there are several studies; however, taken together, the results were not clear. The most complete trial [37] demonstrated that SAMe treatment given for 2 years to patients with alcoholic cirrhosis and less severe hepatic dysfunction decreased significantly the probability of death or need for liver transplantation, and increased GSH levels. Those studies have also shown that treatment with SAMe has no secondary effects on the patients. Several mechanisms [changes in DNA methylation, improved membrane fluidity and changes in the TNFα (tumour necrosis factor α) expression] [29,38,39] have been proposed for SAMe's protective action. Unfortunately, more studies are necessary to support a therapeutic role of SAMe in patients with alcoholic hepatitis.

Mouse models of liver disease with abnormal hepatic SAMe levels

The importance of maintaining adequate hepatic SAMe levels for proper liver function is widely known. For this reason, mouse models of liver disease have been developed in which the enzymes responsible for SAMe synthesis and catabolism, *MAT1A* and *GNMT* respectively, have been inactivated. Both of these knockout mouse models, MAT1A$^{-/-}$ and GNMT$^{-/-}$, develop steatosis and HCC, although only MAT1A$^{-/-}$ develops steatohepatitis, and only GNTM$^{-/-}$ mice develop fibrosis.

Mice deficient in MAT, the enzyme responsible for SAMe synthesis in the adult liver, have decreased hepatic SAMe levels, and spontaneously develop steatosis, NASH (non-alcoholic steatohepatitis) and HCC [40]. By 3 months of age, these mice have hepatomegaly with macrovesicular steatosis. Given these enzyme deficiencies, it is widely believed that oxidative stress plays a key role in the pathogenesis of liver disease in these mice. This has been demonstrated by the increased expression of hepatic CYP2E1 (cytochrome P450, family 2, subfamily E, polypeptide 1) and uncoupling protein 2, the elevated levels of lipid peroxidation and the reduced content of hepatic glutathione. However, several key enzymes involved in cysteine and GSH synthesis are increased, indicative of the liver's attempts at restoring homoeostasis when challenged by this oxidative stress response [40]. These genetically modified mice also exhibit changes in the expression of genes involved in cellular proliferation, as well as lipid and carbohydrate metabolism [41]. As a result, the mice are predisposed to liver injury after CCl_4 and ethanol exposure, and have impaired liver regeneration after partial hepatectomy [42]. Gene expression studies of MAT1A$^{-/-}$ mice and human NASH samples demonstrated the suitability of the mouse model for the study of human disease. In a previous study, a genetic pathway associated with NASH was identified, and the involvement of Sp1 transcription factor in the development of the disease was also assessed [43].

There is also a mouse model that has high levels of SAMe secondary to deficient GNMT expression, the primary enzyme responsible for SAMe catabolism. These GNMT-deficient mice also develop liver disease, although the course and expression of the injury differ from that of MAT1A$^{-/-}$ mice. By 3 months of age, the GNMT-deficient mice present steatosis and fibrosis. The liver pathology becomes more prominent at 8 months of age, with the concomitant appearance of HCC [44]. Oxidative stress and lipid metabolism are the principal processes linked with the pathogenesis of liver injury in these mice. Moreover, these mice have increased susceptibility to injury induced by ethanol and lipopolysaccharide/galactosamine, and have impaired liver regeneration after partial hepatectomy (M. Varela-Rey, unpublished work), similar to what was described for the MAT1A.

These murine models have proved to be very useful for addressing important questions regarding human liver disease. Both models closely replicate the underlying pathologies of select human liver disease, although there are similarities and differences between these two models, as there are in different patients of liver disease. The next big challenge will be to study the molecular mechanisms underlying the disease in these mice, and subsequently classify human patients according to the exact type of liver damage. This will greatly enhance our ability to provide appropriate pharmacological treatment for these diseases.

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References

1 Mato, J.M., Corrales, F.J., Lu, S.C. and Avila, M.A. (2002) *S*-adenosylmethionine: a control switch that regulates liver function. FASEB J. **16**, 15–26
2 Mudd, S.H. and Poole, J.R. (1975) Labile methyl balances for normal humans on various dietary regimens. Metabolism **24**, 721–735
3 Horikawa, S., Ozasa, H., Ota, K. and Tsukada, K. (1993) Immunohistochemical analysis of rat *S*-adenosylmethionine synthetase isozymes in developmental liver. FEBS Lett. **330**, 307–311
4 Gil, B., Casado, M., Pajares, M.A., Bosca, L., Mato, J.M., Martin-Sanz, P. and Alvarez, L. (1996) Differential expression pattern of *S*-adenosylmethionine synthetase isoenzymes during rat liver development. Hepatology **24**, 876–881
5 Mato, J.M. and Lu, S.C. (2007) Role of *S*-adenosyl-L-methionine in liver health and injury. Hepatology **45**, 1306–1312
6 Luka, Z., Capdevila, A., Mato, J.M. and Wagner, C. (2006) A glycine *N*-methyltransferase knockout mouse model for humans with deficiency of this enzyme. Transgenic Res. **15**, 393–397
7 Stipanuk, M.H. (2004) Sulfur amino acid metabolism: pathways for production and removal of homocysteine and cysteine. Annu. Rev. Nutr. **24**, 539–577
8 Lu, S.C. (1999) Regulation of hepatic glutathione synthesis: current concepts and controversies. FASEB J. **13**, 1169–1183
9 Mato, J.M., Alvarez, L., Ortiz, P. and Pajares, M.A. (1997) *S*-adenosylmethionine synthesis: molecular mechanisms and clinical implications. Pharmacol. Ther. **73**, 265–280
10 Prudova, A., Bauman, Z., Braun, A., Vitvitsky, V., Lu, S.C. and Banerjee, R. (2006) *S*-adenosylmethionine stabilizes cystathionine β-synthase and modulates redox capacity. Proc. Natl. Acad. Sci. U.S.A. **103**, 6489–6494
11 Cai, J., Mao, Z., Hwang, J.J. and Lu, S.C. (1998) Differential expression of methionine adenosyltransferase genes influences the rate of growth of human hepatocellular carcinoma cells. Cancer Res. **58**, 1444–1450
12 Lu, S.C. and Mato, J.M. (2005) Role of methionine adenosyltransferase and *S*-adenosylmethionine in alcohol-associated liver cancer. Alcohol **35**, 227–234
13 Shivapurkar, N. and Poirier, L.A. (1983) Tissue levels of *S*-adenosylmethionine and *S*-adenosylhomocysteine in rats fed methyl-deficient, amino acid-defined diets for one to five weeks. Carcinogenesis **4**, 1051–1057
14 Pascale, R.M., Marras, V., Simile, M.M., Daino, L., Pinna, G., Bennati, S., Carta, M., Seddaiu, M.A., Massarelli, G. and Feo, F. (1992) Chemoprevention of rat liver carcinogenesis by *S*-adenosyl-L-methionine: a long-term study. Cancer Res. **52**, 4979–4986
15 Latasa, M.U., Boukaba, A., Garcia-Trevijano, E.R., Torres, L., Rodriguez, J.L., Caballeria, J., Lu, S.C., Lopez-Rodas, G., Franco, L., Mato, J.M. and Avila, M.A. (2001) Hepatocyte growth factor induces MAT2A expression and histone acetylation in rat hepatocytes: role in liver regeneration. FASEB J. **15**, 1248–1250
16 Martinez-Chantar, M.L., Vazquez-Chantada, M., Garnacho, M., Latasa, M.U., Varela-Rey, M., Dotor, J., Santamaria, M., Martinez-Cruz, L.A., Parada, L.A., Lu, S.C. and Mato, J.M. (2006) *S*-adenosylmethionine regulates cytoplasmic HuR via AMP-activated kinase. Gastroenterology **131**, 223–232

17 Wang, W., Fan, J., Yang, X., Furer-Galban, S., Lopez de Silanes, I., von Kobbe, C., Guo, J., Georas, S.N., Foufelle, F., Hardie, D.G. et al. (2002) AMP-activated kinase regulates cytoplasmic HuR. Mol. Cell. Biol. **22**, 3425–3436
18 Wang, W., Caldwell, M.C., Lin, S., Furneaux, H. and Gorospe, M. (2000) HuR regulates cyclin A and cyclin B1 mRNA stability during cell proliferation. EMBO J. **19**, 2340–2350
19 Hardie, D.G. and Hawley, S.A. (2001) AMP-activated protein kinase: the energy charge hypothesis revisited. BioEssays **23**, 1112–1119
20 Hardie, D.G., Hawley, S.A. and Scott, J.W. (2006) AMP-activated protein kinase – development of the energy sensor concept. J. Physiol. **574**, 7–15
21 Pascale, R.M., Simile, M.M., Satta, G., Seddaiu, M.A., Daino, L., Pinna, G., Vinci, M.A., Gaspa, L. and Feo, F. (1991) Comparative effects of L-methionine, *S*-adenosyl-L-methionine and 5′-methylthioadenosine on the growth of preneoplastic lesions and DNA methylation in rat liver during the early stages of hepatocarcinogenesis. Anticancer Res. **11**, 1617–1624
22 Garcea, R., Daino, L., Pascale, R., Simile, M.M., Puddu, M., Frassetto, S., Cozzolino, P., Seddaiu, M.A., Gaspa, L. and Feo, F. (1989) Inhibition of promotion and persistent nodule growth by *S*-adenosyl-L-methionine in rat liver carcinogenesis: role of remodeling and apoptosis. Cancer Res. **49**, 1850–1856
23 Ansorena, E., Garcia-Trevijano, E.R., Martinez-Chantar, M.L., Huang, Z.Z., Chen, L., Mato, J.M., Iraburu, M., Lu, S.C. and Avila, M.A. (2002) *S*-adenosylmethionine and methylthioadenosine are antiapoptotic in cultured rat hepatocytes but proapoptotic in human hepatoma cells. Hepatology **35**, 274–280
24 Borner, C. (2003) The Bcl-2 protein family: sensors and checkpoints for life-or-death decisions. Mol. Immunol. **39**, 615–647
25 Yang, H., Sadda, M.R., Li, M., Zeng, Y., Chen, L., Bae, W., Ou, X., Runnegar, M.T., Mato, J.M. and Lu, S.C. (2004) *S*-adenosylmethionine and its metabolite induce apoptosis in HepG2 cells: role of protein phosphatase 1 and Bcl-X_S. Hepatology **40**, 221–231
26 Misteli, T. (1999) RNA splicing: what has phosphorylation got to do with it? Curr. Biol. **9**, R198–R200
27 Ansorena, E., Berasain, C., Lopez Zabalza, M.J., Avila, M.A., Garcia-Trevijano, E.R. and Iraburu, M.J. (2006) Differential regulation of the JNK/AP-1 pathway by *S*-adenosylmethionine and methylthioadenosine in primary rat hepatocytes versus HuH7 hepatoma cells. Am. J. Physiol. Gastrointest. Liver Physiol. **290**, G1186–G1193
28 Tsukamoto, H. and Lu, S.C. (2001) Current concepts in the pathogenesis of alcoholic liver injury. FASEB J. **15**, 1335–1349
29 Lieber, C.S., Casini, A., DeCarli, L.M., Kim, C.I., Lowe, N., Sasaki, R. and Leo, M.A. (1990) *S*-adenosyl-L-methionine attenuates alcohol-induced liver injury in the baboon. Hepatology **11**, 165–172
30 Villanueva, J.A. and Halsted, C.H. (2004) Hepatic transmethylation reactions in micropigs with alcoholic liver disease. Hepatology **39**, 1303–1310
31 Lu, S.C., Huang, Z.Z., Yang, H., Mato, J.M., Avila, M.A. and Tsukamoto, H. (2000) Changes in methionine adenosyltransferase and *S*-adenosylmethionine homeostasis in alcoholic rat liver. Am. J. Physiol. Gastrointest. Liver Physiol. **279**, G178–G185
32 Kinsell, L.W., Harper, H.A., Barton, H.C., Hutchin, M.E. and Hess, J.R. (1948) Studies in methionine and sulfur metabolism. I. The fate of intravenously administered methionine, in normal individuals and in patients with liver damage. J. Clin. Invest. **27**, 677–688
33 Lee, T.D., Sadda, M.R., Mendler, M.H., Bottiglieri, T., Kanel, G., Mato, J.M. and Lu, S.C. (2004) Abnormal hepatic methionine and glutathione metabolism in patients with alcoholic hepatitis. Alcohol. Clin. Exp. Res. **28**, 173–181
34 Mudd, S.H., Cerone, R., Schiaffino, M.C., Fantasia, A.R., Minniti, G., Caruso, U., Lorini, R., Watkins, D., Matiaszuk, N., Rosenblatt, D.S. et al. (2001) Glycine *N*-methyltransferase deficiency: a novel inborn error causing persistent isolated hypermethioninaemia. J. Inherit. Metab. Dis. **24**, 448–464
35 Tseng, T.L., Shih, Y.P., Huang, Y.C., Wang, C.K., Chen, P.H., Chang, J.G., Yeh, K.T., Chen, Y.M. and Buetow, K.H. (2003) Genotypic and phenotypic characterization of a putative tumor susceptibility gene, GNMT, in liver cancer. Cancer Res. **63**, 647–654
36 Pascale, R.M., Simile, M.M., De Miglio, M.R. and Feo, F. (2002) Chemoprevention of hepatocarcinogenesis: *S*-adenosyl-L-methionine. Alcohol **27**, 193–198
37 Mato, J.M., Camara, J., Fernandez de Paz, J., Caballeria, L., Coll, S., Caballero, A., Garcia-Buey, L., Beltran, J., Benita, V., Caballeria, J. et al. (1999) *S*-adenosylmethionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. J. Hepatol. **30**, 1081–1089
38 Colell, A., Garcia-Ruiz, C., Morales, A., Ballesta, A., Ookhtens, M., Rodes, J., Kaplowitz, N. and Fernandez-Checa, J.C. (1997) Transport of reduced glutathione in hepatic mitochondria and mitoplasts from ethanol-treated rats: effect of membrane physical properties and *S*-adenosyl-L-methionine. Hepatology **26**, 699–708
39 Chawla, R.K., Watson, W.H., Eastin, C.E., Lee, E.Y., Schmidt, J. and McClain, C.J. (1998) *S*-adenosylmethionine deficiency and TNF-α in lipopolysaccharide-induced hepatic injury. Am. J. Physiol. **275**, G125–G129
40 Martinez-Chantar, M.L., Corrales, F.J., Martinez-Cruz, L.A., Garcia-Trevijano, E.R., Huang, Z.Z., Chen, L., Kanel, G., Avila, M.A., Mato, J.M. and Lu, S.C. (2002) Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A. FASEB J. **16**, 1292–1294
41 Lu, S.C., Alvarez, L., Huang, Z.Z., Chen, L., An, W., Corrales, F.J., Avila, M.A., Kanel, G. and Mato, J.M. (2001) Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. Proc. Natl. Acad. Sci. U.S.A. **98**, 5560–5565
42 Chen, L., Zeng, Y., Yang, H., Lee, T.D., French, S.W., Corrales, F.J., Garcia-Trevijano, E.R., Avila, M.A., Mato, J.M. and Lu, S.C. (2004) Impaired liver regeneration in mice lacking methionine adenosyltransferase 1A. FASEB J. **18**, 914–916
43 Rubio, A., Guruceaga, E., Vazquez-Chantada, M., Sandoval, J., Martinez-Cruz, L.A., Segura, V., Sevilla, J.L., Podhorski, A., Corrales, F.J., Torres, L. et al. (2007) Identification of a gene-pathway associated with non-alcoholic steatohepatitis. J. Hepatol. **46**, 708–718
44 Martínez-Chantar, M.L., Vázquez-Chantada, M., Ariz, U., Martínez, N., Varela-Rey, M., Luka, Z., Capdevila, A., Rodríguez, J., Aransay, A.M., Matthiesen, R. et al. (2008) Loss of glycine *N*-methyltransferase gene leads to steatosis and hepatocellular carcinoma in mice. Hepatology **47**, 1191–1199

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