Pleiotropic effects of methionine adenosyltransferases deregulation as determinants of liver cancer progression and prognosis

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Summary

Downregulation of liver-specific *MAT1A* gene, encoding S-adenosylmethionine (SAM) synthesizing isozymes MATI/III, and upregulation of widely expressed *MAT2A*, encoding MATII isozyme, known as *MAT1A:MAT2A* switch, occurs in hepatocellular carcinoma (HCC). Being inhibited by its reaction product, MATII isoform upregulation cannot compensate for MATI/III decrease. Therefore, *MAT1A:MAT2A* switch contributes to decrease in SAM level in rodent and human hepatocarcinogenesis. SAM administration to carcinogen-treated rats prevents hepatocarcinogenesis, whereas *MAT1A*-KO mice, characterized by chronic SAM deficiency, exhibit macrovesicular steatosis, mononuclear cell infiltration in periportal areas, and HCC development. This review focuses upon the pleiotropic changes, induced by *MAT1A/MAT2A* switch, associated with HCC development. Epigenetic control of MATs expression occurs at transcriptional and post-transcriptional levels. In HCC cells, *MAT1A/MAT2A* switch is associated with global DNA hypomethylation, decrease in DNA repair, genomic instability, and signaling deregulation including c-*MYC* overexpression, rise in polyamine synthesis, upregulation of RAS/ERK, IKK/NF-kB, PI3K/AKT, and LKB1/AMPK axis. Furthermore, decrease in *MAT1A* expression and SAM levels results in increased HCC cell proliferation, cell survival, and microvascularization. All of these changes are reversed by SAM treatment *in vivo* or forced *MAT1A* overexpression or *MAT2A* inhibition in cultured HCC cells. In human HCC, *MAT1A:MAT2A* and MATI/III:MATII ratios correlate negatively with cell proliferation and genomic instability, and positively with apoptosis and global DNA methylation. This suggests that SAM decrease and MATs deregulation represent potential therapeutic targets for HCC. Finally, MATI/III:MATII ratio strongly predicts patients' survival length suggesting that *MAT1A:MAT2A* expression ratio is a putative prognostic marker for human HCC.

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Abbreviations: HCC, hepatocellular carcinoma; ASH, alcoholic steatohepatitis; MDD, methyl deficient diet; SAM, S-adenosylmethionine; MAT, methionine adenosyltransferase; SAH, S-adenosylhomocysteine; SAHH, SAH hydroxylase; GSH, reduced glutathione; BHMT, betaine-homocysteine methyltransferase; MTHFHMT, 5-methyltetrahydrofolate homocysteine methyltransferase; 5′-MTA, 5′-methylthioadenosine; CBS, cystathionine β-synthase; PHB1, prohibitin 1; VLDL, very low density lipoproteins; LDL, low density lipoproteins; PH, partial hepatectomy; DN, dysplastic nodule; GNMT, glycine N-methyltransferase; JAK, Janus kinase; STAT1, signal transducer and activator of transcription; LKB1, serine/threonine protein kinase 11; ERK, extracellular signal-regulated kinase; p90RSK, ribosomal protein S6 kinase polypeptide 2; RASGRP3, RAS guanyl releasing protein 3; HGF, hepatocyte growth factor; MAPK, mitogen-activated protein kinase; PI3K, phosphatadylinositol 3-kinase; AKT, V-AKT murine thymoma viral oncogene homolog; SP1, specificity protein 1; c-Mybl2, V-MYB avian myeloblastosis viral oncogene homolog-like 2; NF-kB, nuclear factor kB; AP-1, activator protein-1; TNFα, tumor necrosis factor α; RBP, mRNA-binding proteins; AUF1, AUrich RNA binding factor 1; HuR, Hu antigen R; GI, genomic instability; ODC, ornithine decarboxylase; BAX, BCL2-associated x protein; FAS, tumor necrosis factor receptor superfamily, member 6; AP, apurinic/apyrimidinic; APEX1, endonuclease redox effector APE1/REF-1/APEX1; EGR-1, early growth response protein-1; ROS, reactive oxygen species; CDC2, cell division cycle 2; NOS, nitric oxide synthase; AMPK, AMP activated protein kinase; PFK-2, phosphofructokinase 2; mTORC2, mammalian target of rapamycin complex; TSC1, hamartin; TSC2, tuberin; IKK, inhibitor of kappa light chain gene enhancer in B cells, kinase of; BAK, BCL2 antagonist killer; BCL2, B-cell cell/lymphoma 2; XIAP, inhibitor of apoptosis, x-linked; USP7, Ubiquitin-specific-processing protease 7; MDM2, mouse double minute 2 homolog; NASH, non-alcoholic steatohepatitis; PP2A, protein phosphatase 2A; Spp1, secreted phosphoprotein 1; DUSP1, dual-specificity phosphatase 1; SKP2, S-phase kinase-associated protein 2; CSK1, CDC28 protein kinase b1; FOXM1, forkhead box M1B; HIF-1α, hypoxia-inducible factor 1, alpha subunit; MAFK, V-MAF avian musculoaponeurotic fibrosarcoma oncogene family, protein K; PRMT5, protein arginine methyltransferase 5; JUN, V-JUN avian sarcoma virus 17 oncogene homolog; PIAS1, protein inhibitor of activated STAT1; Mtap, 5′-Methylthioadenosine phosphorylase; HCCB, HCC with better prognosis; HCCP, HCC with poorer prognosis; SL, surrounding liver; ASO, antisense oligonucleotide; Sdc, SAM dacarboxylase; Smr, spermine synthase; Sms, spermidine synthase; PCNA, proliferating cell nuclear antigen.

Introduction

Hepatocellular carcinoma (HCC) is a frequent and fatal human cancer, with 0.25–1 million newly diagnosed cases each year [1–3]. Major risk factors associated with HCC are chronic HBV and HCV infections, alcoholic steatohepatitis (ASH), aflatoxin B1, and some inherited metabolic disorders [2–4]. HCC
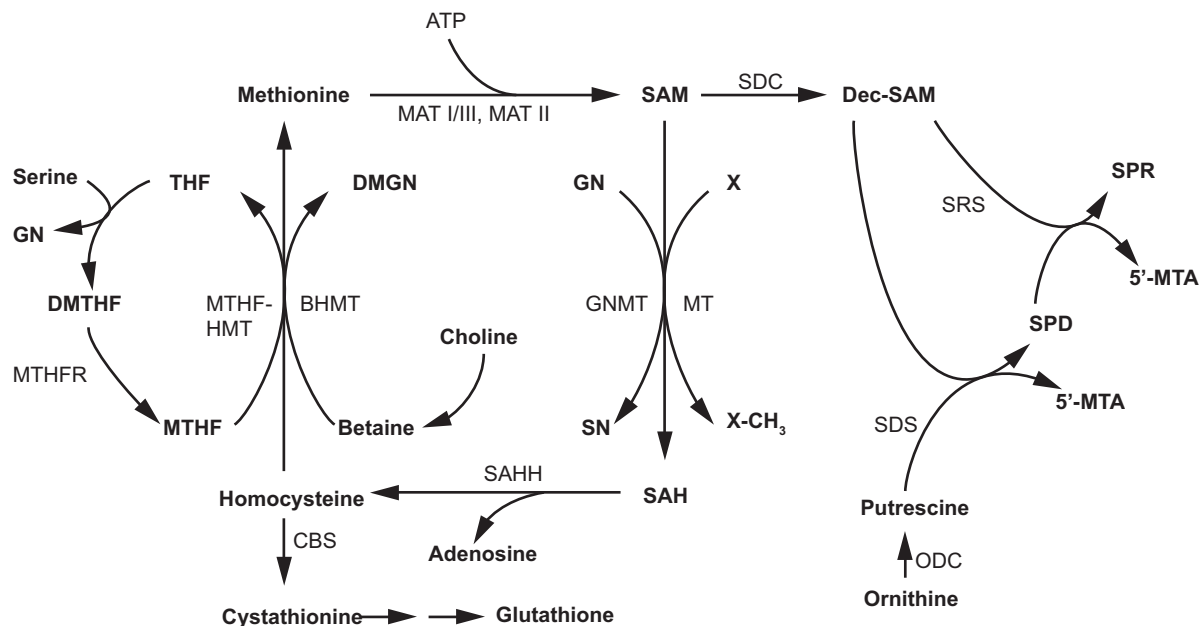
Fig. 1. Methionine metabolism. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; THF, tetrahydrofolate; MTHF, methyl-THF; DMTHF, dimethyl-THF; GN, glycine; DMGN, dimethyl GN; GNMT, glycine N-methyltransferase; SN, sarcosine; Dec-SAM, decarboxylated SAM; SPR, spermine; SPD, spermidine; 5′-MTA, 5′-methylthioedenosine; MAT, methionine adenosyltransferase; MT, methyltransferase; SAHH, SAH hydrolase; CBS, cystathionine beta-synthase; BHMT, betaine-homocysteine methyltransferase; MTHF-HMT, 5-methyltetrahydrofolate homocysteine methyltransferase; MTHFR, methyltetrahydrofolate reductase.; SDC, SAM decarboxylase; SRS, spermine synthase; SDS, spermidine synthase; ODC, ornithine decarboxylase.

incidence exhibits differences related to age, gender, ethnic group, and geographic region [3–5], and shows differences within the human population exposed to risk factors [6], suggesting a pathogenetic role of environmental and/or genetic factors [7–9].

Complex relationships between genetic, etiologic, and environmental risk factors create genotypic and phenotypic heterogeneity within human HCC [2,9]. Consequently, evaluation of pathogenetic mechanisms and identification of prognostic subtypes of HCC are difficult. A valuable contribution to explore HCC pathogenesis is provided by rodent models in which premalignant and malignant lesions exhibit low heterogeneity, without disturbing environmental influences. [10,11]. Studies performed in HCC differently prone to progression, induced in transgenic mice, rodent strains with different susceptibility to hepatocarcinogenesis, and human HCC subtypes, contributed to knowledge of signaling pathways deregulation during hepatocarcinogenesis [12].

Previous observations that ethionine, an antagonist of methionine, causes cancer [13] and methyl-deficient diets (MDDs) [14–16] cause steatohepatitis, followed by HCC development even in absence of carcinogens administration, encouraged studies on mechanisms regulating availability of the major methyl donor S-adenosylmethionine (SAM) and its role in liver injury, including hepatocarcinogenesis. This review provides an interpretive analysis of recent advances on deregulation of SAM metabolism in liver injury predisposing to liver cancer and determining HCC prognosis. We explore the molecular mechanisms involved in SAM antitumor effect and their contribution to identify new putative prognostic markers and opportunities for targeted therapies.

Metabolism of S-adenosylmethionine

Liver is the main source of SAM, synthesized from methionine and ATP in a reaction catalyzed by methionine adenosyltransferases (MATs) [17] (Fig. 1). SAM may be decarboxylated and then channeled into polyamine synthesis, or converted to S-adenosyl-homocysteine (SAH) during transmethylation reactions. A reversible reaction catalyzed by SAHH converts SAH to homocysteine and adenosine. Homocysteine may be channeled into the transsulfuration pathway leading to cystathionine and GSH synthesis. Alternatively, BHMT catalyzes methionine and dimethylglicine synthesis from homocysteine plus betaine. Homocysteine plus 5-methyltetrahydrofolate leads to methionine and tetrahydrofolate synthesis in a reaction catalyzed by MTHF-HMT. SAH and 5′-MTA, a product of polyamine biosynthesis, may inhibit transmethylation reactions. Interestingly, low SAM levels favor homocysteine re-methylation, whereas high SAM levels activate CBS, whose Km for SAM is 1.2–2 mM, much higher than that of MTHF-HMT (60 μM).

Liver-specific *MAT1A* encodes for the isozymes MATI and MATIII, tetramer and dimer of the subunit α1, respectively [18] (Fig. 1). *MAT2A* encodes for a α2-subunit, the widely distributed enzyme MATII isoform. *MAT2A* expression prevails in fetal liver and is substituted by *MAT1A* in adult liver [18,19]. MATI and MATIII isozymes have intermediate (23 μM–1 mM) and high (215 μM–7 mM) Km for methionine, respectively. Thus, physiological liver SAM level (∼60 μM) has low inhibitory effect on MATI and stimulates MATIII activity [18,19]. MATII has the lowest Km (∼4–10 μM) and may be inhibited by the reaction product [18]. A third gene, *MAT2B*, encodes for a β-subunit without catalytic action, which regulates MATII by lowering its Km for

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methionine and Ki for SAM [19]. Therefore, β association with the α-subunit renders MATII more susceptible to inhibition by SAM [19].

Noticeably, the recent discovery of correlations between GNMT, the main enzyme involved in hepatic SAM catabolism (Fig. 1), and MAT1A, and GNMT and BHMT hepatic proteins, supports a coordinate regulation of methionine cycle enzymes as SAM level determinants [20].

Effects of variations of SAM:SAH ratio

Treatment of rats with MDD causes pronounced liver SAM decrease and reduced SAM:SAH ratio [21], lipid peroxidation [15], and fall in phosphatidycholine synthesis because of choline lack and decrease in phosphatidylethanolamine transmethylation. Consequent reduction of lipoprotein assembly and synthesis of membranes involved in lipoprotein secretion leads to steatohepatitis [22]. Lipid peroxidation and SAM decrease also contribute to steatohepatitis by affecting mitochondrial function necessary for fatty acids oxidation. SAM contributes to the stability of PHB1 [23], crucial for maintenance of normal mitochondrial function. SAM pathogenetic role in steatohepatitis is confirmed by the observation that SAM treatment of hepatocytes isolated from MDD-fed rats induces phosphatidycholine synthesis, VLDL and LDL secretion, and decrease in cytoplasmic triacylglycerol [24].

Changes in SAM levels are also involved in ASH pathogenesis. In the pre-fibrotic stage of alcoholic rat liver injury, increase of MATII activity, without change of MATI/III activity, is associated with low SAM/SAH ratio, global DNA hypomethylation, c-Myc upregulation, and DNA strand break [25]. SAM administration protects from ASH [26–28]. Studies on human ASH were inconclusive [29], but long-term treatment with SAM improves survival or delays liver transplantation in patients with alcoholic liver cirrhosis, especially in those with less advanced disease [30]. As concerns chronic hepatitis C, SAM addition with/without betaine to standard therapy with PegIFNa and ribavirin enhances treatment efficacy [31,32]. Further, a biological basis for HCC prevention by SAM in hepatitis B is proposed by the observation that HBx upregulates MAT2A and MAT2β, and reduces MAT1A expression and SAM production in hepatoma cells in vitro [33]. Moreover, parenterally administered SAM protects rodents against D-galactosamine [34], acetaminophen [35], and CCl4 [36,37] toxicity.

Different researches showed low SAM/SAH ratio, global DNA hypomethylation, and c-Myc overexpression 0.5 h after partial hepatectomy (PH) in rats fed adequate diet [38,39]. These changes reached a peak at 5–12 h and then progressively returned to pre-PH levels. Maximum c-Ha-Ras and c-Ki-Ras expression occurred 24–30 h after PH, roughly coincident with DNA synthesis peak [38]. Notably, significant decrease in SAM level and SAM/SAH ratio also occurs in the liver of rats fed adequate diet, during hepatocarcinogenesis induced by different carcinogens and experimental models [40–43], and persists in dysplastic nodules (DN) and HCC several weeks after arresting carcinogen administration [38,43,45]. Furthermore, SAM decrease, with no change in SAH, occurs in human HCC and at a lower extent in the cirrhotic liver surrounding tumor [46].

Normal SAM level and SAM/SAH ratio may be reconstituted by the administration of highly purified SAM during hepatocarcino-genesis [42,44,45]. This treatment results in sharp decrease of preoplastic liver lesions and prevention of DN and HCC development, associated with decrease in labeling index and increase in apoptosis of preneoplastic cells [42,44,45,47]. In diethylnitros-amine-initiated rats, SAM persistently prevents development of preneoplastic lesions promoted by thiobenzamide [43]. Moreover, human HCC cell lines transfected with MAT1A or cultured in the presence of SAM undergo strong proliferation restraint [48,49]. These observations were recently confirmed by Lu et al. [50], which induced orthotropic HCC development by injecting human HCC cell line, H4IIE, in the rat liver parenchyma. Continuous SAM intravenous infusion after tumor cell injection inhibited HCC formation. However, SAM infusion for 24 days did not affect the size of already established tumors. This was explained by a compensatory induction of hepatic GNMT that prevents SAM accumulation. HuH7 cell transfectants, stably overexpressing MAT1A, exhibited higher SAM levels and lower DNA synthesis than control cells [51]. Lower HCC growth rates, microvessel density, and CD31 and Ki-67 staining, and higher apoptosis occurred in MAT1A-transfected than in control tumors [51].

Fall in MAT1A expression associated with MAT2A upregulation occurs in liver cirrhosis and rodent and human HCC, leading to decrease in MAT1A:MAT2A ratio (called MAT1A/MAT2A switch) [46,52,53]. MATI/III downregulation, secondary to oxidation of cysteine residue in ATP binding site, and GSH fall occur in the cirrhotic liver [54,55]. SAM administration reconstitutes the GSH pool, protects MATI/III [54,55], and has beneficial effects against liver fibrosis both in rats and humans [37,54,55,36]. Being inhibited by its reaction product, MATII upregulation cannot compensate for MATI/III decrease [56]. Consequently, decrease in MATI/III:MATII activity ratio strongly contributes, together with the increase in SAM decarboxylation for polyamine synthesis, to sharp SAM decrease [41]. Overall, these important findings suggest that MAT1A/MAT2A switch and fall in SAM level are strongly involved in hepatocarcinogenesis. Accordingly, the MAT1A-KO mouse model, characterized by chronic SAM deficiency, even in the presence of MAT2A induction, exhibits hepatomegaly without histologic abnormalities at 3 months of age, and macrovesicular steatosis involving 25–50% of hepatocytes and mononuclear cell infiltration in periportal areas, at 8 months [57]. HCC develop in many of these mice at 18 months of age [57].

Remarkably, recent findings showed oxidative stress, steatosis, and fibrosis, followed by HCC development [58], and increased susceptibility to aflatoxin B1-related HCC [59] in GNMT-KO mice, characterized by elevated SAM liver levels. Global DNA hypomethylation, aberrant expression of DNA methyltransferases 1 and 3b [60], aberrant hypermethylation of inhibitors of Ras and JAK/STAT pathways [57], and upregulation of Beta-catenin, cyclin D1, and c-Myc [61] occur in these mice during HCC development. Furthermore, Ras-mediated LKB1 overactivation, associated with Erk, p90Rsk, and RasGpr3 expression, promotes the proliferation of GNMT-deficient hepatoma cells [60]. Interestingly, impairment of liver regeneration in GNMT-KO mice stimulates dormant stem/progenitor cells to replicate, a situation that could favor HCC formation [62]. High liver transaminases, liver injury, fibrosis, and HCC development have been documented in children with GNMT mutation [63].

MAT2A upregulation may also contribute to HCC cell proliferation. In H35 hepatocellular carcinoma cells, MAPK and PI3K/AKT pathways are necessary for HGF-induced cell proliferation and MAT2A upregulation [64]. Inhibition of these pathways in H35

cells and fetal liver hepatocytes leads to proliferation restraint, *MAT2A* under-regulation and *MAT1A* overexpression [64]. Moreover, transfection of *MAT2B* in HuH7 cells that do not express this subunit results in β-subunit interaction with α2-subunit, DNA synthesis increase, and SAM production decrease, whereas β-subunit downregulation in HepG2 cells, overexpressing*MAT2B*, diminishes DNA synthesis [65].

Molecular mechanisms underlying the deregulation of MAT genes

The presence of numerous CpGs *MAT1A* and *MAT2A* promoters motivated the evaluation of the epigenetic regulation of their expression in HCC [65–67]. *MAT1A* downregulation in the cirrhotic liver of CCl₄-treated rats and human HepG2 cell line is associated with CCGG sequences methylation in *MAT1A* promoter [66]. In HuH7 cells, *MAT1A* downregulation was attributed to CCGG methylation at +10 and +80 of the coding region [67]. *MAT2A* upregulation in human HCC was associated with CCGG hypomethylation of the gene promoter [68]. Recent work [48] in which the methylation status of all CpGs of *MAT1A* and *MAT2A* promoters was examined in rat and human HCC, confirmed these results and showed *Mat1A/Mat2A* switch and low SAM levels, associated with CpG hypermethylation and histone H4 deacetylation in *Mat1A* promoter, and prevalent CpG hypomethylation and histone H4 acetylation in *Mat2A* promoter of fast growing F344 rats HCC. In slowly growing BN rat HCC, very low changes in *Mat1A:Mat2A* ratio, CpG methylation, and histone H4 acetylation occurred [48]. Furthermore, highest *MAT1A* promoter hypermethylation and *MAT2A* promoter hypomethylation occurred in human HCC with poorer prognosis [48].

Various trans-activating factors such as Sp1, c-Mybl2, NF-kB, and AP-1 participate in *MAT2A* transcriptional upregulation in HCC [18]. The mechanisms regulating *MAT2B* expression are poorly known. Sp1 activates *MAT2B* promoter [19]. *MAT2B* has two dominant splicing variants, variant 1 (V1) and variant 2 (V2), upregulated in HCC. TNFα induces the transcription of only *MAT2B* V1 by mechanisms involving AP-1 and NF-kB [18]. *MAT2B* V1 promoter expression is stimulated by leptin and inhibited by SAM by mechanisms involving ERK and AKT signaling [18].

Accumulating evidence indicates that a class of mRNA-binding proteins (RBPs) plays a pivotal role in post-transcriptional deregulation of gene expression in cancer cells. Among RBPs, AUF1 enhances mRNA decay, whereas HuR selectively binds to AUrich elements promoting mRNA stabilization [69–71]. Remarkably, a recent work [71] showed *Mat1A* mRNA decrease in the fetal rat liver, associated with an increase in its interaction with AUF1 and an increase in *Mat2A* mRNA and its interaction with HuR [71] (Supplementary Fig. 1). Immunofluorescence analysis revealed increased HuR and AUF1 protein levels in human livers with HCC suggesting post-transcriptional regulation of MAT proteins in HCC levels of AUF1 [71]. Based on these findings, we recently demonstrated a sharp increase of AUF1 and HuR in F344 and human HCC associated with a consistent increase in MAT1A-AUF1 and MAT2A-HuR ribonucleoproteins in both HCC types [48]. Interestingly, these changes were very low or absent in slowly progressing HCC of BN rats.

Recent observations attribute reduced *MAT1A* expression to miRNAs upregulation in HCC [72]. Knockdown of miR-664, miR-485-3p, and miR-495 individually in Hep3B and HepG2 cells, induces *MAT1A* expression. Hep3B cells tumorigenesis in nude mice is decreased by stable overexpression and increased by knockdown of miRNAs-664/485-3p/495 [72], suggesting that upregulation of these miRNAs contributes to hepatocarcinogenesis by lowering *MAT1A* expression.

These observations indicate that both transcriptional and post-transcriptional mechanisms contribute to *MAT1A/MAT2A* switch and SAM decrease during hepatocarcinogenesis. Moreover, they suggest that *MAT1A/MAT2A* switch and SAM reduction may have a prognostic value for hepatocarcinogenesis.

Mechanisms of SAM anti-tumor effect

It is widely accepted [73–75] that interaction of DNA with carcinogens and reactive oxygen and nitrogen species, generated during carcinogen metabolism and/or inflammation accompanying early stages of hepatocarcinogenesis, results in genomic instability (GI), leading to somatic point mutations, copy number alterations of individual genes, and gain/loss of chromosomal arms. Several lines of evidence indicate that progressive accumulation of genomic alterations, leading to signaling pathways deregulation, allows initiated cells to evolve to DN and HCC [73–75]. The observation that SAM treatment maintains a high GSH pool, in CCl₄-intoxicated rats [36], suggests a possible chemopreventive role of the SAM antioxidative action. DNA protection from oxidative damage by antioxidants prevents tumor development in various organs, including the liver [76].

A sharp increase in polyamine synthesis may also favor fast proliferation of preneoplastic and neoplastic liver cells. Progressive upregulation of the *ODC* gene and rise in ODC activity and polyamine synthesis occur during rat hepatocarcinogenesis [40,77,78]. Upregulation of polyamine synthesis-related genes also occurs in human HCC [45]. SAM may interfere with polyamine synthesis by inhibiting ODC activity [40].

It should be noted that the effects of SAM on oxidative stress and polyamine synthesis could at least in part depend on accumulation of 5′-MTA [79] (Fig. 1), which can also arise from spontaneous splitting of SAM at physiologic temperature and pH [80]. 5′-MTA could undergo oxidation by microsomal mono-oxygenases or auto-oxidation, with formation of sulfoxide and sulfone derivatives, thus exerting a direct antioxidant effect [81]. 5′-MTA also inhibits CCl₄-induced liver fibrosis [36]. The possibility that ODC inhibition by SAM at least partially depends on its transformation into 5′-MTA is suggested by the observation that SAM preincubation in a cell-free system, in conditions leading to its partial transformation into 5′-MTA, is necessary for strong ODC inhibition to occur in preneoplastic hepatocytes *in vitro* [40]. 5′-MTA is inhibitory even in the absence of preincubation, and its effect is enhanced when its catabolism is blocked by adenine [40].

The possible attribution to 5′-MTA of SAM effects is intriguing, and has been the object of accurate analyses. Indeed, SAM was found to be a stronger inhibitor of DNA synthesis and rat hepatocarcinogenesis than 5′-MTA [41]. The observation that stable transfectants of HuH7 cells overexpressing *MAT1A* exhibit higher SAM levels and no change in 5′-MTA content, and are less tumorigenic *in vivo* than control cells [50], strongly supports an anti-tumorigenic effect of SAM independent of 5′-MTA. Furthermore, SAM deficiency during hepatocarcinogenesis is associated with global DNA hypomethylation [37] that is not reversed by

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5′-MTA, whereas SAM-induced inhibition of the development of preneoplastic foci in rat liver carcinogenesis is associated with complete recovery of DNA hypomethylation [41], and is prevented by the hypomethylating agent 5-azacytidine [82].

Global DNA hypomethylation induces GI during hepatocarcinogenesis [83]. AP sites represent the most frequent DNA lesions in cells [84]. Together with other DNA repair proteins, APEX1 participates in base excision repair [85]. Moreover, APEX1 is also involved in the regulation of gene expression as a redox co-activator of different transcription factors, such as EGR-1, p53, and AP-1 [86]. The induction of *APEX1* gene by ROS at the transcriptional level [87] is part of the defense mechanism against GI [88].

A recent work [89] showed increased GI in livers of 1-month-old *MAT1A*-KO mice, compared to wild type mice, whereas Apex1 mRNA and protein levels were reduced by 20% and 50%, respectively, in these mice of all ages. These changes were correlated with increase in AP sites and reduced expression of APEX1 targets *Bax*, *Fas*, and *p21* [89]. In cultured human and mouse hepatocytes, *MAT1A* mRNA decreased whereas *APEX1* and c-*MYC* mRNAs increased. However, APEX1 protein level decreased to 60% of baseline [89] (Supplementary Fig. 2). SAM prevented these changes in cultured hepatocytes, indicating that although SAM inhibits *APEX1* transcription, it stabilizes APEX1 protein [89] (Supplementary Fig. 2). This SAM effect on APEX1 regulation might contribute to SAM chemopreventive action and in part explains why chronic SAM deficiency predisposes to HCC.

The mechanism of APEX1 stabilization by SAM is not known. Recent reports envisage proteasome inhibition by SAM. Simultaneous overexpression of ubiquitin-9 and *APEX1* in HeLa cells dramatically lowers APEX1 protein, suggesting ubiquitin-9 is involved in APEX1 protein degradation [90]. SAM inhibits chymotrypsin-like and caspase-like activities in 26S proteasome and causes degradation of some of the 26S proteasomal subunits, which is blocked by the proteasome inhibitor MG132 [91]. Furthermore, SAM and 5′-MTA lower CDC2 expression, upregulated in several cancers, resulting in decreased ubiquitin-9 phosphorylation and expression [91].

Nitric oxide (NO) is a product of L-arginine to L-citrulline conversion by NOS. Calcium-independent, inducible iNOS is present in hepatocytes, Kupffer and stellate cells, and cholangiocytes, whereas calcium-dependent eNOS is present in endothelial cells [92]. NO may favor HCC development by inducing DNA mutations, in hepatocytes surviving to oxidative stress, and vasodilatation providing premalignant and malignant cells with sufficient metabolites and oxygen. Overproduction of inflammatory cytokines and growth factors during early stages of hepatocarcinogenesis deregulates iNOS [92]. Reactive nitrogen species produced via iNOS during chronic hepatitis may play a key role in carcinogenesis by causing DNA damage. iNOS suppression by aminoguanidine results in decreased HCC cell growth, NF-kB and RAS/ERK downregulation, and increased apoptosis *in vivo* and *in vitro* [93]. eNOS activation by AMPK during hepatocarcinogenesis may also contribute to NO production, which is in turn an endogenous AMPK activator [94], and lowers SAM level by inactivating MATI/III [95] (Fig. 2). On the other hands, survival of SAM-deficient cells in *MAT1A*-KO mice requires LKB1/AMPK activation. HGF is mitogenic for hepatocytes through LKB1/AMPK activation, which is blocked by SAM [95] (Fig. 2).

Recent observations indicate that LKB1/AMPK axis activation may contribute to hepatocarcinogenesis through other mechanisms. Consequent to AMPK activation in hepatocytes is nuclear
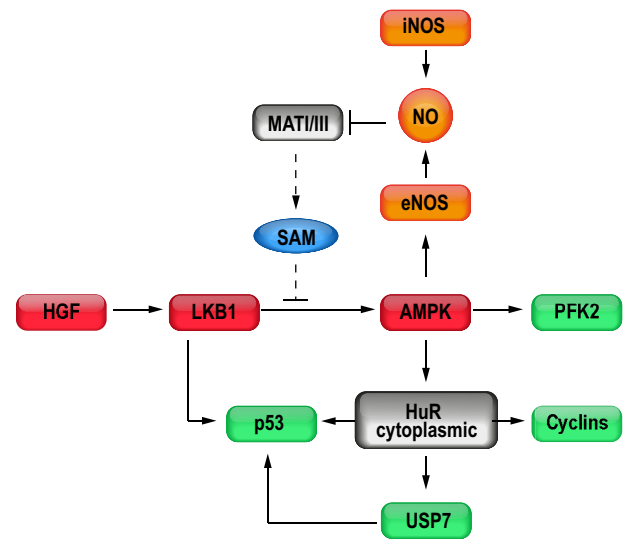

Fig. 2. Effect of SAM on HGF/LKB1/AMPK axis. The HGF/LKB1/AMPK axis enhances NO production via eNOS activation, upregulates the glycolytic key enzyme PFK-2, and induces the nuclear to cytoplasmic HuR translocation, resulting in stabilization of *cyclins*, *p53*, and *USP7* mRNAs. Hyperactive LKB1 induces p53 hyperphosphorylation. The interaction of phosphorylated p53 with USP7 blocks the negative regulation of p53 by MDM2. SAM inhibits LKB1. This effect is controlled by MATI/III inhibition operated by NO.

to cytoplasmic HuR translocation, resulting in cyclin mRNAs stabilization. Increased basal LKB1/AMPK axis leads to a rise in cytoplasmic HuR levels, cyclin D1 expression, and cell proliferation [96] (Fig. 2). Furthermore, AMPK upregulation can contribute to the glycolytic metabolism of cancer cells [97] through activation of PFK-2, a key enzyme for glycolysis [98].

LKB1 may also regulate AKT-mediated cell survival independently of PI3K, AMPK, and mTORC2 [99]. A critical role is played by the deubiquitinating enzyme USP7. USP7 contributes to the stability of MDM2, a negative p53 regulator, impairing its self-ubiquitination and degradation. In SAM-deficient hepatocytes, p53 is mostly cytosolic and hyperphosphorylated by several kinases, including hyperactive LKB1 [100]. p53 hyperphosphorylation and its interaction with USP7 block the negative regulation by MDM2. Furthermore, active LKB1-induced HuR cytosolic translocation, stabilizes *p53* and *USP7* mRNAs [96] (Fig. 2). Thus, LKB1 controls apoptotic response through phosphorylation and cytoplasmic retention of p53, regulation of the de-ubiquitination enzyme USP7, and HuR nucleo-cytoplasmic shuttling. Notably, cytoplasmic staining of p53 and p-LKB1 (Ser428) occurs in a NASH-HCC animal model (from *MAT1A*-KO mice) and in liver biopsies obtained from human HCC derived from both ASH and NASH [99]. These findings, however, contrast with the report of a loss of LKB1, identified as an oncosuppressor gene, in cancer cells, including HCC [100]. AMPK, activated by LKB1, inhibits AKT signaling turning off mTOR by activating the tumor oncosuppressor complex TSC2/TSC1 [101]. Moreover, AMPK α2 catalytic subunit downregulation is statistically associated with undifferentiated HCC and poor patient prognosis, and AMPK inactivation promotes hepatocarcinogenesis by destabilizing p53 in a p53 deacetylase (SIRTUIN 1)-dependent manner [102]. In complex, the effects of LKB1/AMPK signaling on HCC development are contradictory and probably a comparison between different
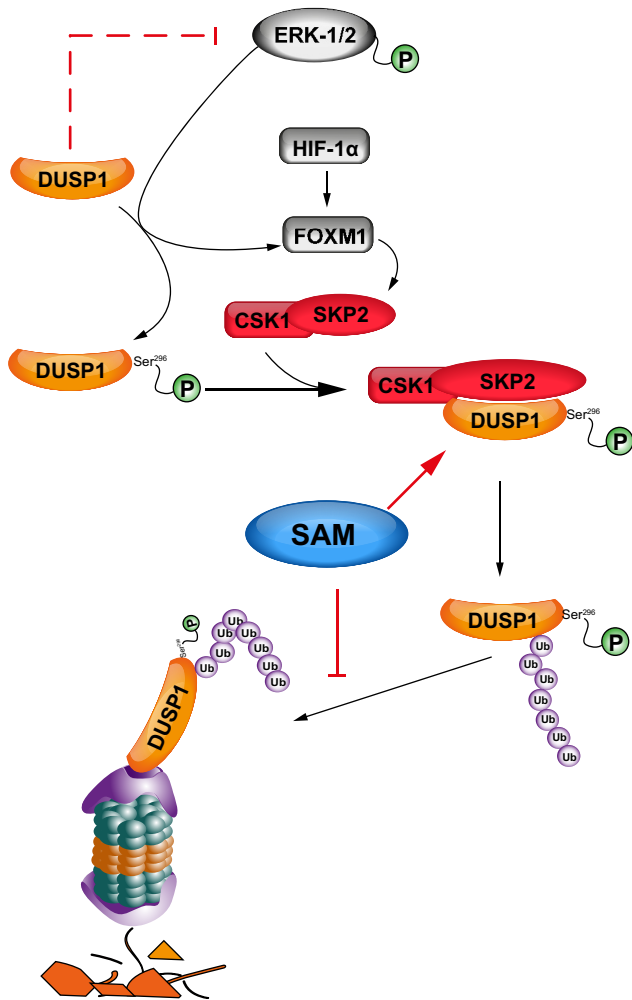
Fig. 3. Interference of SAM with ERK1/2 inhibition by DUSP1. The inhibition of ERK1/2 activity by DUSP1 is controlled by DUSP1 phosphorylation of Ser296 residue, followed by its ubiquitination by the SKP2–CKS1 ubiquitin ligase and proteasomal degradation, as well as by SKP2–CKS1 activation operated by FOXM1, a major target of ERK1/2 and HIF-1α. SAM enhances DUSP1 inhibitory effect by increasing *DUSP1* mRNA at transcriptional level, and by contributing to the increase in DUSP1 protein at post-translational levels, probably through inhibition of its proteasomal degradation.

SAM level can influence ERK1/2 activity by interfering with DUSP1, a specific ERK inhibitor (Fig. 3). DUSP1 downregulation and ERK1/2 upregulation occur in fast progressing DN and HCC of F344 rats and human HCC [108,109]. Conversely, active ERK1/2 phosphorylates the Ser296 residue of DUSP1, thus contributing to its ubiquitination by the SKP2-CKS1 ubiquitin ligase, followed by proteasomal degradation [108,109]. On the other hand, ERK1/2 sustains SKP2-CKS1 activity through its target FOXM1 [110] (Fig. 3). Notably, *DUSP1* mRNA and protein levels are markedly reduced in livers of MAT1A-KO mice and in cultured mouse and human hepatocytes, with protein decreasing to lower levels than mRNA [111]. SAM treatment protects against the fall in DUSP1 mRNA and protein in cultured mouse and human hepatocytes, and SAM administration to *MAT1A*-KO mice results in increase in SAM and Dusp1 mRNA and protein levels, and decrease in Erk activity [111]. These observations show a control of MAPK by SAM. SAM treatment increases *DUSP1* mRNA at transcriptional level, and contributes to increase in DUSP1 protein at post-translational levels, probably through inhibition of its proteasomal degradation [111] (Fig. 3). Interestingly, TNF-α/HIF-1α axis sustains the expression of FOXM1 [112], which mediates ERK1/2 effects on cell cycle, cell survival, and angiogenesis [110]. Hypoxia could contribute to ERK1/2 upregulation by reducing SAM level of HCC cells through HIF-1α binding to *MAT2A* promoter [113]. These findings support a suppressive effect of SAM on malignant transformation through ERK1/2 inhibition.

Changes of MATs expression may affect cancer cell growth by interfering with protein methylation. A recent study [114] identified two partially overlapping areas at the C-terminal end of the protein involved in cytoplasmic retention and nuclear localization of MATI/III in most rat tissues. Nuclear accumulation of the active enzyme was correlated with histone H3K27 trimethylation, an epigenetic modification associated with DNA methylation, therefore pointing to the need of MATI/III to guarantee SAM supply for specific methylations and, eventually, additional roles. Interestingly, MATIIα also provides SAM locally on chromatin by interacting with chromatin-related proteins involved in histone modification, chromatin remodeling, transcription regulation, and nucleo-cytoplasmic transport [115,116]. This mechanism can regulate MAFK, a member of MAF oncoproteins, which interacts with both MATIIα and MATIIβ [114,115]. MAFK functions as transcription activator and repressor by forming diverse heterodimers to bind to MAF recognition elements of DNA [115,117]. However, the oncogenic role of MAFK and its targets in HCC is unknown. Moreover, ERK1/2 activation, elicited by particular growth factors in different cell lines including HCC cells, may be limited by arginine methylation of RAF proteins by PRMT5 [117]. Expression of RAF mutants that cannot be methylated affects the amplitude and duration of ERK activation by growth factors [117]. However, PRMT5 accelerates cell cycle progression through the G1 phase, activates PI3K/AKT and suppresses JNK/c-Jun signaling in lung cancer [118]. Apparent discrepancies could depend on PRMT5 localization. PRMT5 and p44/MED50/WD45/WDR77 cytoplasmic co-localization is required for prostate cancer cell growth. In contrast, nuclear PRMT5, present in benign prostate epithelium, inhibits cell growth in a methyltransferase activity-independent manner [119].

Finally, HCV protein impairs JAK-STAT signaling by inhibiting STAT1 methylation, which favors STAT1 binding by its inhibitor PIAS1 [120]. Remarkably, SAM and betaine restore STAT1 methylation and improve IFNalpha antiviral effect in cell culture [120].

experimental models and human HCC subtypes may contribute to their complete understanding.

Other mechanisms of SAM antitumor effects have been envisaged. Forced expression of *MAT1A* in HCC cell lines results in downregulation of *cyclin D1*, *E2F1*, *IKK*, *NF-kB*, and antiapoptotic *BCL2* and *XIAP* genes, and upregulation of proapoptotic *BAK* and *BAX* genes [48]. SAM counteracts NF-kB activation in rat preneoplastic foci [103] and upregulates the oncosuppressor PP2A that dephosphorylates and inactivates AMPK, pAKT, and pERK [104,105]. The lowest SAM levels and PP2A expression occur in both rat and human HCC exhibiting the highest pAKT and pERK expression and proliferation rates [[45,48,106] and Frau *et al.*, unpublished results]. ERK and PI3K pathways may be also activated by binding of SPP1 (osteoponin) to integrin receptors in cancer [107]. Reduction of SPP1 expression in *MAT1A* transfected tumors [50] may contribute to ERK and PI3K downregulation.
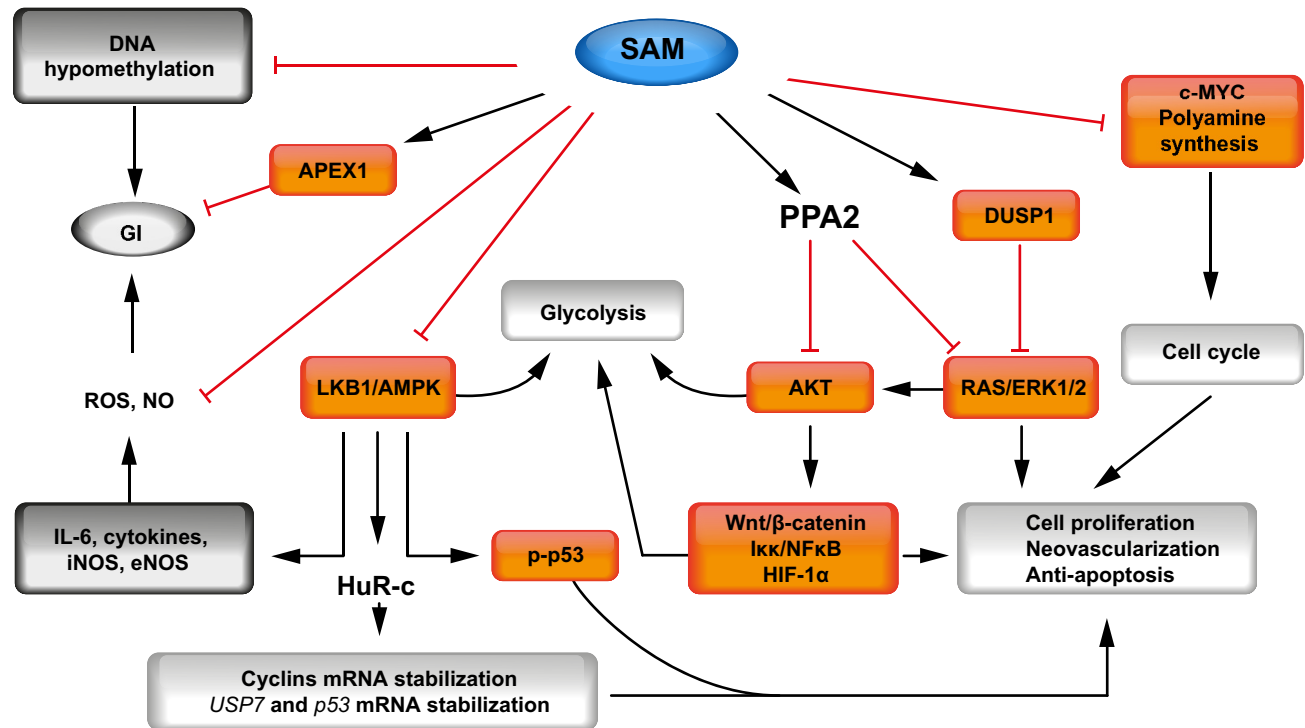
Fig. 4. Pleiotropic effects of SAM treatment during hepatocarcinogenesis. SAM capacity to methylate DNA and stabilize the DNA repair enzyme APEX1, and SAM antioxidant activity reduce genomic instability (GI). The inhibition by SAM of LKB1/AMPK axis increases cytoplasmic concentration of HuR, which stabilizes *p53* and *USP7* mRNAs. Activation of LKB1 leads to p53 hyperphosphorylation and its interaction with USP7 with consequent block of the negative regulation of p53 by MDM2. Thus, LKB1 controls apoptotic response through phosphorylation and retention of p53 in the cytoplasm, regulation of the de-ubiquitination enzyme USP7, and HuR nucleo-cytoplasmic shuttling. SAM also controls cell growth and survival by inducing PPA2 expression that phosphorylates and inactivates AKT and its targets. Moreover, PPA2 activation and DUSP1 stabilization inhibit RAS/ERK pathway. Finally, SAM affects cell cycle by inhibiting c-MYC expression and polyamine synthesis.

Changes in methionine metabolism and HCC prognosis

Increasing evidence indicates that the deregulation of various signaling pathways progressively increases with HCC progression and has a prognostic value [98]. The comparative analysis of c-Myc and c-Myc/TGFα transgenic mice and of genetically susceptible F344 and genetically resistant BN rats recapitulates the main pathogenetic mechanisms of human HCC, with c-Myc and BN tumors approaching human HCC characterized by better prognosis, and c-Myc/TGFα and F344 HCCs resembling those with shorter survival [98,106,121,122].

Decrease in MatI/III:MatII activity ratio occurs in c-Myc and c-Myc/TGFα transgenics, with the lowest values in c-Myc transgenics [45]. *Sahh* gene expression increases in HCC of c-Myc transgenics and in DN and HCC of the double transgenics, suggesting a relatively high production of homocysteine, presumably not associated with rise in GSH because of decreased *Cbs* levels in tissues of both transgenic models [45] (Fig. 1). *Bhmt* expression decreases in dysplastic and neoplastic liver of both transgenic lines, whereas *Mthf-hmt* expression does not change in c-Myc lesions, showing a sharp increase in HCC of double transgenics [45]. As concerns polyamine synthesis, progressive increase in *Sdc*, *Odc*, *Smr*, and *Sms* mRNAs occurs in dysplastic and neoplastic lesions of both transgenic models, with the highest levels in the lesions of c-Myc/Tgf-a transgenics (Fig. 1). Finally, the expression of *Mtap1*, encoding a key enzyme for methionine re-synthesis through the salvage pathway [123], increases only in the lesions of double transgenics [45].

Similar results were found in HCC with better prognosis (survival >3 years after partial liver resection; HCCB) and poorer prognosis (survival <3 years; HCCP) and their corresponding surrounding liver (SL) [45]. In these lesions, MATI:MATII ratio progressively decreases from SL to HCC with the lowest values in HCCP, reflecting the changes in *MAT1A:MAT2A* expression ratio. A slight increase or no change in *SAHH* expression and a marked decrease of *CBS* mRNA occur in human HCCs with better and poorer prognosis and their corresponding SL, with respect to the control liver. As concerns the genes encoding key enzymes of methionine synthesis, *BHMT* expression decreases in liver lesions of all subgroups, *MTHF-HMT* does not change and *MTAP1* decreases in HCCP. Finally, levels of *SDC*, *ODC*, *SMR*, and *SMS* progressively increase from SL to HCC, with highest values in HCCP. These observations indicate the association of *MAT1A/MAT2A* switch, decrease in methionine resynthesis, and increase in SAM utilization for polyamine synthesis with HCC progression. Accordingly, cell proliferation rate of transgenic mice and human HCC are positively correlated with global DNA hypomethylation and GI [45]. These observations suggest that deregulated methionine metabolism and MATI/III:MATII ratio are implicated in HCC progression and prognosis.

In support of the above suggestion, a recent work [48] showed that MATI/III:MATII ratio and *Mat1A:Mat2A* expression ratio are negatively correlated with DNA synthesis and DNA methylation and positively correlated with apoptosis of F334 and BN rat liver lesions. Furthermore, the analysis of human HCCs showed a progressive decrease in MAT1A expression and MATI/III activity and

progressive increase in MAT2A expression and MATII activity from SL to HCC. These changes were paralleled by progressive increase in proliferation rate, and decrease in DNA methylation and apoptosis from SL to HCC. Correlation analysis revealed an inverse correlation of *MAT1A:MAT2A* and MATI/III:MATII ratios with PCNA expression and GI, and a direct correlation with apoptosis and DNA methylation. Correlation experiments clearly indicated the presence of two patient subgroups with significantly different MATI/III:MATII ratio and survival length, with the HCC subset showing the lower MATI/III:MATII ratio being associated with shorter survival, and Cox analysis showed that MATI/III:MATII ratio significantly predicts patient survival length. In the multivariate analysis model, MATI/III:MATII ratio remained significantly associated with overall survival, together with patients' age, tumor etiology and grade, PCNA expression, DNA methylation, and GI.

duction of oxygen reactive and nitroactive species, and activation of LKB1/AMPK axis, which may induce GI; (b) cell cycle activation following upregulation of c-*MYC* and genes involved in polyamine synthesis; (c) RAS/ERK, IKK/NF-kb, PI3K/AKT, and NF-kB signaling upregulation, leading to increase in cell proliferation, cell survival, and microvascularization.

Most of these changes were discovered in rodent models [37,41,78,89,99,105]. Previous research on comparative functional genomics to evaluate rodent models for human liver cancer [106,121,122] and other cancers [124,125] indicated that molecular pathways associated with specific cancer phenotypes are evolutionarily conserved [126]. Accordingly, the progression of both human and rodent HCC prognostic subtypes is correlated with upregulation of MAPK, IKK/NF-kB, JAK/STAT, WNT/FZD, and PI3K/AKT pathways, and cell cycle key genes, downregulation of cell cycle inhibitors [98], and decrease in *MAT1A*

Key Points

- The deficiency of labile methyl groups results in pronounced liver SAM decrease, without significant changes in SAH content, decrease in SAM:SAH ratio, lipid peroxidation and fall in phosphatidycholine synthesis, impaired mitochondrial function necessary for fatty acids oxidation, and steatohepatitis followed by HCC development. Decrease in SAM level and SAM/SAH ratio also occurs, in liver of rats fed adequate diet, during hepatocarcinogenesis induced by different carcinogens and experimental models, and in dysplastic nodules and HCC of rats and humans

- SAM is synthesized by the liver-specific MATI/III and the ubiquitous MATII encoded by *MAT1A* and *MAT2A* genes, respectively. Decrease in *MAT1A* expression and increase in *MAT2A* expression, with consequent fall in *MAT1A:MAT2A* expression ratio (*MAT1A*/*MAT2A* switch), occurs in rodent HCC and human liver cirrhosis and HCC. Being inhibited by its reaction product, MATII isoform upregulation cannot compensate for MATI/III isozyme decrease. Decrease in MATI/III:MATII activity ratio strongly contributes to the sharp decrease in SAM level and hepatocarcinogenesis. Accordingly, SAM administration to carcinogen-treated rats strongly prevents hepatocarcinogenesis. *MAT1A*-KO mice, characterized by chronic SAM deficiency, exhibit macrovesicular steatosis and mononuclear cell infiltration in periportal areas, at 8 months, and HCC development, in many mice, at 18 months of age

- *Mat1A*/*Mat2A* switch and low SAM levels are under epigenetic control at transcriptional and post-transcriptional levels. CpG hypermethylation and histone H4 deacetylation of *Mat1A* promoter, and prevalent CpG hypomethylation and histone

H4 acetylation in *Mat2A* promoter occur in fast-growing HCC of F344 rats, genetically susceptible to hepatocarcinogenesis. In slow-growing HCC of genetically resistant BN rats, changes in the *Mat1A:Mat2A* ratio, CpG methylation, and histone H4 acetylation are very low. Highest *MAT1A* promoter hypermethylation and *MAT2A* promoter hypomethylation occur in human HCC with poorer prognosis. Furthermore, the levels of AUF1 protein, which destabilizes *MAT1A* mRNA, *MAT1A*-AUF1 ribonucleoprotein, HuR protein, which stabilizes *MAT2A* mRNA, and *Mat2A*-HuR ribonucleoprotein sharply increase in F344 and human HCC, and undergo low/no increase in BN HCC

- In HCC cells, *MAT1A*/*MAT2A* switch is associated with global DNA hypomethylation, decrease in DNA repair, genomic instability, and signaling upregulation including c-MYC overexpression, rise in polyamine synthesis, upregulation of RAS/ERK, IKK/NF-kB, PI3K/AKT, and NF-kB patways, and of LKB1/AMPK axis. Furthermore, decrease in *MAT1A* expression and SAM levels is associated with increase in HCC cell proliferation, cell survival, and microvascularization. All of these changes are reversed by SAM treatment *in vivo* or forced *MAT1A* overexpression or *MAT2A* inhibition in cultured HCC cells

- *MAT1A:MAT2A* expression ratio is a putative prognostic marker for human HCC. Indeed, in human HCC, *MAT1A:MAT2A* and MATI/III:MATII ratios correlate negatively with cell proliferation and genomic instability, and positively with apoptosis and global DNA methylation. Moreover, MATI/III:MATII ratio strongly predicts patients' survival length

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Conclusions and future perspectives

Pleiotropic effects on signal transduction (Fig. 4), associated with decrease in *MAT1A* expression and SAM levels, favoring hepatocarcinogenesis, include: (a) global DNA hypomethylation, pro-

expression and SAM levels [45,48]. Some of these changes, including *MAT1A*/*MAT2A* switch, are putative prognostic markers of HCC [48].

The interference of changes in *MAT1A* expression and SAM level with several pathways, during hepatocarcinogenesis, opens

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new therapeutic perspectives. SAM chemopreventive effect for experimental hepatocarcinogenesis has been well documented [41–44,49,127]. In humans, according to recent observations [29,127–129], HCC could be prevented by a SAM curative effect on ASH and hepatitis C. An ongoing phase II clinical trial is evaluating SAM as a potential chemopreventive agent in hepatitis C and cirrhosis [129].

No evidence of SAM therapeutic effect against HCC is available [49]. Preliminary approaches should test the effects of stable *MAT1A* overexpression or *MAT2A/MAT2B* inhibition in experimental HCC *in vivo*. Silencing of *MAT2A* or *MAT2B* in HepG2 cells inhibits proliferative response to leptin [130]. However, intracellular transduction of viral vectors *in vivo* still presents numerous limitations [131]. In this context, the therapeutic effect for HCC of a family of fluorinated N,N-dialkylaminostilbene agents, which inhibits colorectal cancer cells proliferation *in vitro* and *in vivo* [132], should be texted. These molecules bind to MATIIα catalytic subunit and inhibit SAM synthesis. They also inhibit WNT/β-catenin signaling [132], therefore, they could be particularly effective against β-catenin mutated HCC. Furthermore, a recent observation of *MAT1A* downregulation by miRNAs-664/485-3p/495 overexpression in HCC [72], suggests a therapeutic effect of specific anti-miRNAs oligonucleotides. Liver vessels allow entering molecules up to 200 nm in diameter, including antisense oligonucleotides (ASOs) inhibiting or decoying miRNAs. ASOs delivery using pegylated liposomes, biodegradable polymers, lipid nanoparticles is an alternative. However, novel modification, conjugation or formulation strategies may improve effective and safe delivery of anti-miRNAs oligonucleotides. Nonetheless, different miRNAs inhibitors are in preclinical studies, 15 nt ASOs are in clinical trials and 8 nt versions show promise in non-human primates [133,134].

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Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2013.04.031.

References

[1] Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology 2008;48:1312–1327.

[2] Feo F, Pascale RM, Simile MM, De Miglio MR, Muroni MR, Calvisi DF. Genetic alterations in liver carcinogenesis. Crit Rev Oncog 2000;11:19–62.

[3] Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology 2004;127:S5–S16.

[4] McGlynn KA, London WT. Epidemiology and natural history of hepatocellular carcinoma. Best Pract Res Clin Gastroenterol 2005;19:3–23.

[5] El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004;127:S27–S34.

[6] Indulski JA, Lutz W. Metabolic genotype in relation to individual susceptibility to environmental carcinogens. Int Arch Occup Environ Health 2000;73:71–85.

[7] Shih WL, Yu MW, Chen PJ, Wu TW, Lin CL, Liu CJ, et al. Evidence for association with hepatocellular carcinoma at the PAPSS1 locus on chromosome 4q25 in a family-based study. Eur J Hum Genet 2009;1:1250–1259.

[8] Kumar V, Kato K, Urabe Y, Takahashi A, Muroyama R, Hosono N, et al. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. Nat Genet 2001;43:455–458.

[9] Feo F, De Miglio MR, Simile MM, Muroni MR, Calvisi DF, Frau M, et al. Hepatocellular carcinoma as a complex polygenic disease. Interpretive analysis of recent developments on genetic predisposition. Biochim Biophys Acta 2006;1765:126–147.

[10] Feo F, Pascale RM, Calvisi DF. Models for liver cancer. In: Alison MR, editor. The cancer handbook, 2nd ed., vol. 2. New York: Wiley; 2007. p. 1118–1133.

[11] Newell P, Villanueva A, Friedman SL, Koike K, Llovet JM. Experimental models of hepatocellular carcinoma. J Hepatol 2008;48:858–879.

[12] Lee JS, Grisham JW, Thorgeirsson SS. Comparative functional genomics for identifying models of human cancer. Carcinogenesis 2005;26:1013–1020.

[13] Farber DE. Ethionine carcinogenesis. Adv Cancer Res 1963;7:383–474.

[14] Mikol YB, Hoover KL, Creasia D, Poirier LA. Hepatocarcingenesis in fats fed methyl-deficient, aminoacid-defined diets. Carcinogenesis 1983;4:1619–1629.

[15] Goshal AK, Farber E. Induction of liver cancer by a diet deficient of choline and methionine without added carcinogens. Carcinogenesis 1984;5:1367–1370.

[16] Yohoyama S, Sells MA, Reddy TV, Lombardi B. Hepatocarcinogenetic and promoting action of a choline-devoid diet in the rat. Cancer Res 1985;4:2834–2842.

[17] Finkelstein JD. Methionine metabolism in mammals. J Nutr Biochem 1990;1:228–237.

[18] Ramani K, Mato JM, Lu SC. Role of methionine adenosyltransferase genes in hepatocarcinogenesis. Cancers 2011;3:1480–1497.

[19] LeGros L, Halim AB, Chamberlin ME, Geller A, Kotb M. Regulation of the human MAT2B gene encoding the regulatory b subunit of methionine adenosyltransferase, MAT II. J Biol Chem 2001;276:24918–24924.

[20] Ji Y, Nordgren KK, Chai Y, Hebbring SJ, Jenkins GD, Abo RP, et al. Human liver methionine cycle: MAT1A and GNMT gene resequencing, functional genomics, and hepatic genotype-phenotype correlation. Drug Metab Dispos 2012;40:1984–1992.

[21] Shivapurka N, Poirier LA. Tissue levels of S-adenosylmethionine and S-adensylhocysteine in rats fed choline-deficient, aminoacid-defined diets for one to five weeks. Carcinogenesis 1983;4:1051–1057.

[22] Oler A, Lombardi B. Further studies on a defect in the intracellular transport and secretion of proteins by the liver of choline-deficient rats. J Biol Chem 1970;245:1282–1288.

[23] Santamaría E, Avila MA, Latasa MU, Rubio A, Martín-Duce A, Lu SC, et al. Functional proteomics of non, 46ic steatohepatitis: mitochondrial proteins as targets of S-adenosylmethionine. Proc Natl Acad Sci U S A 2003;100:3065–3070.

[24] Pascale R, Pirisi L, Daino L, Zanetti S, Satta A, Bartoli E, et al. Role of phosphatidylethanolamine methylation in the synthesis of phosphatidylcholine by hepatocytes isolated from choline-deficient rats. FEBS Lett 1982;14:293–297.

[25] Lu SC, Huang Z-Z, Yang H, Mato JM, Avila MA, Tsukamoto H. Changes in methionine adenosyltransferase and S-adenosylmethionine homeostasis in alcoholic rat liver. Am J Physiol Gastrointest Liver Physiol 2000;279:G178–G185.

[26] Feo F, Pascale R, Garcea R, Daino L, Pirisi L, Frassetto S, et al. Effect of the variations of S-adenosyl-L-methionine liver content on fat accumulation and ethanol metabolism in ethanol-intoxicated rats. Toxicol Appl Pharmacol 1986;83:331–341.

[27] Lieber CS, Casini A, DeCarli LM, Kim CI, Lowe N, Sasaki R, et al. S-adenosyl-L-methionine attenuates alcohol-induced liver injury in the baboon. Hepatology 1990;11:165–172.

[28] Gong Z, Yan S, Zhang P, Huang Y, Wang L. Effects of S-adenosylmethionine on liver methionine metabolism and steatosis with ethanol-induced liver injury in rats. Hepatol Int 2008;2:346–352.

[29] Halsted CH, Medici V. Aberrant hepatic methionine metabolism and gene methylation in the pathogenesis and treatment of alcoholic steatohepatitis. Int J Hepatol 2012;2012, 959746.

[30] Mato JM, Camara J, Fernandez de Paz J, Caballeria L, Coll S, Caballero A, et al. S-adenosylmethionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. J Hepatol 1999;30:1081–1089.

[31] Filipowicz M, Bernsmeier C, Terracciano L, Duong FHT, Heim MH. S-adenosyl-methionine and betaine improve early virological response in chronic hepatitis C patients with previous nonresponse. PLoS One 2010;5:e15492.

[32] Feld JJ, Modi AA, El-Diwany R, Rotman Y, Thomas E, Ahlenstiel G, et al. S-adenosyl methionine improves early viral responses and interferon-stimulated gene induction in hepatitis C nonresponders. Gastroenterology 2011;140:830–839.

[33] Liu Q, Chen J, Liu L, Zhang J, Wang D, Ma L, et al. The X protein of hepatitis B virus inhibits apoptosis in hepatoma cells through enhancing the methionine adenosyltransferase 2A gene expression and reducing S-adenosylmethionine production. J Biol Chem 2011;286:17168–17180.

[34] Stramentinoli G, Gualano M, Ideo G. Protective role of S-adenosyl-l-methionine on liver injury induced by D-galactosamine in rats. Biochem Pharmacol 1978;27:1431–1433.

[35] Stramentinoli G, Pezzoli C, Galli-Kienle M. Protective role of S-adenosyl-l-methionine against acetaminophen induced mortality and hepatotoxicity in mice. Biochem Pharmacol 1979;28:3567–3571.

[36] Corrales F, Giménez A, Alvarez L, Caballeria J, Pajares MA, Andreu H, et al. S-adenosylmethionine treatment prevents carbon tetrachloride-induced S-adenosylmethionine synthetase inactivation and attenuates liver injury. Hepatology 1992;16:1022–1027.

[37] Simile MM, Banni S, Angioni E, Carta G, De Miglio MR, Muroni MR, et al. 5-Methylthioadenosine administration prevents lipid peroxidation and fibrogenesis induced in rat liver by carbon-tetrachloride intoxication. J Hepatol 2001;34:386–394.

[38] Garcea R, Daino L, Pascale RM, Simile MM, Puddu M, Ruggiu ME, et al. Protooncogene methylation and expression in regenerating liver and preneoplastic liver nodules induced in the rat by diethylnitrosamine: effect of variations of S-adenosylmethionine: S-adenosylhomocysteine ratio. Carcinogenesis 1989;10:1183–1192.

[39] Huang ZZ, Mao Z, Cai J, Lu SC. Changes in methionine adenosyltransferase during liver regeneration in the rat. Am J Physiol 1998;275: G14–G21.

[40] Feo F, Garcea R, Pascale RM, Pirisi L, Daino L, Donaera A. The variations of S-adenosyl-l-methionine content modulate hepatocyte growth during phenobarbital promotion of diethylnitrosamine-induced rat liver carcinogenesis. Toxicol Pathol 1987;15:109–113.

[41] Garcea R, Pascale RM, Daino L, Frassetto S, Cozzolino P, Ruggio ME, et al. Variations in ornithine decarboxylase activity and S-adenosyl-l-methionine and S-methylthioadenosine contents during the development of diethylnitrosamine-induced liver hyperplastic nodules and hepatocellular carcinomas. Carcinogenesis 1987;8:653–658.

[42] Pascale RM, Simile MM, Satta G, Seddaiu MA, Daino L, Pinna G, et al. 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 1991;11:1617–1624.

[43] Simile MM, Saviozzi M, De Miglio MR, Muroni MR, Nufris A, Pascale RM, et al. Persistent chemopreventive effect of S-adenosyl-L-methionine on the development of liver putative preneoplastic lesions induced by thiobenzamide in diethylnitrosamine-initiated rats. Carcinogenesis 1996;17:1533–1537.

[44] Garcea R, Daino L, Pascale RM, Simile MM, Puddu M, Frassetto S, et al. Inhibition of promotion and persistent nodule growth by S-adenosyl-L-methionine in rat liver carcinogenesis: role of remodeling and apoptosis. Cancer Res 1989;49:1850–1856.

[45] Pascale RM, Simile MM, De Miglio MR, Nufris A, Daino L, Seddaiu MA, et al. Chemoprevention by S-adenosyl-L-methionine of rat liver carcinogenesis initiated by 1,2-dimethylhydrazine and promoted by orotic acid. Carcinogenesis 1995;16:427–430.

[46] Calvisi DF, Simile MM, Ladu S, Pellegrino R, De Murta V, Pinna F, et al. Altered methionine metabolism and global DNA methylation in liver cancer: relationship with genomic instability and prognosis. Int J Cancer 2007;121:2410–2420.

[47] Pascale RM, Simile MM, De Miglio MR, Feo F. Chemoprevention of hepatocarcinogenesis: S-adenosyl-L-methionine. Alcohol 2002;27:193–198.

[48] Cai J, Mao Z, Hwang JJ, Lu SC. Differential expression of methionine adenosyltransferase genes influences the rate of growth of human hepatocellular carcinoma cells. Cancer Res 1998;58:1444–1450.

[49] Frau M, Tomasi ML, Simile MM, Demartis MI, Salis F, Latte G, et al. Role of transcriptional and posttranscriptional regulation of methionine adenosyltransferases in liver cancer progression. Hepatology 2012;56:165–175.

[50] Lu SC, Ramani K, Ou X, Lin M, Yu V, Ko K, et al. S-adenosylmethionine in the chemoprevention and treatment of hepatocellular carcinoma in a rat model. Hepatology 2009;50:462–471.

[51] Li J, Ramani K, Sun Z, Zee C, Grant EG, Yang H, et al. Forced expression of methionine adenosyltransferase 1A in human hepatoma cells suppresses in vivo tumorigenicity in mice. Am J Pathol 2010;176:2456–2466.

[52] Mato JM, Corrales FJ, Lu SC, Avila MA. S-Adenosylmethionine: a control switch that regulates liver function. FASEB J 2002;16:15–26.

[53] Lu SC, Mato JM. Role of methionine adenosyltransferase and S-adenosyl-methionine in alcohol-associated liver cancer. Alcohol 2005;35:227–234.

[54] Mato JM, Alvarez L, Ortiz P, Mingorance J, Durán C, Pajares MA. S-adenosyl-L-methionine synthetase and methionine metabolism deficiencies in cirrhosis. Adv Exp Med Biol 1994;368:113–117.

[55] Mato JM, Corrales F, Martin-Duce A, Ortiz P, Pajares MA, Cabrero C. Mechanisms and consequences of the impaired trans-sulphuration pathway in liver disease: part I. Biochemical implications. Drugs 1990;40:58–64.

[56] Finkelstein JD. Metabolic regulatory properties of S-adenosylmethionine and S-adenosylhomocysteine. Clin Chem Lab Med 2007;45:1694–1699.

[57] Lu SC, Alvarez L, Huang ZZ, Chen L, An W, Corrales FJ, et al. 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 2001;98:5560–5565.

[58] Martinez-Chantar M, Vázquez-Chantada M, Ariz U, Martínez N, Varela M, Luka Z, et al. Loss of the GNMT gene leads to steatosis and hepatocellular carcinoma in mice. Hepatology 2008;47:1191–1199.

[59] Liu SP, Li YS, Lee CM, Yen CH, Liao YJ, Huang SF, et al. Higher susceptibility to aflatoxin B(1)-related hepatocellular carcinoma in glycine N-methyltransferase knockout mice. Int J Cancer 2011;128:511–523.

[60] Liao YJ, Liu SP, Lee CM, Yen CH, Chuang PC, Chen CY, et al. Characterization of a glycine N-methyltransferase gene knockout mouse model for hepatocellular carcinoma: Implications of the gender disparity in liver cancer susceptibility. Int J Cancer 2009;124:816–826.

[61] Martínez-López N, García-Rodríguez JL, Varela-Rey M, Gutiérrez V, Fernández-Ramos D, Beraza N, et al. Hepatoma cells from mice deficient in glycine N-methyltransferase have increased RAS signaling and activation of liver kinase B1. Gastroenterology 2012;143:787–798.

[62] Martinez-Chantar ML, Lu SC, Mato JM, Luka Z, Wagner C, French BA, et al. The role of stem cells/progenitor cells in liver carcinogenesis in glycine N-methyltransferase deficient mice. Exp Mol Pathol 2010;88:234–237.

[63] Lu SC, Mato JM. S-adenosylmethionine in liver health, injury, and cancer. Physiol Rev 2012;92:1515–1542.

[64] Pañeda C, Gorospe I, Herrera B, Nakamura T, Fabregat I, Varela-Nieto I. Liver cell proliferation requires methionine adenosyltransferase 2A mRNA up-regulation. Hepatology 2002;35:1381–1391.

[65] Martínez-Chantar ML, García-Trevijano ER, Latasa MU, Martín-Duce A, Fortes P, Caballería J, et al. Methionine adenosyltransferase II beta subunit gene expression provides a proliferative advantage in human hepatoma. Gastroenterology 2003;124:940–948.

[66] Torres L, Avila MA, Carretero MV, Latasa MU, Caballería J, López-Rodas G, et al. Liver-specific methionine adenosyltransferase MAT1A gene expression is associated with a specific pattern of promoter methylation and histone acetylation: implications for MAT1A silencing during transformation. FASEB J 2000;14:95–102.

[67] Tomasi ML, Li TW, Li M, Mato JM, Lu SC. Inhibition of human methionine adsenosyltransferase 1A transcription by coding region methylation. J Cell Physiol 2012;227:1583–1591.

[68] Yang H, Huang ZZ, Zeng Z, Chen C, Selby RR, Lu SC. Role of promoter methylation in increased methionine adenosyltransferase 2A expression in human liver cancer. Am J Physiol Gastrointest Liver Physiol 2001;280:184–190.

[69] Lal A, Mazan-Mamczarz K, Kawai T, Yang X, Martindale JL, Gorospe M. Concurrent versus individual binding of HuR and AUF1 to common labile target mRNAs. EMBO J 2004;23:3092–3102.

[70] Kim ME, Hur J, Jeo S. Emerging roles of RNA and RNA-binding protein network in cancer cells. BMB Rep 2009;42:125–130.

Review

[71] Vázquez-Chantada M, Fernández-Ramos D, Embade N, Martínez-Lopez N, Varela-Rey M, Woodhoo A, et al. HuR/-methyl-HuR and AUF1 regulate the MAT expressed during liver proliferation, differentiation, and carcinogenesis. Gastroenterology 2010;138:1943–1953.

[72] Yang H, Cho ME, Li TW, Peng H, Ko KS, Mato JM, et al. MicroRNAs regulate methionine adenosyltransferase 1A expression in hepatocellular carcinoma. J Clin Invest 2012;123:285–298.

[73] Ha HL, Shin HJ, Feitelson MA, Yu DY. Oxidative stress and antioxidants in hepatic pathogenesis. World J Gastroenterol 2010;16:6035–6043.

[74] Parola M, Leonarduzzi G, Robino G, Albano E, Poli G, Dianzani MU. On the role of lipid peroxidation in the pathogenesis of liver damage induced by long-standing cholestasis. Free Radic Biol Med 1996;20:351–359.

[75] Hernandez-Munoz R, Diaz-Munoz M, Lopez V, Lopez-Barrera F, Yanez L, Vidrio S, et al. Balance between oxidative damage and proliferative potential in an experimental rat model of CCl4-induced cirrhosis: protective role of adenosine administration. Hepatology 1997;26:1100–1110.

[76] De Flora S, Izzotti A, D'Agostin F, Balansky RM. Mechanisms of N-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking-related end-points. Carcinogenesis 2001;22:999–1013.

[77] Pascale RM, Simile MM, Gaspa L, Daino L, Seddaiu MA, Pinna G, et al. Alterations of ornithine decarboxylase gene during the progression of rat liver carcinogenesis. Carcinogenesis 1993;14:1077–1080.

[78] Feo F, Garcea R, Daino L, Pascale RM, Pirisi L, Frassetto S, et al. Early stimulation of polyamine biosynthesis during promotion by phenobarbital of diethylnitrosamine-induced rat liver carcinogenesis. The effects of variations of the S-adenosyl-L-methionine cellular pool. Carcinogenesis 1985;6:1713–1720.

[79] Heby O. Role of polyamines in the control of cell proliferation and differentiation. Differentiation 1981;19:1–20.

[80] Wu SE, Huskey WP, Borchardt RT, Schowen REL. Chiral instability at sulfur of S-adenosylmethione. Biochemistry 1983;22:2828–2832.

[81] Brunmark A, Cadenas E. Redox and addition chemistry of quinoid compounds and its biological implications. Free Radic Biol Med 1989;7:435–477.

[82] Pascale RM, Simile MM, Ruggiu ME, Seddaiu MA, Satta G, Sequenza MJ, et al. Reversal by 5-azacytidine of the S-adenosyl-L-methionine-induced inhibition of the development of putative preneoplastic foci in rat liver carcinogenesis. Cancer Lett 1991;56:259–265.

[83] Mann CD, Neal CP, Garcea G, Manson MM, Dennison AR, Berry DP. Prognostic molecular markers in hepatocellular carcinoma: a systematic review. Eur J Cancer 2007;43:979–992.

[84] Boiteux S, Guillet M. AP sites in DNA: repair and biological consequences in Saccharomyces cerevisiae. DNA Repair (Amst) 2004;3:1–12.

[85] Izumi T, Brown DB, Naidu CV, Bhakat KK, Macinnes MA, Saito H, et al. Two essential but distinct functions of the mammalian AP endonuclease. Proc Natl Acad Sci U S A 2005;102:5739–5743.

[86] Tell G, Damante G, Caldwell D, Kelley MR. The intracellular localization of APE1/Ref-1: more than a passive phenomenon? Antioxid Redox Signal 2005;7:367–384.

[87] Ramana CV, Boldogh I, Izumi T, Mitra S. Activation of apurinic/apyrimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals. Proc Natl Acad Sci U S A 1998;95:5061–5066.

[88] Grösch S, Fritz G, Kaina B. Apurinic endonuclease (Ref-1) is induced in mammalian cells by oxidative stress and involved in clastogenic adaptation. Cancer Res 1998;58:4410–4416.

[89] Tomasi ML, Iglesias-Ara A, Yang H, Ramani K, Feo F, Pascale MR, et al. S-adenosylmethionine regulates apurinic/apyrimidinic endonuclease 1 stability: implication in hepatocarcinogenesis. Gastroenterology 2009;136:1025–1036.

[90] Yan MD, Xu WJ, Lu LR, Sun LY, Liu XY, Zheng ZC. Ubiquitin conjugating enzyme Ubc9 is involved in protein degradation of redox factor-1 (Ref-1). Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) 2000;32:63–68.

[91] Tomasi ML, Tomasi I, Ramani K, Pascale RM, Xu J, Giordano P, et al. S-adenosyl methionine regulates ubiquitin-conjugating enzyme 9 protein expression and sumoylation in murine liver and human cancers. Hepatology 2012;56:982–993.

[92] Pascale RM, Frau M, Feo F. Prognostic significance of iNOS in hepatocellular carcinoma. In: Bonavida B, editor. Nitric oxide (NO) and cancer. New York: Springer; 2010. p. 309–328, ch. 17.

[93] Calvisi DF, Pinna F, Ladu S, Pellegrino R, Muroni MR, Simile MM, et al. Aberrant iNOS signalling is under genetic control in rodent liver cancer and potentially prognostic for human disease. Carcinogenesis 2008;29:1639–1647.

[94] Zhang J, Xi Z, Dong Y, Wang S, Liu C, Zou MH. Identification of nitric oxide as an endogenous activator of the AMP-activated protein kinase in vascular endothelial cells. J Biol Chem 2008;283:27452–27461.

[95] Vázquez-Chantada M, Ariz U, Varela-Rey M, Embade N, Martínez-Lopez N, Fernández-Ramos D, et al. Evidence for an LKB1/AMPK/eNOS cascade regulated by HGF, S-adenosylmethionine and NO in hepatocyte proliferation. Hepatology 2009;49:608–617.

[96] Martínez-Chantar ML, Vázquez-Chantada M, Garnacho M, Varela-Rey M, Dotor J, Santamaría M, et al. S-adenosylmethionine regulates cytoplasmic HuR via AMP-activated kinase. Gastroenterology 2006;131:223–232.

[97] Almeida A, Moncada S, Bolaños JP. Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway. Nat Cell Biol 2004;6:45–51.

[98] Calvisi DF, Frau M, Tomasi ML, Feo F, Pascale RM. Deregulation of signalling pathways in prognostic subtypes of hepatocellular carcinoma: novel insights from interspecies comparison. Biochim Biophys Acta 2012;1826:215–237.

[99] Martínez-López N, Varela-Rey M, Fernández-Ramos D, Woodhoo A, Vázquez-Chantada M, Embade N, et al. Activation of LKB1-Akt pathway independent of PI3 Kinase plays a critical role in the proliferation of hepatocellular carcinoma from NASH. Hepatology 2010;52:1621–1631.

[100] Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer 2001;11:85–95.

[101] Hezel AF, Bardeesy N. LKB1; linking cell structure and tumor suppression. Oncogene 2008;27:6908–6919.

[102] Lee CW, Wong LL, Tse EY, Liu HF, Leong VY, Lee JM, et al. AMPK promotes p53 acetylation via phosphorylation and inactivation of SIRT1 in liver cancer cells. Cancer Res 2012;72:4394–4404.

[103] García-Román R, Salazar-González D, Rosas S, Arellanes-Robledo J, Beltrán-Ramírez O, Fattel-Fazenda S, et al. The differential NF-kB modulation by S-adenosyl-L-methionine, acetylcysteine and quercetin on the promotion stage of chemical hepatocarcinogenesis. Free Radic Res 2008;42:331–343.

[104] Millward TA, Zolnierowicz S, Hemmings BA. Regulation of protein kinase cascades by protein phosphatase 2A. Trends Biochem Sci 1999;24:186–191.

[105] Eichhorn PJ, Creyghton MP, Bernards R. Protein phosphatase 2A regulatory subunits and cancer. Biochim Biophys Acta 2009;179:1–15.

[106] Frau M, Simile MM, Tomasi ML, Demartis MI, Daino L, Seddaiu MA, et al. An expression signature of phenotypic resistance to hepatocellular carcinoma identified by cross-species gene expression analysis. Cell Oncol 2012;35:163–173.

[107] Zhao J, Dong L, Lu B, Wu G, Xu D, Chen J, et al. Down-regulation of osteopontin suppresses growth and metastasis of hepatocellular carcinoma via induction of apoptosis. Gastroenterology 2008;135:956–968.

[108] Calvisi DF, Pinna F, Ladu S, Pellegrino R, Sanna V, Sini M, et al. Ras-driven proliferation and apoptosis signalling during rat liver carcinogenesis is under genetic control. Int J Cancer 2008;23:2057–2064.

[109] Calvisi DF, Pinna F, Meloni F, Ladu S, Pellegrino R, Sini L, et al. Dual-specificity phosphatase 1 ubiquitination in extracellular signal-regulated-kinase-mediated control of growth in human hepatocellular carcinoma. Cancer Res 2008;68:4192–4200.

[110] Calvisi DF, Pinna F, Ladu S, Pellegrino R, Simile MM, Frau M, et al. Forkhead box M1B is a determinant of rat susceptibility to hepatocarcinogenesis and sustains ERK activity in human HCC. Gut 2009;58:679–687.

[111] Tomasi ML, Ramani K, Lopitz-Otsoa F, Rodríguez MS, Li TW, Ko K, et al. S-adenosylmethionine regulates dual-specificity mitogen-activated protein kinase phosphatase expression in mouse and human hepatocytes. Hepatology 2010;51:2152–2161.

[112] Xia L, Mo P, Huang W, Zhang L, Wang Y, Zhu H, et al. The TNF-α/ROS/HIF-1-induced upregulation of FoxMI expression promotes HCC proliferation and resistance to apoptosis. Carcinogenesis 2012;33:2250–2259.

[113] Liu Q, Liu L, Zhao Y, Zhang J, Wang D, Chen J, et al. Hypoxia induces genomic DNA demethylation through the activation of HIF-1α and transcriptional upregulation of MAT2A in hepatoma cells. Mol Cancer Ther 2011;10:1113–1123.

[114] Reytor E, Pérez-Miguelsanz J, Alvarez L, Pérez-Sala D, Pajares MA. Conformational signals in the C-terminal domain of methionine adenosyltransferase I/III determine its nucleocytoplasmic distribution. FASEB J 2009;23:3347–3360.

[115] Katoh Y, Ikura T, Hoshikawa Y, Tashiro S, Ito T, Ohta M, et al. Methionine adenosyltransferase II serves as a transcriptional corepressor of Maf oncoprotein. Mol Cell 2011;41:554–566.

[116] Igarashi K, Katoh Y. Metabolic aspects of epigenome: coupling of S-adenosylmethionine synthesis and gene regulation on chromatin by SAMIT module. Subcell Biochem 2012;61:105–118.

Review

[117] Andreu-Pérez P, Esteve-Puig R, de Torre-Minguela C, López-Fauqued M, Bech-Serra JJ, Tenbaum S, et al. Protein arginine methyltransferase 5 regulates ERK1/2 signal transduction amplitude and cell fate through CRAF. Sci Signal 2011;4:ra58.

[118] Wei TY, Juan CC, Hisa JY, Su LJ, Lee YC, Chou HY, et al. Protein arginine methyltransferase 5 is a potential oncoprotein that upregulates G1 cyclins/cyclin-dependent kinases and the phosphoinositide 3-kinase/AKT signaling cascade. Cancer Sci 2012;103:1640–1650.

[119] Gu Z, Li Y, Lee P, Liu T, Wan C, Wang Z. Protein arginine methyltransferase 5 functions in opposite ways in the cytoplasm and nucleus of prostate cancer cells. PLoS One 2012;7:e44033.

[120] Duong FH, Christen V, Filipowicz M, Heim MH. S-Adenosylmethionine and betaine correct hepatitis C virus induced inhibition of interferon signaling in vitro. Hepatology 2006;43:796–806.

[121] Lee JS, Chu IS, Mikaelyan A, Calvisi DF, Heo J, Reddy JK, et al. Application of comparative functional genomics to identify best-fit mouse models to study human cancer. Nat Genet 2004;36:1306–1311.

[122] Andersen JB, Loi R, Perra A, Factor VM, Ledda-Columbano GM, Columbano A, et al. Progenitor-derived hepatocellular carcinoma model in the rat. Hepatology 2011;51:1401–1409.

[123] Albers E. Metabolic characteristics and importance of the universal methionine salvage pathway recycling methionine from 5'-methylthioadenosine. IUBMB Life 2009;61:1132–1142.

[124] Ellwood-Yen K, Graeber TG, Wongvipat J, Iruela-Arispe ML, Zhang J, Matusik R, et al. Myc-driven murine prostate cancer shares molecular features with human prostate tumors. Cancer Cell 2003;4:223–238.

[125] Sweet-Cordero A, Mukherjee S, Subramanian A, You H, Roix JJ, Ladd-Acosta CC, et al. An oncogenic KRAS2 expression signature identified by cross-species gene-expression analysis. Nat Genet 2005;37:48–55.

[126] Lee JS, Thorgeirsson SS. Comparative and integrative functional genomics of HCC. Oncogene 2006;25:3089–3801.

[127] Anstee QM, Day CP. S-adenosylmethionine (SAMe) therapy in liver disease: a review of current evidence and clinical utility. J Hepatol 2012;57:1097–1109.

[128] Cholongitas E, Papatheoroditis GV. Review aryicle: novel therapeuic options for chronic hepatitis C. Aliment Phramacol Ther 2008;27:884.

[129] Morgan TR. Chemoprevention of hepatocellular carcinoma in chronic hepatitis C. Recent Results Cancer Res 2011;188:85–99.

[130] Ramani K, Yang HP, Xia M, Iglesias Ara A, Mato JM, Lu SC. Leptin's mitogenic effect in human liver cancer cells requires induction of both methionine adenosyltransferase 2A and 2β. Hepatology 2008;47:521–531.

[131] Calvisi DF, Pascale RM, Feo F. Dissection of signal transduction pathways as a tool for the development of targeted therapies of hepatocellular carcinoma. Rev Recent Clin Trials 2007;2:217–236.

[132] Zhang W, Sviripa V, Chen X, Shi J, Yu T, Hamza A, et al. Fluorinated N, N-Dialkylaminostilbenes repress colon cancer by targeting methionine S-adenosyltransferase 2A. ACS Chem Biol 2013;8:796–803.

[133] Broderick JA, Zamore PD. MicroRNA therapeutics. Gene Ther 2011;18:1104–1110.

[134] Guo J, Friedman SL. The expression patterns and clinical significance of microRNAs in liver diseases and hepatocellular carcinoma. Curr Pharm Des 2013;19:1262–1272.