Methylthioadenosine phosphorylase gene expression is impaired in human liver cirrhosis and hepatocarcinoma

Carmen Berasain a,1, Henar Hevia a,1, Jokin Fernández-Irigoyen a, Esther Larrea a, Juan Caballería b, José M. Mato c, Jesús Prieto a, Fernando J. Corrales a,2, Elena R. García-Trevijano a,2, Matías A. Avila a,2,*

a Division of Hepatology and Gene Therapy, CIMA, Facultad de Medicina, Universidad de Navarra. 31008 Pamplona, Spain
b Servicio de Hepatología, Hospital Clínico y Provincial, 08036 Barcelona, Spain
c CIC-Biogune, Metabolomics Unit, Technological Park of Bizkaia, 48710 Zamudio, Spain

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Abstract

Methylthioadenosine phosphorylase (MTAP) is a key enzyme in the methionine and adenine salvage pathways. In mammals, the liver plays a central role in methionine metabolism, and this essential function is lost in the progression from liver cirrhosis to hepatocarcinoma. Deficient MTAP gene expression has been recognized in many transformed cell lines and tissues. In the present work, we have studied the expression of MTAP in human and experimental liver cirrhosis and hepatocarcinoma. We observe that MTAP gene expression is significantly reduced in human hepatocarcinoma tissues and cell lines. Interestingly, MTAP gene expression was also impaired in the liver of CCl 4-cirrhotic rats and cirrhotic patients. We provide evidence indicating that epigenetic mechanisms, involving DNA methylation and histone deacetylation, may play a role in the silencing of MTAP gene expression in hepatocarcinoma. Given the recently proposed tumor suppressor activity of MTAP, our observations can be relevant to the elucidation of the molecular mechanisms of multistep hepatocarcinogenesis.

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1. Introduction

Methylthioadenosine phosphorylase (MTAP) (5′-deoxy-5′-methylthioadenosine: orthophosphate methylthio-ribosyltransferase, EC 2.4.2.28) is the rate-limiting enzyme in the methionine salvage pathway [1–3]. MTAP catalyzes the phosphorolytic cleavage of 5′-methylthioadenosine (MTA), a sulfur-containing adenosyl nucleoside that is formed from S-adenosylmethionine (AdoMet) in the polyamine biosynthetic pathway [4–6]. The reaction products, adenine and 5-methylthioribose 1-phosphate are reused: adenine is metabolized to adenine nucleotide pools via adenine phosphoribosyltransferase [7], and 5-methylthioribose 1-phosphate is metabolized to methionine through a series of reactions [8]. This methionine salvage pathway has been studied in detail in Klebsiella pneumoniae [9], but has also been demonstrated in mammalian tissues such as rat liver [8]. MTAP is expressed in all normal tissues and in non-transformed cell lines, however, many tumors and malignant cell lines, including breast, lung, colon and ovarian carcinomas, glioblastoma, leukemia and melanoma lack MTAP activity [1,10–15]. In most of these tumors and transformed cells, the complete absence of MTAP activity has been attributed to total or partial deletions of the MTAP gene, located on human chromosome 9p21 in a region frequently deleted in human malignancies [16,17]. Additionally, MTAP promoter hypermethylation has also been associated with reduced MTAP expression in human melanoma cell lines [18].
Interestingly, it has been recently found that expression of MTAP in an MTAP-deficient breast adenocarcinoma cell line resulted in a dramatic inhibition of tumorigenicity, showing that MTAP can function as a tumor suppressor gene [19].

Consistent with its central metabolic role, the liver parenchyma is one of the normal human tissue types where MTAP gene expression is higher [17]. We have previously shown that the expression of genes involved in methionine metabolism is impaired in human liver cirrhosis and hepatocarcinoma (HCC) and in experimental models of chronic liver damage [20,21], and that these alterations may contribute to the progression to the transformed phenotype [22,23]. However, to our knowledge, there are no studies addressing MTAP status in human liver disease. The aim of this work was to characterize the expression of MTAP in human and experimental liver cirrhosis and hepatocarcinoma.

2. Materials and methods

2.1. Patients

We obtained specimens of liver tissue from three groups of subjects: (a) control individuals (n=19; 11 males; mean age 58, range 45–70). Control human liver tissue was obtained from patients in whom a cholecystectomy was performed for the treatment of a symptomatic cholelithiasis and who consented to be submitted to a liver biopsy during the surgical procedure. In the control group, both the liver function tests and liver histology were normal or showed minimal changes. (b) Patients with liver cirrhosis of different etiology (n=10; nine males; mean age 60, range 42–77): six patients with hepatitis C viral (HCV) cirrhosis, two with hepatitis B viral (HBV) cirrhosis and two alcoholic cirrhosis. Cirrhotic liver samples were obtained at the time of liver transplantation. (c) Patients with primary hepatocellular carcinoma (HCC) (n=9; all males; mean age 67, range 39–79). Cancerous liver tissues were obtained during surgical resection. All tissues were immediately frozen in liquid nitrogen for subsequent isolation of RNA. This study was approved by the human research review committee of the University of Navarra. Studies were conducted in compliance with the ethical standards formulated at the Helsinki Declaration of 1975 (revised in 1983).

2.2. Experimental model of cirrhosis

Male Wistar rats (Harlan, Barcelona, Spain) (150 g of weight at the onset of treatment), fed ad libitum a standard laboratory diet, were used. Cirrhosis was induced with CCl4 injected intraperitoneally (0.15 ml/100 g body weight) twice a week for 9 weeks. Animals were sacrificed 24 h after the last injection. Liver samples were taken, snap-frozen in liquid nitrogen and stored at −80 °C until analysis. The establishment of cirrhosis was monitored by histological examination of liver sections from treated animals. We performed all experimental procedures in conformity with our Institution’s guidelines for the use of laboratory animals.

2.3. Cell culture

The human hepatocarcinoma cell lines HuH7, PLC, SK-Hep1 and Hep3B were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, glutamine, and antibiotics (Gibco). HuH7 and PLC cells were treated with 5-aza-2′-deoxycytidine (5-aza-dC) (Sigma, St. Louis, MO, USA) at 10 µM for 48 h. 5-Aza-dC was added directly to complete culture medium from a 10 mM stock solution in distilled water. Trichostatin A (TSA) (Sigma) was added at a final concentration of 100 nM.

2.4. RNA isolation, RT-PCR and real-time PCR

We extracted total RNA using TRI Reagent (Sigma). We treated RNA (2 µg) with DNaseI (Gibco-BRL, Paisley, UK) prior to reverse transcription with M-MLV Reverse Transcriptase (Gibco-BRL) in the presence of RNaseOUT (Gibco-BRL), to avoid amplification of MTAP pseudogene [24]. We designed all primers to distinguish between genomic and cDNA amplification and sequenced all PCR products to confirm the specificity. Real-time PCR was performed with 1/20 of the RT reaction using an iCycler (BioRad) and the iQ SYBR Green Supermix (BioRad). The primers used for MTAP amplification were: 5′-TGGAA-TAATTGTTGAGACAGGC-3′ and 5′-TGCCACA-CTCCTCTGGC-3′. MTAP mRNA levels were normalized according to β-actin quantitation in the same sample. The primers used for β-actin amplification were: human 5′-AGCTGCTGTTGGCCA-3′ and 5′-CTGGTCCTGGGGG-3′, rat 5′-CAACCTCTTGT-GACGTC-3′ and 5′-CTGGTGCTAGGGCG-3′. To monitor the specificity, final PCR products were analyzed by melting curves and electrophoresis. The amount of MTAP transcript was calculated and expressed as the difference relative to the control gene β-actin (2ΔCt, where ΔCt represents the difference in threshold cycles between the target and control genes) essentially as described [25]. Total RNA extracted from human peripheral blood cells and the human lung adenocarcinoma cell line A549 [14] were used as positive and negative controls for the real-time PCR analyses of MTAP gene expression.

2.5. Genomic DNA isolation and analysis of MTAP promoter methylation

Genomic DNA was isolated from control and cirrhotic liver samples, HCC tissue and HCC cell lines as previously reported [20,21]. Where indicated, aliquots of genomic DNA (0.5 µg) were digested with the restriction
enzyme NotI (New England Biolabs, Beverly, MA, USA) (10 U/μg of DNA) at 37 °C for 18 h. Subsequently, a 606 bp region of MTAP was amplified by PCR using the ImmoGene DNA polymerase from Bioline (Randolph, MA, USA) (32 cycles, 58 °C annealing temperature), and the following primers: 5’-CGTCCAGGCTATTTGCAAGC-3’ and 5’-CCTCAC-CAAGGCCGGTACTG-3’. This region of human MTAP promoter contains a CpG island (nucleotides −461 to −441) [18], harboring an internal NotI restriction site. NotI digestion is inhibited by cytosine methylation, consequently PCR amplification of this region will not occur in unmethylated DNA samples. MTAP gene promoter methylation was further examined by methylation-specific PCR (MSP) analysis. Genomic DNA samples (1 μg) were treated with sodium bisulfite and subsequently purified using the CpGenome DNA modification kit from Qbiogene, Inc. (Carlsbad, CA, USA) following the manufacturer’s recommendations. Two sets of primers were used to amplify each region of interest (35 cycles, 58 °C annealing temperature), one pair recognized a sequence in which CpGs are unmethylated and the other recognized a sequence with methylated CpGs. The primers used were: unmethylated sense 5’-TTTGTCTTTTGTTGTTGTTGTTG-3’ antisense 5’-TTAACCAATATTAAATACATC-3’, methylated sense 5’-TTTGTCTTTTGTTGTTGTTGTTG-3’ antisense 5’-TTAACCAATATTAAATACATC-3’, and included the NotI site in the MTAP promoter mentioned above.

2.6. Assessment of allelic status of MTAP

Allelic status (no deletion, mono-allelic deletion, bi-allelic deletion) of MTAP was studied in control (n=9) and cirrhotic (n=6) human liver samples, in human HCC samples (n=5), and in the HCC cell lines mentioned above, using a real-time quantitative PCR method previously described [26]. This method of gene dosage has been successfully applied for the assessment of the allelic status of MTAP in human acute lymphoblastic leukemia. A single-copy sequence S9ribP mapping at 19q13.4 [27], a region that is not deleted in HCC [28], is used as reference sequence. The relative copy number of MTAP was obtained by calculating the ratio of the value obtained for MTAP to the S9ribP value as described [26]. The normalized ratio of MTAP to S9ribP values is expected to be close to 1 if no deletion is present, around 0.5 in the case of mono-allelic deletion and 0 for bi-allelic deletion [25]. Real-time PCR was performed using an iCycler (BioRad) and the iQ SYBR Green Supermix (BioRad). The primers used were 5’-GATGAGAAGGACCACCGCGTCTTCCGTCCTTG-3’ and 5’-GCAAAGACTGAATCGATACAC-3’ for S9ribP, and 5’-TGGATTGTTGGAGCACAGGC-3’ and 5’-CACCTTCTCAGCTGATAAGC-3’ for MTAP. The sense primer for MTAP was an exonic primer while the antisense MTAP primer was intronic, to avoid amplification of MTAP pseudogene [24].

2.7. Measurement of AdoMet and MTA in rat liver samples

AdoMet and MTA concentrations were determined by high-pressure liquid chromatography according to the procedure previously described [29]. Briefly, liver samples were homogenized in 1 ml of 0.4 M perchloric acid. Homogenates were centrifuged at 10,000×g and 4 °C for 15 min and 100 μl of the supernatant were analyzed on a Bio-Sil®-ODS-5S column equilibrated in 0.01 M ammonium formate, 4 mM heptanesulfonic acid, pH 4.0. AdoMet and MTA were eluted with a two-step gradient of acetonitrile in the same buffer (0–25% in 31 min and 25–100% in 20 min). Chromatograms were analyzed with the Beckman System Gold software.

2.8. Measurement of MTAP activity

Liver specimens were homogenized in five volumes of 50 mM Tris/HCl, pH 7.4 with protease inhibitors. The cytosolic fraction was obtained by centrifugation at 100,000×g and 4 °C during 90 min. The activity assay was performed according to the conditions described before with minor modifications [5]. Briefly, the reaction mixture contained 50 mM potassium phosphate buffer, pH 7.4, 200 μM MTA and the amount of cytosol required for a protein concentration of 1 mg/ml in a final volume of 400 μl. The assay mixture was incubated at 37 °C and the reaction was stopped after 0 and 30 min by adding perchloric acid to a final concentration of 0.4 M. MTAP activity was calculated as the consumption of MTA during the activity assay. Consumption of MTA was estimated by subtracting the MTA remaining after 30 min incubation from the initial concentration measured at time 0. MTA was measured in 100 μl aliquots as described above.

2.9. Statistics

Data obtained in experiments performed with hepatoma cell lines are mean±S.E. of at least three independent experiments performed in triplicate. Statistical significance was estimated using the Mann–Whitney unpaired test. The statistical analysis was performed with the SPSS 6.0 program.

3. Results

3.1. Expression of MTAP in healthy and diseased human liver

We examined MTAP mRNA levels by real-time PCR in 19 normal liver samples, 9 HCC and 10 cirrhotic liver samples (Fig. 1). As mentioned before, the expression of MTAP is diminished or lost in many types of human malignancies, our present data indicate that MTAP gene expression is markedly reduced in human HCC with respect to control livers. It is known that a significant number of
circumstantial liver can be considered as a pre-cancerous condition in which molecular alterations involved in the development of the malignant phenotype take place [21,30–32]. Interestingly, we observe that the mRNA levels of MTAP are already significantly reduced in cirrhotic liver tissue as compared to healthy controls, and that there are no differences between cirrhotic liver and HCC in terms of MTAP gene expression. MTAP gene expression was similarly reduced in all cirrhotic liver samples regardless of the etiology (data not shown). In concordance with the reduced mRNA levels of MTAP observed in the cirrhotic liver samples, MTAP specific activity was also decreased when compared to healthy controls (123±17 vs. 47±1 pmol/min/mg of protein. Values are mean±S.E. P<0.05).

The allelic status of MTAP was studied in randomly selected control, cirrhotic and HCC samples according to the recently described method of Bertin et al. [26]. This real-time PCR-based method determines the dosage of a target gene using a reference gene not deleted in the condition studied [25]. The theoretical target/reference ratios are 0, 0.5 and 1 for samples with bi-allelic deletion, mono-allelic deletion and no deletion, respectively. The mean values (±S.D.) obtained for MTAP gene in this assay were: 1.00±0.05 in control liver samples (n=9), 0.88±0.18 in cirrhotic liver samples (n=6) and 0.96±0.17 in HCC samples (n=5). The data obtained in cirrhotic and HCC samples were close to the theoretical value of 1, observed in the control samples, suggesting that no deletional events occurred in MTAP in our cirrhotic or tumoral liver samples.

3.2. Expression of MTAP in experimental liver cirrhosis

The impairment in MTAP gene expression observed in cirrhotic human liver was confirmed in the experimental model of CCl4-induced liver cirrhosis in rats. As shown in Fig. 2, the mRNA levels of MTAP were markedly reduced in the liver of rats with established hepatic cirrhosis. Given the central role played by MTAP in MTA metabolism, we measured the hepatic contents of this metabolite in control and cirrhotic rat livers. We found that in chronically injured rat liver MTA levels were reduced by 47% when compared to control rats (6.65±0.5 vs. 3.53±0.2 pmol/mg of protein, n=6 animals per group. Values are mean±S.E. P<0.05). Thus, in spite of a compromised expression of MTAP, MTA is not accumulated in the cirrhotic liver. As indicated before, MTA is produced during polyamine biosynthesis from AdoMet, its metabolic precursor. We determined AdoMet contents in control and cirrhotic livers, and found that these were dramatically reduced in CCl4-treated rats (80.3% decrease) (43.4±12.7 vs. 8.53±1.67 pmol/mg of protein, n=6 animals per group. Values are mean±S.E., P<0.05).

3.3. MTAP promoter is hypermethylated in the cirrhotic and transformed human liver. Involvement of epigenetic mechanisms in the downregulation of MTAP gene expression

Loss of MTAP gene expression in transformed cell lines and tissues has been mainly attributed to homozygous deletions and translocations at the chromosome 9p21 region [17]. We examined the expression of MTAP in four different human hepatocarcinoma cell lines by real-time PCR. As depicted in Fig. 3A, MTAP gene expression was detected in all of them, with the exception of SK-Hep1 cells. However, MTAP mRNA levels were markedly reduced, but not completely absent, when compared to levels found in healthy human liver. In addition, heterozygous deletions were not detected in these HCC cell lines, except in Sk-Hep1 cells in which MTAP was homozygously deleted. This is in agreement with the reduced expression of MTAP found in HCC tissues and our previous observation showing the absence of allelic losses of MTAP in HCC. Altogether, these data suggest the involvement of alternative mechanisms for the impaired expression of MTAP. Methylation at CpG dinucleotides in the promoter region of genes contributes to the silencing of many genes during cellular transformation [33–35]. It has been recently reported that hypermethylation of a CpG island (nucleotides −461 to −441) in MTAP gene

![Fig. 1. Expression of MTAP in control, HCC and cirrhotic human liver.](Image 1)

![Fig. 2. Expression of MTAP in control and CCl4-cirrhotic rat liver.](Image 2)
promoter occurs in human melanoma cells, and is responsible for the reduced expression of MTAP in this transformed cell type [18]. We examined the methylation status of MTAP gene promoter in three samples of cirrhotic human liver, two human HCC samples, three HCC cell lines and three samples of normal human liver. For this purpose, genomic DNA was incubated with the methylation-sensitive restriction enzyme NotI, and subsequently PCR amplified using primers flanking the CpG island containing the NotI restriction site. PCR amplification in HCC cells indicates the hypermethylation of MTAP promoter. Lower panel: MSP analysis of MTAP promoter methylation in control human liver and the HCC cell lines Hep3B and PLC (M: methylated, U: unmethylated). (C) Effect of 5-aza-dC treatment (10 μM for 48 h, solid bars) on MTAP gene expression in HuH7 and PLC cells as determined by real-time PCR. Data are mean±S.E. of two experiments performed in triplicate. Differences between control and treated cultures were statistically significant (*P<0.05). (D) Effect of TSA (100 nM) on MTAP gene expression in PLC cells. Data are mean±S.E. of two experiments performed in triplicate. Differences between control and treated cultures were statistically significant (*P<0.05).

Fig. 3. (A) Expression of MTAP in human HCC cell lines. MTAP mRNA levels were determined in Hep3B, HuH7, PLC and SK-Hep1 cell lines by real-time PCR. Values found in control human liver (mean±S.E., n=19) are shown. (B) Upper panel: methylation analysis of human MTAP promoter in samples from control human liver, cirrhotic human liver, human HCC and the HCC cell lines Hep3B, HuH7 and PLC. Genomic DNA samples were treated or not with the methylation-sensitive NotI restriction enzyme and subsequently amplified by PCR using primers flanking the CpG island containing the NotI restriction site. PCR amplification in HCC cells indicates the hypermethylation of MTAP promoter. Lower panel: MSP analysis of MTAP promoter methylation in control human liver and the HCC cell lines Hep3B and PLC (M: methylated, U: unmethylated). (C) Effect of 5-aza-dC treatment (10 μM for 48 h, solid bars) on MTAP gene expression in HuH7 and PLC cells as determined by real-time PCR. Data are mean±S.E. of two experiments performed in triplicate. Differences between control and treated cultures were statistically significant (*P<0.05). (D) Effect of TSA (100 nM) on MTAP gene expression in PLC cells. Data are mean±S.E. of two experiments performed in triplicate. Differences between control and treated cultures were statistically significant (*P<0.05).

4. Discussion

In this work we have evaluated the expression of MTAP in human HCC tissues and cell lines by real-time PCR, and found it to be significantly reduced when compared to normal human liver. It has been extensively reported that MTAP gene expression is lost in a wide variety of human transformed cell lines and tumors [12–17]. The MTAP gene has been mapped to the chromosome 9p21 region [17,36,37]. This region also contains the cell cycle regulatory genes p16INK4A and p14ARF, and is frequently
homozgyously deleted in primary tumors [38]. These deletions are quite large and often involve multiple genes, as evidenced by the high incidence of co-deletion of MTAP and p16INK4A in malignant cells [39]. Our data revealed that although reduced, MTAP gene expression was detected in the human HCC tissues and cell lines examined, and that allelic deletions were not detected in human liver cirrhosis, HCC samples or cell lines. These facts, together with previous reports demonstrating that the p16INK4A locus is not frequently deleted in human HCC [40,41], suggested the involvement of alternative mechanisms in MTAP down-regulation. The hypermethylation of CpG islands in the promoter regions of genes is associated with the aberrant silencing of transcription observed in cancer. Methylated CpG dinucleotides are bound by methyl cytosine-binding proteins, such as MeCP2, that can in turn recruit protein complexes including HDACs. Histone deacetylation alters the configuration of nucleosomes, promoting chromatin condensation and impairing gene transcription [34,35,42]. Interestingly, de novo hypermethylation of p16INK4A promoter is the most frequent somatic alteration of this gene found in human HCC [41]. Our present data show that MTAP promoter is also hypermethylated in human HCC cell lines. We were able to upregulate MTAP gene transcription in HCC cells using 5-aza-dC, a demethylating agent, and TSA, an inhibitor of HDACs. Together, these observations suggest that epigenetic mechanisms are likely to participate in the downregulation of MTAP expression in HCC, as has been previously demonstrated in melanoma cells [18].

Chronic liver injury, inflammation, fibrosis and finally liver cirrhosis often precede the development of HCC. Liver cirrhosis is thus considered a preneoplastic stage in this multistep process, and certain genetic and epigenetic alterations found in HCC, such as p16INK4A hypermethylation, can be detected in this condition [20,21,30,31,43]. In this context we have previously shown that the hepatic mRNA levels of the main enzymes involved in methionine metabolism are reduced in the cirrhotic human liver and HCC [20]. In particular, the expression of methionine adenosyltransferase 1A gene (MAT1A), responsible for AdoMet synthesis in the liver, is reduced in human and experimental cirrhosis, and silenced in HCC, through mechanisms involving chromatin remodeling [20,21,23]. These findings are in line with our current observations showing that MTAP gene expression is already impaired in human and CCl4-induced rat liver cirrhosis.

The expression of MTAP in human liver cirrhosis seemed lower than in HCC tissues. Although this difference did not reach statistical significance, there was indeed a tendency towards reduced expression in the first condition. It is worth mentioning that we have previously observed a similar situation for other genes involved in methionine metabolism. For example, the expression of betaine-homocysteine methyltransferase and methionine synthase were more compromised in a higher number of samples of liver cirrhosis than in HCC tissues [20]. It seems that the expression of methionine metabolism-related genes would be especially sensitive to the environmental conditions of liver cirrhosis.

It has been shown that re-expression of MTAP in cancer cell lines devoid of MTAP activity results in reduced cellular levels of MTA [44]. One could expect that the impairment in MTAP gene expression observed in our experimental model of liver cirrhosis could lead to the accumulation of this metabolite. However, the consequences of manipulating MTAP gene expression in a cancer cell line may be different from what occurs in chronic liver injury. In a similar model of CCl4-induced cirrhosis, it was previously reported that hepatic MTA levels were diminished, and that MTA treatment reduced liver damage [45]. In agreement with this, we have observed a substantial decrease in MTA contents in the liver of cirrhotic rats. One possible explanation for this situation may lay in the reduced ability of the cirrhotic liver to efficiently convert methionine into AdoMet, which is the metabolic precursor of MTA [23]. This is supported by our present findings showing a dramatic reduction in AdoMet levels in our model of rats chronically treated with CCl4.

Taken together, our findings suggest that in the chronically injured liver the metabolic flow through the methionine and adenine salvage pathways is impaired due to the combined deficiency in MTA availability and reduced MTAP gene expression and enzymatic activity. There are a number of potential pathogenic consequences for this situation. Considering that the endogenous production of adenine and adenine nucleotides is significantly dependent on MTAP activity [46], our present observations may contribute to explain the significant reduction in total adenine nucleotides found in the cirrhotic rat liver [47]. In addition, it has been recently reported that the reduction in downstream products of the methionine salvage pathway due to MTAP deletion may lead to ornithine decarboxylase (ODC) activation in human tumors [19]. ODC is the rate-limiting enzyme in the production of polyamines [4]. Elevated ODC activity has been reported in many human tumors and its overexpression is sufficient to cause transformation [4,48]. Re-expression of MTAP in a human mammary tumor cell line resulted in decreased ODC activity and tumorigenesis [19,49]. Consequently, our current observations may help to explain the elevation of ODC activity found not only in human HCC, but also in human and experimental chronically injured liver [50–52]. Hepatocarcinogenesis is a complex and multistep process and the precise genomic alterations that drive its development are not completely understood [28]. Aberrant promoter methylation and transcriptional inactivation of tumor suppressor genes is emerging as a major mechanism in the development of human cancer, and HCC is one of the tumor types with a high frequency of CpG island methylation [53–55]. Furthermore, CpG island hypermethylation is frequently observed in chronic liver disease and accumulates during the different stages of human hepatocarcinogenesis [43,55]. This situation has been described so far for a
number of genes that may be directly related to the control of cell growth [43,53–55]. The relevance of a persistent downregulation of MTAP gene expression for the development of cancer can be inferred from the recently observations showing the tumor suppressor activity of this gene [49]. However, in the case of hepatocarcinogenesis, the early loss of MTAP expression may have further significance. It is known that impairment of methionine metabolism in rodents by feeding a diet deficient in methionine and choline leads to the development of HCC [56], and we have recently shown that MATIA knockout mice spontaneously develop HCC [57]. These facts support a strong link between a defective methionine metabolism in the liver and the development of neoplasia. Our present observations on the hypermethylation and impairment of MTAP gene expression in preneoplastic stages support this notion.

In summary, we have shown that MTAP gene expression is compromised in liver cirrhosis and HCC probably through epigenetic mechanisms. The reversible nature of these epigenetic modifications [33–35], make MTAP a candidate gene for the application of alternative therapeutic and preventive interventions in liver cancer [59]. Indeed, clinical trials using low doses of demethylating agents have yielded encouraging responses in hematopoietic malignancies (reviewed in Ref. [35]). Inhibitors of HDACs have been shown to reactivate tumor suppressor genes, and are currently being developed for their potential use in cancer therapy in combination with demethylating agents [35,58]. Finally, strategies aimed at the direct inhibition of the enzymatic activity of the different DNA methyltransferases, using compounds that do not have to be incorporated into DNA, are also being considered [35].

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