RESEARCH ARTICLE

5-Aza-2'-deoxycytidine Induces Hepatoma Cell Apoptosis via Enhancing Methionine Adenosyltransferase 1A Expression and Inducing S-Adenosylmethionine Production

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Abstract

   In hepatocellular cancer (HCC), lack of response to chemotherapy and radiation treatment can be caused by a loss of epigenetic modifications of cancer cells. Methionine adenosyltransferase 1A is inactivated in HCC and may be stimulated by an epigenetic change involving promoter hypermethylation. Therefore, drugs releasing epigenetic repression have been proposed to reverse this process. We studied the effect of the demethylating reagent 5-aza-2'-deoxycitidine (5-Aza-CdR) on MAT1A gene expression, DNA methylation and S-adenosylmethionine (SAMe) production in the HCC cell line Huh7. We found that MAT1A mRNA and protein expression were activated in Huh7 cells with the treatment of 5-Aza-CdR; the status of promoter hypermethylation was reversed. At the same time, MAT2A mRNA and protein expression was significantly reduced in Huh7 cells treated with 5-Aza-CdR, while SAMe production was significantly induced. However, 5-Aza-CdR showed no effects on MAT2A methylation. Furthermore, 5-Aza-CdR inhibited the growth of Huh7 cells and induced apoptosis and through down-regulation of Bcl-2, up-regulation of Bax and caspase-3. Our observations suggest that 5-Aza-CdR exerts its anti-tumor effects in Huh7 cells through an epigenetic change involving increased expression of the methionine adenosyltransferase 1A gene and induction of S-adenosylmethionine production.

Keywords: 5-Aza-2'-deoxycytidine - MAT1A - S-adenosylmethionine - methylation - hepatocellular carcinoma

Introduction

   Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world; there is no drug intervention effective for the treatment of HCC (Huang et al., 2011; Villanueva et al., 2011). In the complex multistage process of hepatocarcinogenesis, the accumulation of epigenetic alterations that inactivate tumor suppressor genes and activate oncogenes is required to form a fully malignant tumor (Baylin et al., 1998). DNA hypermethylation is an alternative mechanism that disrupts tumor suppressor gene function (Fan et al., 2007).

   Methionine adenosyltransferase (MAT) is the only enzyme that can catalyze the biosynthesis of S-adenosylmethionine, which is the principal biological methyl donor in the liver cell (Lu et al., 2008). SAMe can regulate hepatocyte growth and apoptosis (Mato et al., 2007; Lu et al., 2008). Exogenous SAMe inhibits the growth of hepatoma cells and prevents development of HCC (Cai et al., 1998; Martínez-Chantar et al., 2006; Lu et al., 2009).

SAMe also induces apoptosis in human hepatoma cell lines via the mitochondrial death pathway (Yang et al., 2004), while SAMe protected against okadaic-induced apoptosis in normal hepatocytes (Ansorena et al., 2002). In mammals, two genes (MAT1A and MAT2A), encode for two homologous MAT catalytic subunits, MAT1A is expressed mostly in the liver, while MAT2A predominates in the fetal liver and is progressively replaced by MAT1A during development (Lu et al., 2008; Mato et al., 2008; Markham et al., 2009). Thus MAT1A can be considered a marker of differentiated liver phenotype. Our previous studies showed that hypermethylation of the MAT1A promoter may be one of events in the development of HCC (Zhang et al., 2013). Low expression of MAT1A is likely involved in the progression of the tumor and was found to be an independent factor for poor prognosis of patients with HCC. Therefore, it is a reasonable strategy to reverse MAT1A gene epigenetic change by using drugs releasing epigenetic repression.

   The demethylating reagent 5-aza-2'-deoxycitidine

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(5-Aza-CdR) inhibits DNA methyltransferases (DNMTs) and reverses DNA methylation (Christman., 2002). It has been found that 5-Aza-CdR can inhibit cancer cell growth, particularly the leukemia cells, and it has been applied for the treatment of myelodysplastic syndromes (Garcia-Manero., 2012). 5-Aza-CdR can inhibit hepatocellular carcinoma cells growth by inhibiting the telomerase activity and inducing p16 expression (Liu et al., 2001; Tao et al., 2012). However, the mechanisms underlying its anticancer activity and other biological effects are not fully understood.

In this study, we investigated the molecular mechanism underlying the effects of 5-aza-2'-deoxycytidine on MAT1A: MAT2A expression and SAMe production, meanwhile, we explored the effect of 5-Aza-CdR on apoptosis pathway in the Huh7 cell line. Our results demonstrated 5-aza-2'-deoxycytidine can reactivate MAT1A and inhibit MAT2A expression, induce S-Adenosylmethionine production of human hepatoma Huh7 cells through reversing the hypermethylation of Mat1A promoter, and it induced apoptosis through down-regulation of Bcl-2, up-regulation of Bax and caspase-3 in Huh7 cells.

Materials and Methods

Cell line culture

Human hepatocellular carcinoma cell line Huh7 were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in the recommended media supplemented with 10% (v/v) fetal bovine serum at 37°C in an incubator with 5% CO_2.

5-Aza-CdR treatment

Cells were seeded at a density of 5×10^5 cells per well in 6-well tissue culture plates and were allowed to attach over a 24 h period. The demethylating reagent 5-Aza-CdR (Sigma, USA) was added to a final concentration of 5 μmol/L,10 μmol/L and the cells grew for 48 h. The medium was changed 24 h after drug treatment.

RNA extraction and quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted with Trizol (Invitrogen, USA) from cells. 1μg total RNA was used for producing cDNA using the ReverTra Ace (Toyobo, Japan). Real-time PCR was performed using the All-in-oneTm qPCR Mix (GeneCopoeia, USA) in a MX3000P instrument (Stratagene, USA) according to the manufacturer's protocol. β-actin mRNA levels were used for normalization. PCR primer sequences are shown in Table1. The real-time PCR reaction (total volume: 20 μl) contained 1 μl cDNA, 10 μl All-in-oneTm qPCR Mix, 0.6 μl of each 10 μM forward primer and reverse primer, 0.4 μl 50×ROX reference dyes and 7.4 μl deionized water. The real-time PCR cycle was performed as follows: 95°C for 10 min, 40 cycles of 95°C for 10 s , 55°C for 20 s and 72°C for 15 s, and a dissociation stage.

Western blotting analysis

Cells were lysed in RIPA lysis buffer (Beyotime, China) with 1% ProteoBlockTm Protease Inhibitor Cocktail (Fermentas, Canada). An equal amount of protein (30 μg) was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto the nitrocellulose membrane. After blocking with 5% bovine serumal bumin, the membrane was probed with anti-MATα1 and anti-MATα2 (Santa Cruz, USA), developed with the BeyoECL Plus substrate system (Beyotime, China). Blots were stripped and re-probed with β-actin antibody (Santa Cruz, USA) to confirm equal protein loading. The anti-Bcl-2, anti-Bax (Santa Cruz, USA) and anti-cleaved-caspase-3 (Beyotime, China) were used to western blotting analysis on the same way.

Quantification of the Levels of SAMe by HPLC

SAMe contents were determined by reverse phase-HPLC, as described previously (Jiang et al., 2009). The contents were quantified using an Dionex ultimate 3000 (USA) system. The compounds were separated on a reversed-phase UltimateTM AQ-C18 column (5 μm, 4.6×250 mm; Welch, China) connected to an guard column (5 μm; Scienhome, China) at 26°C. The two mobile phases consist of (A) 5mM ammonium formate and 0.2% (v/v) formic acid aqueous solution (pH 3.0) and (B) HPLC-grade methanol (TEDIA, USA). The HPLC-grade ammonium formate, formic acid, SAM and N-(2-mercaptopropionyl)-glycine (internal standard, MPG) standards were purchased from Sigma-Aldrich (USA). The wavelength for detection was 254 nm. The column was equilibrated with 80% A : 20% B. The flow rate was 0.5 mL/min. The sample injection volume was 10 μl. The data were acquired and processed using Chromeleon software (Dionex, USA).

The collected cells were put in the solvents consisting of 50% A and 50% B at a density of 7.5×10^6 cells. The briefly vortex-mixed samples were stored at -20°C for 10 min. An following Ultrasonic Process (VCX130; Sonics, USA) were applied. The power intensity was 100W, frequency was 20KHz, reaction time was per 3 s with a 3 s interval for 3 times. The samples were centrifuged at $12,000 \times g$ for 15 min at 4°C and filtered through 0.45μM Millex-HV filters (Millipore, USA).

Calibration curves were constructed of six standard concentrations of SAMe and analyzed for five runs. For each curve, the absolute peak area ratios of SAMe to MPG were calculated and plotted against the nominal SAMe concentration. The concentrations of SAMe were determined using the equations of linear regression obtained from the calibration curves. Method validation including intra-day precision, inter-day precision, extraction recoveries and stabilities were analysed as described previously (Jiang et al., 2009; Krijt et al., 2009).

Methylation-specific polymerase chain reaction

Genomic DNA was extracted from cells using AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen, USA) according to the manufacturer's protocol. The gDNA was subjected to sodium bisulfite treatment using an EpiTect Bisulfite Kit (Qiagen, Germany). Fifty ng of bisulfitemodified DNA was subjected to methylation-

Table 1. Primer Sequences for qRT-PCR and MSP

Gene	Primer Sequence (5'-3')	Product size (bp)
β-actin-F	TCAGCAAGCAGGAGTATG	97
β-actin-R	GTCAAGAAAGGGTGTAACG	
MAT1A-F	AGAGTGCTTGTCCAGGTT	97
MAT1A-R	GCTCTCGCTCTGTCTTCT	
MAT2A-F	ATCTACCACCTACAGCCAAG	180
MAT2A-R	CCAACGAGCAGCATAAGC	
MAT1A-MF	TAAGTTGGAGGGAGGGATATATTTC	130
MAT1A-MR	CAAACTTATCTTTAACAAAACCACGA	
MAT1A-UF	TAAGTTGGAGGGAGGGATATATTTT	128
MAT1A-UR	AACTTATCTTTAACAAAACCACAAA	
MAT2A-MF	TTTGAATAGAGGATTGAAATATCGA	140
MAT2A-MR	TCGTAAACTTAAAAATAAAAACAACG	
MAT2A-UF	TTTGAATAGAGGATTGAAATATTGA	139
MAT2A-UR	CATAAACTTAAAAATAAAAACAACACT	

specific PCR (MSP) amplification, the methylation patterns of the MAT1A and MAT2A gene were analyzed. Methylated and unmethylated DNA primer pairs were designed as described previously (Wang et al., 2011; Frau et al., 2012; Zhang et al., 2013). Detailed sequences are listed in Table 1. The PCR reaction (total volume: 50 μl) contained 25 μl Specific™ Taq Master Mix (SinoBio, China), 2 μl bisulfite-treated gDNA, 2 μl of each forward primer and reverse primer (10 μM) and 19 μl deionized water. The PCR conditions were as follows: an initial denaturation at 94°C for 1 min 30 s, followed by 40 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 60 s, a final extension at 72°C for 5 min and a hold step at 4°C to cool. Amplified PCR products were fractionated using a 2.5% agarose gel containing ethidium bromide.

3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay and cells apoptosis analysis The Huh7 cells were seeded in 96-well plates and cultured with 5-Aza-CdR for 48 h. The cells were examined by a colorimetric 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL, Sigma) assay. The spectrophotometric absorbance was measured using a plate reader at 570 nm. To determine whether 5-Aza-CdR could effectively induce apoptosis of Huh7 cells, we treated it with various doses of 5-Aza-CdR for 48 h. The morphology of apoptosis was observed using Annexinv/PI apoptosis kit (MultiSciences, China) according to the manufacturer's protocol. The cells were repeatedly detected for five times with flow cytometry (Epics AltraII; Beckman Coulter, USA) and were analyzed with Expo32 V1.2 (Beckman Coulter, USA).

Statistical analysis

The relative mRNA expression of MAT1A and MAT2A analysis was performed using REST 2009 (Qiagen, Germany), Statistical analysis were performed using SPSS version 18.0 for Windows (IBM Software, USA), *P*-values of <0.05 were considered significant. GraphPad Prism 5 (GraphPad Software, USA) were used for graphs.

Results

5-Aza-CdR inhibited MAT2A expression and activated MAT1A expression in Huh7 cells

To investigate the effects of 5-Aza-CdR on MAT1A and MAT2A mRNA expression, Huh7 cells were cultured
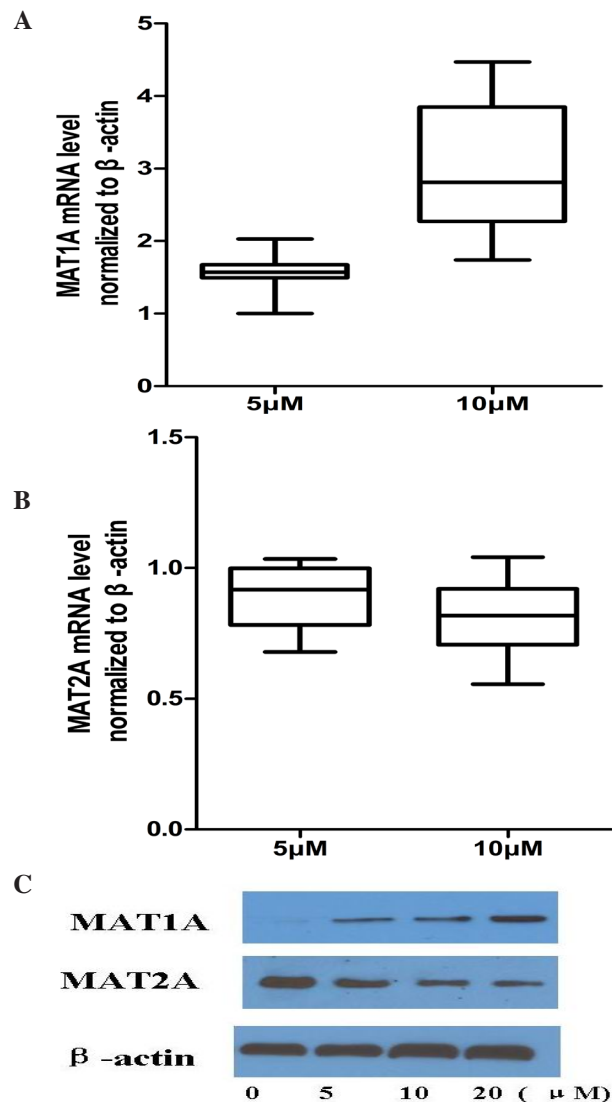

Figure 1. 5-Aza-CdR Inhibited MAT2A Expression and Activated MAT1A Expression in Huh7 Cells. The relative mRNA expression of MAT1A and MAT2A in Huh7 cells treated with 5-Aza-CdR to blank control group: A. Up-regulation of MAT1A mRNA expression was observed in Huh7 cells treated with 5-Aza-CdR, by maximal expression at 10 μmol/L 5-Aza-CdR. There was a 1.536-fold expression treated with 5 μmol/L 5-Aza-CdR (*P* =0.001), and a 2.863-fold expression with 10 μmol/L 5-Aza-CdR (*P*=0.001). B. Down-regulation of MAT2A mRNA expression was observed too, by minimum expression at 10 μmol/L 5-Aza-CdR. There was a 0.880-fold expression treated with 5 μmol/L 5-Aza-CdR, and a 0.799-fold expression treated with 10 μmol/L 5-Aza-CdR (*P* = 0.021). The protein expression of MAT1A (MATα1) and MAT2A (MATα2) in Huh7 cells treated with different 5-Aza-CdR concentration: C. Up-regulation of MAT1A protein expression was observed in Huh7 cells treated with 5-Aza-CdR, by maximal expression at 10 μmol/L 5-Aza-CdR. Down-regulation of MAT2A protein expression was observed too, by minimum expression at 10 μmol/L 5-Aza-CdR

with 0 μmol/L, 5 μmol/L and 10 μmol/L 5-Aza-CdR for 48 h. Up-regulation of MAT1A mRNA expression was observed in Huh7 cells treated with 5-Aza-CdR, by maximal expression at 10 μmol/L 5-Aza-CdR. There was a 1.536-fold expression treated with 5 μmol/L 5-Aza-CdR (*P* = 0.001), and a 2.863-fold expression with 10 μmol/L 5-Aza-CdR (*P* = 0.001). Down-regulation of MAT2A
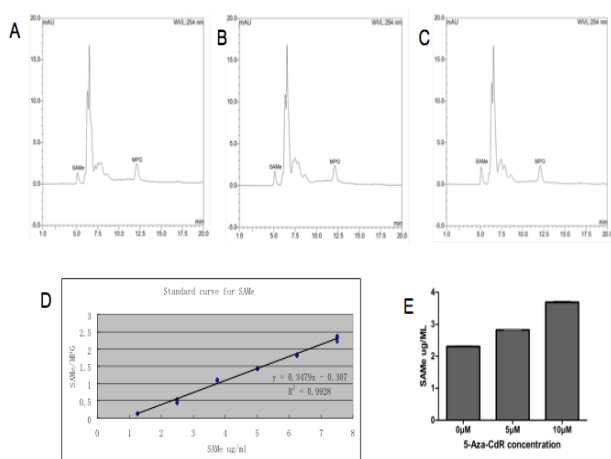
Figure 2. 5-Aza-CdR Induced S-Adenosylmethionine Production in Huh7 Cells. A. The HPLC curve of lysed Huh7 cells in blank control group containing 100 μg/ml MPG. B. The HPLC curve of lysed Huh7 cells treated with 5μmol/L 5-Aza-CdR containing 100 μg/ml MPG. C. The HPLC curve of lysed Huh7 cells treated with 10 μmol/L 5-Aza-CdR containing 100 μg/ml MPG. D. The regression equation of the curve and its correlation coefficient (R^2) was calculated as follows: y=0.3479x-0.307, R^2=0.9928. E. SAMe production were significantly induced in Huh7 cells treated with 5-Aza-CdR, by maximal production at 10 μmol/L 5-Aza-CdR. The increased concentration of SAMe was about 23% in the cells treated with 5 μmol/L 5-Aza-CdR (2.3037±0.006 vs 2.8226±0.010, $P<0.01$) and about 60% in the cells treated with 10μmol/L 5-Aza-CdR (2.3037±0.006 vs 3.688±0.013, $P<0.01$)

mRNA expression was observed too, by minimum expression at 10 μmol/L 5-Aza-CdR. There was a 0.880-fold expression treated with 5 μmol/L 5-Aza-CdR, and a 0.799-fold expression treated with 10 μmol/L 5-Aza-CdR ($P = 0.021$). The results revealed that the effect of 5-Aza-CdR on MAT1A and MAT2A mRNA expression varied in different 5-Aza-CdR concentration (Figure 1).

The results of Western blotting are analogous in three independent experiments.

Up-regulation of MAT1A protein expression was observed in Huh7 cells treated with 5-Aza-CdR, by maximal expression at 10 μmol/L 5-Aza-CdR. Down-regulation of MAT2A protein expression was observed too, by minimum expression at 10 μmol/L 5-Aza-CdR. The results revealed that the effect of 5-Aza-CdR on MAT1A and MAT2A protein expression varied in different 5-Aza-CdR concentration, and kept a consistency with mRNA expression (Figure 1).

5-Aza-CdR induced S-Adenosylmethionine production in Huh7 cells

SAMe production were observed by HPLC. Calibration curves was constructed. The linear regression analysis was constructed by plotting the SAMe/MPG peak-area ratio versus SAMe concentration (μg/mL). The range of concentrations was found to be suitable for the analysis of samples. The regression equation of the curve and its correlation coefficient (R^2) was calculated as follows: y=0.3479x-0.307, R^2=0.9928. The error for back-calculated concentration of each calibration point was within 10%. SAMe production were significantly induced
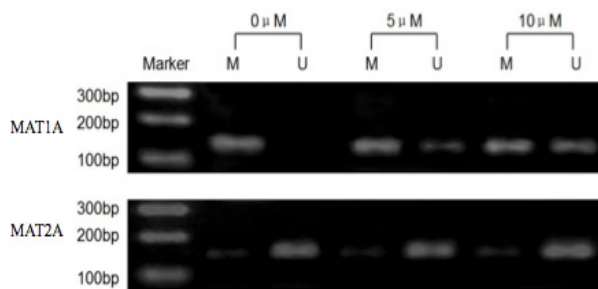
Figure 3. 5-Aza-CdR Increased Demethylation of MAT1A Promoter and Had No Obvious Effect on MAT2A Promoter Methylation in Huh7 Cells. The demethylation of MAT1A promoter was found in Huh7 cells treated with 5-Aza-CdR. However, 5-Aza-CdR showed no effects on the methylation sites of MAT2A promoter in Huh7 cells

in Huh7 cells treated with 5-Aza-CdR, by maximal production at 10 μmol/L 5-Aza-CdR. The increased concentration of SAMe was about 23% in the cells treated with 5 μmol/L 5-Aza-CdR ($P<0.01$) and about 60% in the cells treated with 10 μmol/L 5-Aza-CdR ($P<0.01$) (Figure 2).

5-Aza-CdR increased demethylation of MAT1A promoter and had no obvious effect on MAT2A promoter methylation in Huh7 cells

Since promoter methylation may be involved in MAT1A repression in HCC cells, we observed the effects of 5-Aza-CdR on promoter methylation of MAT1A and MAT2A gene using MSP. According to MSP analysis, the MAT1A promoter was found to be hypermethylated in Huh7 cells. The demethylation of MAT1A promoter was found in Huh7 cells treated with 5-Aza-CdR. However, 5-Aza-CdR showed no effects on the methylation sites of MAT2A promoter in Huh7 cells (Figure 3). These data suggested that the demethylation of MAT1A promoter by 5-Aza-CdR played an important role in up-regulation of MAT1A expression.

5-Aza-CdR inhibited cells growth and induced cells apoptosis by Bcl-2, Bax and caspase-3 pathway in Huh7 cells

To determine whether 5-Aza-CdR could effectively inhibit Huh7 cells growth, we treated it with various doses of 5-Aza-CdR for 48 h. We found that 5-Aza-CdR inhibited the growth of Huh7 cells. The growth inhibition was about 11% in the cells treated with 5 μmol/L 5-Aza-CdR ($P<0.01$) and about 19% in the cells treated with 10 μmol/L 5-Aza-CdR ($P<0.01$). With the administration of 5-Aza-CdR, more cells revealed nuclear condensation and fragmentation of apoptotic cell death. These results were confirmed by Annexin-V and propidium iodide staining and FACS analysis. The cells apoptosis of Huh7 cells was significantly enhanced treated with 5 μmol/L ($P<0.01$) and 10 μmol/L 5-Aza-CdR ($P<0.01$). These results suggested that 5-Aza-CdR could inhibit cells growth and induce cells apoptosis of Huh7 cells (Figure 4).

Meanwhile, we investigated the apoptosis pathway of Huh7 cells which 5-Aza-CdR induced. Up-regulation of Bax and cleaved-caspase-3 and down-regulation of Bcl-
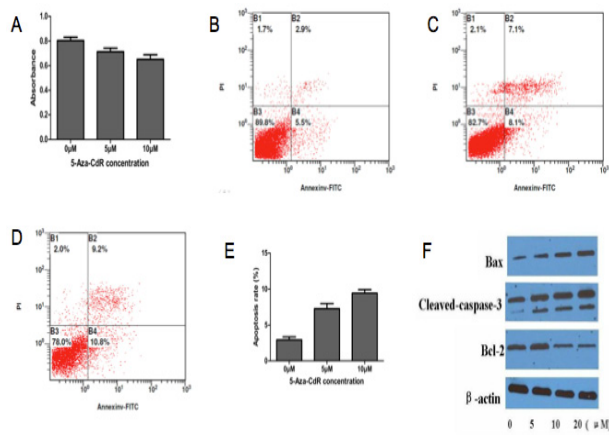
Figure 4. 5-Aza-CdR Inhibited Cells Growth and Induced Cells Apoptosis by Bcl-2, Bax and Caspase-3 Pathway in Huh7 Cells. 5-Aza-CdR inhibited the growth of Huh7 cells: A. The growth inhibition was about 11% in the cells treated with 5 μmol/L 5-Aza-CdR (*P*<0.01) and about 19% in the cells treated with 10μmol/L 5-Aza-CdR (*P*<0.01). 5-Aza-CdR induced apoptosis of Huh7 cells: B. The average apoptosis rate of Huh7 cells in blank control group was 2.92 %. C. The average apoptosis rate of Huh7 cells treated with 5 μmol/L 5-Aza-CdR was 7.28%. D. The average apoptosis rate of Huh7 cells treated with 10μmol/L 5-Aza-CdR was 9.44%. E. The apoptosis rate of Huh7 cells was significantly enhanced treated with 5-Aza-CdR (*P*<0.01). The protein expression of Bax, cleaved-caspase-3 and Bcl-2 in Huh7 cells treated with different 5-Aza-CdR concentration: F. Up-regulation of Bax and cleaved-caspase-3 and down-regulation of Bcl-2 protein expression was observed in Huh7 cells treated with 5-Aza-CdR

2 protein expression was observed in Huh7 cells treated with 5-Aza-CdR. The results revealed 5-Aza-CdR induced apoptosis in Huh7 cells through down-regulation of Bcl-2, up-regulation of Bax and caspase-3 (Figure 4). The results of Western blotting are analogous in three independent experiments.

Discussion

HCC is one of the most common malignancies in the world, there is no drug intervention effective for the treatment of HCC. Most malignancies exhibit an aberrant methylation of promoter regions of genes associated with loss of function (Baylin et al., 1998). This epigenetic process leads to malignant transformation (Fan et al., 2007). DNA methylation normally occurs at the cytosine residues within CpG dinucleotides through action of DNMTs. Methylation of CpG island is associated with delayed DNA replication, condensed chromatin and inhibition of transcription initiation (Tazi et al., 1990; Jones et al., 1999). Alterations in methylation status have been recognized as an epigenetic mechanism of selection during tumorigenesis in this type of cancer and a correlation between methylation patterns of HCC and their pathological features have been suggested (Lee et al., 2003). In addition, the methylation of specific genes is a possible way for the tumor to block some key pathways (Ueki et al., 2000; Xie et al., 2006).

The most widely used demethylating agent, 5-Aza-CdR, was first characterized 30 years ago and it functions as a mechanism-dependent suicide inhibitor of DNA methyltransferases, with which genes silenced by hypermethylation can be reactivated (Watanabe et al., 2010). It has been found that Mat1A:Mat2A switch and low SAM levels, associated with CpG hypermethylation of Mat1A promoter, and prevalent CpG hypomethylation in Mat2A promoter of fast growing HCC (Frau et al., 2012). Forced MAT1A overexpression in HepG2 and Huh7 cells led to rise in SAMe level, decreased cell proliferation, increased apoptosis (Li et al., 2010; Frau et al., 2012).

However, there is not much research on the correlation of SAMe level and methylation status of MAT1A with 5-Aza-CdR. In this study, we first examined the mRNA level of MAT1A and MAT2A using qRT-PCR. Our results showed that MAT1A and MAT2A mRNA expression were significant respectively up-regulated and down-regulated in Huh7 cells treated with 5-Aza-CdR. Subsequently, MAT1A and MAT2A protein expression were significant respectively up-regulated and down-regulated in Huh7 cells treated with 5-Aza-CdR. Our results demonstrated that MAT1A expression was reactivated and MAT2A expression was inhibited in Huh7 cells treated with 5-Aza-CdR, which is consistent with previous studies (Torres et al., 2000). We then investigated SAMe level in Huh7 cells by HPLC. We found SAMe production was significantly induced in Huh7 cells treated with 5-Aza-CdR. Furthermore, We investigated the gene promoter methylation status in Huh7 cells using MSP. The MAT1A promoter is hypermethylation in HCC cells, company with hypomethylation of MAT2A promoter (Yang et al., 2001). We investigated some methylation sites of MAT1A and MAT2A, The demethylation of MAT1A promoter was found in Huh7 cells treated with 5-Aza-CdR, However, 5-Aza-CdR showed no effects on the methylation sites of MAT2A promoter in Huh7 cells. We inferred 5-Aza-CdR could affect Mat1A promoter, and had no effects on Mat2A promoter, the inhibited MAT2A expression maybe caused by the induced Mat1A expression and need a further study. Meanwhile, we investigated whether 5-Aza-CdR could effectively inhibit Huh7 cells growth and apoptosis. We found that 5-Aza-CdR inhibited the growth of Huh7 cells and induced apoptosis in Huh7 cells. Furthermore, We investigated 5-Aza-CdR induced apoptosis in Huh7 cells through down-regulation of Bcl-2, up-regulation of Bax and caspase-3, we inferred increased SAMe production by 5-Aza-CdR caused the result (Frau et al., 2012).

In conclusion, the demethylating reagent 5-Aza-CdR could reactivate MAT1A and inhibit MAT2A expression, induce SAMe production of human hepatoma cell through reversing the hypermethylation of Mat1A promoter, it inhibited the growth of Huh7 cells and induced apoptosis in Huh7 cells through down-regulation of Bcl-2, up-regulation of Bax and caspase-3. Our findings may provide insights into one of the mechanisms through which 5-Aza-CdR exerts growth-inhibitory effects on HCC cells.

Acknowledgements

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