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Arsenic silences hepatic PDK4 expression through activation of histone H3K9 methyltransferase G9a

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Abstract

It is well established that increased liver cancer incidence is strongly associated with epigenetic silencing of tumor suppressor genes; the latter is contributed by the environmental exposure to arsenic. Pyruvate dehydrogenase kinase 4 (PDK4) is a mitochondrial protein that regulates the TCA cycle. However, the epigenetic mechanisms mediated by arsenic to control PDK4 expression remain elusive. In the present study, we showed that histone methyltransferase G9a and Suv39H-mediated histone H3 lysine 9 (H3K9) methylations contributed to PDK4 silencing in hepatic cells. The PDK4 expression was induced by G9a inhibitor BRD4770 (BRD) and Suv39H inhibitor Chaetocin (CHA). In contrast, arsenic exposure decreased PDK4 expression by inducing G9a and increasing H3K9 di- and tri-methylations levels (H3K9me_{2/3}). In addition, arsenic exposure antagonizes the effect of BRD by enhancing the enrichment of H3K9me_{2/3} in the PDK4 promoter. Moreover, knockdown of G9a using siRNA induced PDK4 expression in HCC cells. Furthermore, arsenic decreased hepatic PDK4 expression as well as diminished the induction of PDK4 by BRD in mouse liver and hepatocytes. Overall, the results suggest that arsenic causes aberrant repressive histone modification to silence PDK4 in both HCC cells and in mouse liver.

Graphical abstract

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Keywords

PDK4; Arsenic; H3K9 methylation; G9a; BRD4770

Introduction

Pyruvate dehydrogenase kinase isoenzyme 4 (PDK4) aids in regulation of the pyruvate dehydrogenase complex through inactivation of pyruvate dehydrogenase (PDH) via phosphorylation (1). The PDK4 gene encodes for the PDK4 protein within the mitochondria and has recently been linked to tumor proliferation due to its function in glucose metabolic pathways (2). Several types of cancers exhibited low expression of PDK4. For instance, PDK4 was significantly down-regulated in prostate cancer and was suggested as a potential marker for the diagnosis of prostate cancer (3). Aberrant PDK4 expression resulting from epigenetic change was also observed in colon cancer (4). Many epigenetic modulators associated with histone lysine methylations (e.g. LSD1, G9a and Suv39H1/2 and EZH2) have been shown to be highly expressed in liver tumor tissues promoting liver cancer progression (5–7). Hence, a goal of this study is to understand the molecular regulation of PDK4 expression by epigenetic modulators.

Arsenic (sodium arsenite, NaAsO_2) is commonly found in drinking water worldwide. Arsenic exposure has been associated with alterations of tumor suppressor function, which is frequently downregulated by epigenetic mechanisms in hepatocellular carcinoma (HCC) (8–10). Over the past decade, a growing body of evidence has shown that epigenetic modifications play a significant role in arsenic-induced adverse effects on human health (11, 12). Arsenic was shown to increase histone modifications in both *in vitro* and *in vivo* studies (13, 14). A second goal of this study is to examine a crosstalk between arsenic and epigenetic modulators to control PDK4 expression in HCC cells.

In this study, we showed that inhibiting H3K9 methyltransferases G9a and Suv39H1 by BRD and CHA, respectively, induced PDK4 expression in HCC cells. In addition, arsenic exposure decreased PDK4 expression, which correlated with the increased levels of H3K9me2/me3. Moreover, arsenic diminished the induction of PDK4 by BRD in HCC cells

and in mouse liver, and enhanced H3K9me2/me3 recruitment to the PDK4 promoter. Our results provide a mechanistic insight into the role of arsenic in modulating PDK4 expression.

Materials and Methods

Cell culture and treatment

Human HCC cell lines Huh7, Hep3B, MH97H and PDK4-MH97H cells with stable PDK4 overexpression were maintained in DMEM (Gibco, NY) with 10% fetal bovine serum (Gibco) and 100 U/ml penicillin-streptomycin (Mediatech, VA) (15–17). PDK4-MH97H cells were generated by retroviral gene transfer with recombinant MSCV expression vectors (pMSCVPDK4). Isolation and culturing of primary hepatocytes were described previously (18, 19). For small-molecule inhibitor treatment, Huh7, Hep3B and MH97H cells were treated with vehicle control (DMSO), 2 and 10 μ M of BRD4770 (Sigma, MO) or 0.1 and 0.5 μ M of Chaetocin (Enzo Life Sciences, NY) for 48 h. For arsenic exposure, Huh7, Hep3B and PDK4-MH97H cells were cultured in growth medium containing various concentrations of NaAsO₂ (Sigma) (0.1–50 μ M) for 3 ~ 24 h. For a combination treatment, cells were cultured in medium containing DMSO control (Ctrl group), 24h 1.0 μ M NaAsO₂ (As group), 48h 10 μ M BRD4770 (BRD group) and 10 μ M BRD4770 plus 1.0 μ M NaAsO₂ (BRD + As group). G9a siRNA (sc-43777) was purchased from Santa Cruz.

Mouse study and treatment

Wild-type C57BL/6 mice were purchased from the Jackson Lab, ME. Eight-week-old male mice were randomly assigned to four treatment groups (n=5/group), including control (Ctrl), arsenic exposure (As), BRD injection (BRD), and arsenic exposure plus BRD injection (BRD + As). Mice in Ctrl and As were injected i.p. 3X weekly with vehicle solution 1% DMSO / PBS (20 ml/kg). Mice in BRD and BRD + As were injected i.p. 3X weekly with BRD (1 mg/kg b.w.) diluted in 1% DMSO / PBS. Mice in As and BRD + As were fed with 50 ppm or 200 ppm NaAsO₂ (Sigma) via distilled drinking water. The arsenic water was replaced every other day. All mice were maintained on animal chow with free access to food and water for the duration of the study. Mice were sacrificed four-weeks later, at which point blood and liver tissues were collected for further analysis (20, 21). Protocols for the animal studies were approved by the Institutional Animal Care and Use Committee at the University of Connecticut.

Protein isolation and analysis

Whole-cell lysate and liver lysates were prepared using RIPA buffer (50 mM TrisHCl pH7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS) containing a protease inhibitor cocktail (Roche, IN) (22). The extracted proteins were boiled in SDS sample buffer and analyzed by Western blotting (23, 24). The anti-PDK4 antibody was purchased from Abgent, CA and from Dr. Robert Harris, a Professor Emeritus of Indiana University; anti-G9a antibody from Sigma; antitotal H3 and anti-H3K9me3 antibodies from Abcam, MA; anti-H3K9me2 antibody from Active Motif, CA, and anti-GAPDH antibody from Cell Signaling, MA.

Quantitative real-time RT-PCR (qPCR)

Total RNAs of cells and liver tissues were extracted using TRIzol reagent (Thermo Fisher, NY) (19, 25). One microgram of isolated RNA was reverse transcribed using an iScript RT reagent kit (Bio-Rad, MA). Human and mouse PDK4 mRNAs were measured using the specific primer sets listed in Supplementary Table S1 and iTaq Universal SYBR Green Supermix (Bio-Rad). Human and mouse GAPDH mRNAs were also measured as internal controls for normalization.

Quantitative chromatin immunoprecipitation (qChIP)

qChIP analysis was performed essentially as described previously (18, 26). Briefly, cells or minced liver tissues were crosslinked with 1% formaldehyde (Sigma) for 10 min at room temperature, and the reaction was stopped by 125 mM glycine. Nuclei were isolated by resuspending the cells in swelling buffer containing 5 mM PIPES pH 8.0, 85 mM KCl, 1% NP-40 and complete protease inhibitors for 20 min at 4 °C. The isolated nuclei were resuspended in nuclei lysis buffer (50 mM Tris pH 8.0, 10 mM EDTA, 1% SDS) and sonicated using a bioruptor sonicator (Thermo Fisher). Samples were pooled and immunoprecipitated with 4 g of H3K9me2 (Active Motif), H3K9me3 (Abcam), G9a (Abcam) and IgG (Cell Signaling) antibodies overnight at 4°C. Immunoprecipitates were washed twice with dialysis buffer (50 mM Tris pH 8.0, 2 mM EDTA, 0.2% Sarkosyl) and four times with IP wash buffer (100 mM Tris pH 8.0, 500 mM LiCl, 1% NP-40, and 1% deoxycholic acid sodium salt). After reverse crosslinking was performed, the DNA was eluted and purified using a PCR purification kit (Qiagen, Hilden, Germany). The primer sequences used for qChIP analyses are listed in Supplementary Table S2 and the PDK4 promoter region amplified by qChIP analyses are shown in Supplementary Fig. S1 & S2.

Cell viability measurements

All cell viability experiments were conducted with propidium iodide (PI) (27). In brief, BRD, CHA or arsenic treated cells were fixed in cold and stained in 50 mg/ml PI (Becton Dickinson, NJ) containing 100 mg/ml DNase-free RNase A (EMD Millipore, Darmstadt, Germany) for 30 min. Cells were then analyzed immediately via FACS analyzer (Becton Dickinson).

Statistical analysis

All the experiments were performed in triplicate and repeated at least two times. The data presented as the mean values $\pm$ standard error of the mean (SEM). Statistical analysis was carried out using the Student *t* test for unpaired data to compare the values between the two groups. *P* < 0.05 was considered statistically significant.

Results

H3K9me2/3 inhibitors BRD and CHA induce PDK4 expression in HCC cells

In the past decade, numerous selective small-molecule inhibitors of chromatin modulators were developed. Given the importance of epigenetic mechanisms in the regulation of gene transcription and the previous findings that four epigenetic modulators LSD1, G9a,

Suv39H1 and EZH2 were overexpressed in HCC tissues (5–7), we assessed their effects on PDK4 expression in HCC cells. BRD (BRD4770, inhibitor of H3K9 methyltransferase G9a) and CHA (Chaetocin, inhibitor of H3K9 methyltransferases G9a and Suv39H1) (Table 1) induced PDK4 mRNA in Huh7, Hep3B and MH97H cells (Fig. 1A-1B). The highest concentration of BRD (10 μ M) and CHA (0.5 μ M) did not affect cell viability (data not shown). In contrast, Trichostatin A (TSA) (inhibitor of H3K4 demethylase LSD1) and EPZ-6438 (inhibitor of H3K27 methyltransferase EZH2) showed no effect to activate PDK4 expression (data not shown). Consistently, the PDK4 protein levels were also significantly increased in BRD and CHA treated Huh7, Hep3B and MH97H cells (Fig. 1C-1D). BRD and CHA selectively inhibit the catalytic activity of the H3K9 methyltransferases G9a and SUV39H1, which mainly mediate H3K9me2 and H3K9me3 on histone proteins (28). Taken together, these results suggest that methyltransferases of H3K9, other than H3K4 or H3K27, may contribute to PDK4 silencing in liver cancer cells.

In eukaryotic species, H3K9me2/3 are predominant marks of heterochromatin formation and subsequent gene silencing. We reasoned that BRD- and CHA-mediated H3K9me2/3 inhibition could contribute to PDK4 induction. Whole cell lysates prepared from untreated and treated HCC cells were analyzed by Western blot with H3K9me2/3 and H3 antibodies. BRD (10 μ M) significantly diminished both global histone H3K9me2 and H3K9me3 levels whereas CHA (0.5 μ M) decreased only H3K9me3 levels (Fig. 1C-1D). In addition, we observed a substantial decrease of G9a protein in BRD treated cells, but not in CHA treated cells. The results confirmed that the inhibition of H3K9me2 and/or H3K9me3 by BRD or CHA was associated with upregulation of PDK4. Considering the fact that CHA inhibits enzymatic activities of both Suv39h and G9a (29) whereas BRD exhibits a higher selectivity (G9a only) and stronger inhibition towards G9a, we decided to focus on BRD in the subsequent studies.

Arsenic induces global H3K9 me2/3 levels and reduces PDK4 expression in HCC cells

Arsenic exposure can drive H3K9 methylations causing epigenetic silencing of tumor suppressor genes, which leads to tumorigenesis (30, 31). Based on our results that PDK4 expression was mediated by histone H3K9me2/3 in HCC cells, we speculated that arsenic exposure would deregulate the expression of PDK4 through the alteration of H3K9me2/3.

In Huh7 and Hep3B cells, high-dose arsenic exposure resulted in a significant, time dependent decrease of PDK4 mRNA and protein (Fig. 2A). In addition, the ectopically expressed PDK4 in MH97H-PDK4 cells (Supplementary Fig. S3) was downregulated by arsenic in a time-dependent fashion (Fig. 2B, left). Interestingly, G9a protein was transiently induced in cells treated with arsenic (3h, 6 h), whereas H3K9me2,me3 levels were constantly elevated in arsenic treated cells (Fig. 2B, right).

Because the environmental levels of arsenic that humans are normally exposed to are at a lower level (less than 1 μ M in drinking water) (32), we next tested the effect of arsenic on PDK4 expression under a lower dose range (0.1 ~ 2.5 μ M) and longer exposure time (24 h). We found that arsenic at 0.5 μ M (Hep3B) or 1.0 μ M (Huh7) showed an effective reduction of PDK4 mRNA (Fig. 2C) and protein (Fig. 2D). Interestingly, the H3K9me2 and H3K9me3 levels were increased by arsenic in a concentration dependent manner in Huh7 and Hep3B

cells (Fig. 2D). The G9a protein showed an induction by 0.1 μ M arsenic in Huh7 and Hep3B cells, which subsequently declined following higher doses of arsenic exposure (Fig. 2D). Overall, the results suggest that arsenic-mediated transcriptional silencing of PDK4 is likely associated with the elevated H3K9me2/3 levels in HCC cells.

Arsenic enhances the recruitment of H3K9me2/3 in the PDK4 promoter in HCC cells

Next, we determined whether there was a crosstalk between G9a and arsenic to regulate PDK4 expression. To further determine how arsenic inhibits PDK4 gene transcription, we conducted qChIP assays (27). We first identified several potential regions within the 3.0K upstream of the human PDK4 gene based on information obtained from the chromatin immunoprecipitation data (<http://genome.ucsc.edu/>) (Supplementary Fig. S1 and Fig. S2). Because we could not find publically available H3K9me2/3 database for human cells we used H3K27 mark as a reference. The size of chromatin fragment is presented in Supplementary Fig. S4A. qPCR did not detect any signal from an unrelated site (Supplementary Fig. S4B). Our pilot qChIP results showed that the 3 amplicons from TSS to the distal promoter region (+0.5, 1.4 and 3.0 K TSS) detected strong enrichment for H3K9me2/3 and G9a. Therefore, these three regions were analyzed further. Overall, the H3K9me2 (Fig. 3A) and H3K9me3 (Fig. 3B) marks (Ctrl) were enriched in the proximal PDK4 promoter region as well as the distal region. The G9a binding signal was also enriched in these areas, especially in the 3.0K upstream region of the transcription start site (+3.0K TSS) (Fig. 3C).

Arsenic treatment markedly increased the accumulation of H3K9me2/3 in the human PDK4 promoter regions (+0.5, 1.4 and 3.0 K TSS), whereas G9a was enriched by arsenic in +1.4 and 3.0K regions from the TSS. The recruitment of H3K9me2/3 and G9a to the human PDK4 promoter was diminished to a certain extent by BRD. The enrichment of BRD was fully or partially prevented by arsenic.

qPCR and Western blot analysis confirmed that the induction of PDK4 mRNA and protein by BRD was completely suppressed by arsenic exposure in Huh7 cells (Fig. 3D-3E). The decreased G9a protein by BRD was in agreement with the reduced recruitment of G9a to the PDK4 promoter. Next, we conducted G9a knockdown experiment to further examine its direct involvement in the regulation of PDK4. G9a knockdown increased PDK4 expression in HCC cells (Fig. 3F). Overall, the results suggest that arsenic inhibits PDK4 gene transcription by increasing H3K9me2/3 marks to the PDK4 promoter.

Arsenic exposure inhibits BRD-mediated induction of PDK4 in mouse liver

To further assess the cross-regulation of BRD and arsenic on PDK4 expression *in vivo*, C57BL/6J mice were exposed to arsenic (50 ppm) in drinking water for four weeks or received BRD by i.p. injection alone or in combination with arsenic. As shown in Fig. 4A, hepatic PDK4 mRNA expression was significantly induced by BRD compared with the control (Ctrl group). Although arsenic alone did not decrease PDK4 mRNA, it effectively prevented the induction of PDK4 by BRD. Similarly, the induction of PDK4 protein in BRD treated liver was decreased by arsenic (Fig. 4B). In addition, the G9a protein as well as H3K9me2/m3 levels were decreased by BRD to varying extents, which were partially or

fully restored by arsenic. The results from a higher concentration of arsenic treatment (200 ppm) for two weeks were compatible to the low dose arsenic treatment (Fig. 4C). A similar result was observed in primary hepatocytes (Fig. 4D).

qChIP assay was used to determine H3K9me2/3 marks in the mouse PDK4 promoter *in vivo* (Supplementary Fig. S2 for primer locations). Similar to what were observed in HCC cells, arsenic markedly enhanced the enrichment of H3K9me2/3 marks in the mouse PDK4 promoter as well as lessened the effect of BRD (Figs. 4E-4F).

Overall, our results provide evidence for a crosstalk between arsenic and BRD to control PDK4 expression *in vivo* in mouse liver and *in vitro* in hepatic cells.

Discussion

Despite conflicting evidence defining the role of PDK4 in tumorigenesis, either promoting (2, 33) or repressing carcinogenesis (3, 4); it is certain that PDK4 is an important factor to be considered in tumorigenesis. Hepatic PDK4 expression is regulated by insulin, fatty acids and hormone receptors (34–36). PDK4 expression in liver cancer cells is also regulated by lipopolysaccharide (LPS) (37). A major finding of our study is the discovery of arsenic-mediated epigenetic control of PDK4 expression involving the histone H3K9 di- and tri-methylations.

Chronic environmental exposure to arsenic contributes to the initiation of liver disease including HCC (32, 38). Previous studies have shown that arsenic disrupts a wide range of epigenetic modifications (11). In the present study, we have shown that arsenic exposure results in a transient activation of G9a but a sustained elevation of H3K9m2/m3 methylation, which contributes to PDK4 gene silencing.

Histone methylation represents one of the most frequent epigenetic modifications to control gene expression during the development of cancer. H3K9 di-methylation catalyzed by G9a and Glp (G9a-like protein) is elevated in arsenic treated lung cancer cells A549 (14). In mouse myoblast cells, arsenic represses myogenin expression by enhancing promoter recruitment of Glp/EZH2/DNMT (31). In human HCC, several histone methylation modulators including G9a, EZH2, Suv39H1/2 and LSD1 are overexpressed (5–7). Using specific inhibitors of H3K9me2/3, we show that methylation of H3K9, but not H3K4 and K27, is the histone methylation required for silencing PDK4 in HCC cells. BRD was previously discovered as a substrate-competitive inhibitor that selectively decreases G9a enzymatic activity and reduces cellular levels of H3K9me2 & me3 (39, 40). In contrast, CHA is a non-selective natural inhibitor with more specificity against Suv39H1/2 and H3K9me3 (29). We found that both drugs induced PDK4 expression in HCC cells. More intriguingly, BRD repressed G9a protein expression in HCC cells and in mouse liver, in addition to its known function to inhibit G9a enzymatic activity and H3K9me2/3. On the other hand, CHA showed a striking inhibition of H3K9me3 with little effect on the global levels of G9a and H3K9me2. Therefore, at least in the cellular context of HCC cells, BRD exhibits a broader substrate specificity than CHA. The H3K9me2/3 marks catalyzed by G9a

serves as a potential molecular mechanism in arsenic-mediated reduction of PDK4 expression.

Thus far, arsenics effect *in vivo* on histone modification has not been well characterized. One study reported that 6-month exposure to 50 ppm of arsenic in drinking water induced H3K9me2 which was associated with an increase in G9a recruitment to chromatin (13). Arsenic levels in the liver were greatly increased after one month of arsenic exposure at the dose of 100 ppm (41). In our study, one month of 50 ppm arsenic exposure enhanced H3K9me2 and me3 marks in the PDK4 promoter region, which was accompanied by the reduction of PDK4 protein. Therefore, arsenic is involved in modulating BRD-mediated PDK4 expression by enhancing the occupancy of H3K9me2 and me3 marks in PDK4 promoter thus altering the state of chromatin.

In conclusion, we elucidated an epigenetic mechanism that controls PDK4 expression via arsenic-mediated modulation of H3K9 methylation. Our findings shed light on a better understanding of the carcinogenicity of arsenic in HCC. Future studies are required to further elucidate the function of PDK4 in HCC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Histone methyltransferase G9a inhibitor BRD induces PDK4 expression
- Arsenic decreases PDK4 expression and increases H3K9me2 and me3 levels
- Arsenic enhances H3K9me2/me3 enrichment in the PDK4 promoter
- Arsenic antagonizes the activation of PDK4 by BRD

Figure 1. PDK4 expression was induced by H3K9me2/3 inhibitors BRD and CHA

A-B qPCR analysis of PDK4 mRNA expression (normalized by GAPDH) in DMSO control (Ctrl), BRD (A) and CHA (B) treated HCC cells. Data are represented as means $\pm$ S.D. * $P < 0.05$, ** $P < 0.01$ vs Ctrl.

C-D Western blot analysis of PDK4 and G9a protein and H3K9me2/me3 levels in DMSO control (Ctrl) and BRD (10 μ M) (C) and CHA (0.5 μ M) (D) treated HCC cells. GAPDH and total H3 were used as loading controls.

Figure 2. PDK4 expression was suppressed by arsenic via induction of H3K9me2/me3

A qPCR and Western blot analysis of endogenous PDK4 mRNA and protein expression in arsenic treated HCC cells. Data are represented as means $\pm$ S.D. * P < 0.05, ** P < 0.01 vs Ctrl.

B Left: qPCR of PDK4 mRNA expression in PDK4-MH97H cells treated with arsenic. Data are represented as means $\pm$ S.D. * P < 0.05, ** P < 0.01 vs Ctrl. Right: Western blot analysis of PDK4 and G9a protein, as well as H3K9me2 and m3 levels in PDK4-MH97H cells treated with arsenic.

C qPCR analysis of PDK4 mRNA in HCC cells treated with low doses of arsenic. Data are represented as means $\pm$ S.D. * P < 0.05, ** P < 0.01 vs Ctrl.

D Western blot of PDK4 and G9a protein expression and H3K9me2/me3 levels in HCC cells treated with low doses of arsenic. GAPDH and total H3 were used as loading controls.

Figure 3. Arsenic enhanced H3K9me2/me3 marks in the PDK4 promoter in HCC cells

A-C ChIP assays to examine histone H3K9me2 (A), H3K9me3 (B) and G9a (C) occupancy in the PDK4 promoter in Huh7 cells. Ctrl, DMSO control; As, arsenic exposure (1.0 μ M, 24h); BRD, BRD treatment (10 μ M, 48h); BRD + As, BRD plus arsenic. The results were quantitated as the mean ratio of precipitated DNA to input DNA as determined by qPCR. Data are represented as means $\pm$ S.D. * P < 0.05, ** P < 0.01 vs Ctrl; # p < 0.05, ## p < 0.01 vs BRD.

D qPCR analysis of PDK4 mRNA expression in Huh7 cells treated with arsenic (1.0 μ M) without or with BRD (10 μ M). Data are represented as means $\pm$ S.D. * P < 0.05, ** P < 0.01 vs Ctrl.

E Western blot analysis of PDK4 and G9a proteins in Huh7 cells treated with arsenic (1.0 μ M) without or with BRD (10 μ M). GAPDH was used as a loading control.

F Western blot analysis of PDK4 and G9a protein in Huh7 cells with G9a knockdown (100 nM siRNA). GAPDH was used as a loading control.

Figure 4. Arsenic enhanced H3K9me2/me3 marks in the PDK4 promoter in mouse liver

A qPCR analysis of PDK4 mRNA expression in mouse liver. Ctrl, DMSO control; As, arsenic exposure (50 ppm, 4 weeks); BRD, BRD treatment (1 mg/kg b.w.); BRD + As, BRD plus arsenic. Data are represented as means $\pm$ S.D. ** $P < 0.01$ vs Ctrl; ## $p < 0.01$ vs BRD.

B Western blot analysis of PDK4 and G9a proteins and H3K9me2/me3 levels in mouse liver. GAPDH and total H3 were used as loading controls. $n=5$ /group. Equal amount of proteins pooled from five individual mice were loaded in duplicate.

C Western blot analysis of PDK4 protein in mouse liver and primary hepatocytes.

D-E ChIP assays to examine histone H3K9me2 (C) and H3K9me3 (D) occupancy in the PDK4 promoter in mouse liver. Ctrl, DMSO control; As, arsenic exposure (50 ppm, 4 weeks); BRD, BRD treatment (1 mg/kg b.w.); BRD + As, BRD plus arsenic. The results were quantitated as the mean ratio of precipitated DNA to input DNA as determined by qPCR. Data are represented as means $\pm$ S.D. * $P < 0.05$, ** $P < 0.01$ vs Ctrl; # $p < 0.05$, ## $p < 0.01$ vs BRD.

Table 1

Information of small-molecule inhibitors

Target enzyme	Enzymatic activity	Methylated lysine residue	Transcriptional function of the enzyme	Small-molecule inhibitor
LSD1	Demethylation	H3K4	Repression	Trichostatin A (TSA)
G9a	Methylation	H3K9	Repression	BRD4770 (BRD)
Suv39H	Methylation	H3K9	Repression	Chaetocin (CHA)
EZH2	Methylation	H3K27	Repression	EPZ-6438