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Epigenetic loss of CDH1 correlates with multidrug resistance in human hepatocellular carcinoma cells

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ABSTRACT

Promoter CpG hypermethylation of tumor suppressor genes is an essential step in cancer progression but little is known about its effect on cancer multidrug resistance. In this study, we showed that CDH1 promoter was hypermethylated in drug resistance of a doxorubicin-induced multidrug resistant hepatocellular carcinoma cell line R-HepG2. Transfection of CDH1 cDNA into R-HepG2 cells led to increased amount of doxorubicin uptake, decreased cell viability, decreased P-glycoprotein expression and increased apoptotic population of cells exposed to doxorubicin. Proto-oncogene tyrosine-protein kinase FYN was over-expressed in R-HepG2 cells which displayed a negative correlation with the expression of CDH1. FYN was knocked down in R-HepG2 cells, leading to less drug resistance by increased cell viability, increased doxorubicin uptake and attenuated P-glycoprotein expression. Our findings identified epigenetic silencing of CDH1 in cancer cells might be a new molecular event of multidrug resistance.

1. Introduction

One of the main causes of treatment failure in chemotherapy is the acquirement of multidrug resistance (MDR) [1]. Mechanistic studies showed that MDR can limit the effectiveness of chemotherapeutics through decreased drug uptake, increased drug efflux, increased DNA damage repair and/or reduced ability to undergo apoptosis [2]. It has been identified activation of gene expressions such as *MDR1* (P-glycoprotein) [3] and *BCRP* [4] as crucial molecular events in MDR. Therefore, researchers suggested that MDR is the consequence of polygenic expression changes involving multiple genes and pathways including the epigenetic mechanisms [5,6]. Reports focused on cancer epigenetics have been proposed to explain the relationship between chemosensitivity and epigenetic alterations such as aberrant promoter CpG methylation [7,8], histone modifications [9,10] and chromatin changes [11]. We explored the mechanism behind a doxorubicin-induced multidrug resistant human hepatocellular carcinoma cell line (R-HepG2) and investigated *CDH1* gene promoter hypermethylation in the cells.

CDH1 encodes a protein, namely E-cadherin (CDH1), which is a trans-membrane glycoprotein important for the maintenance of normal epithelial phenotypes [12]. It acts as a tumor and invasion suppressor and the loss of CDH1 function is an essential event in tumorigenesis and epithelial tumor invasion. In this study, we used R-HepG2 and its parental HepG2 cell lines to study the relationship between *CDH1* promoter methylation and MDR in R-HepG2 cells. We also investigated the possible involvement of FYN with CDH1 on drug sensitivity in these cells. Our results revealed that loss of CDH1 by promoter hypermethylation was an important feature of MDR in R-HepG2 cells through regulation of P-glycoprotein expression level. CDH1 expression might also influence the expression of FYN which is also a recently reported regulator of MDR.

2. Materials and methods

2.1. Cell lines and cell culture

HepG2, human hepatocellular carcinoma cell line, was purchased from American Type Culture Collection (ATCC, HB-8065). R-HepG2 is a doxorubicin-induced MDR sub-lineage cell line of HepG2. It was established in our laboratory by long-term culturing HepG2 cell with increasing concentrations of doxorubicin and found to be highly resistant to doxorubicin, vincristine and methotrexate [13]. Both cell lines were cultured in RPMI-1640 culture medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. For culturing R-HepG2 cells, additional 1.2 μM of doxorubicin was added for every sub-cultivation.

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2.2. CDH1 promoter cloning and bisulfite sequencing

387 bp *CDH1* promoter region (−186 to + 176 bp) was cloned for bisulfite sequencing. The bisulfite modified DNA samples were used as templates for PCR. PCR products were analyzed by 2% agarose gel electrophoresis and purified by gel extraction. Each purified PCR product (10 ng) was ligated into TA cloning vector pCR2.1 (Invitrogen, CK2020) and transformed into the JM109 bacterial strain. Ten resultant plasmids were sequenced using M13 forward and reverse primers which were performed by Tech Dragon Ltd. (Hong Kong, China). The methylation patterns of the sites of interest were analyzed. Primers for *CDH1* promoter cloning were: CDH1-S: TAGTAATTTTAGGTTAGAGGGTTAA, CDH1-A: AAA TACCTACAACAACAACAACAAC.

2.3. Reverse transcription polymerase chain reaction (RT-PCR) and Western blot analysis

Total RNA was extracted from cells and the first strand cDNA was synthesized using TaqMan Reverse Transcription Reagents (Applied Biosystems, N808-0234) following the manufacturer's manuals by using 2 μg RNA template in each reaction. Thermal cycling program was set for cDNA synthesis as 25 °C for 10 min, 37 °C for 60 min and 95 °C for 5 min. cDNA synthesized was immediately used as template for second round PCR. Cell lysis and Western blot were carried out as described previously [14]. All antibodies were purchased from Cell Signaling Technology.

2.4. Transient transfection

Plasmid containing the full length cDNA of *CDH1* in the vector pCMV6-XL4 (pCMV6-XL4-CDH1) was purchased from Origene Technologies Incoporation (SC117413). Transient transfection of CDH1 clone in R-HepG2 cells was performed using Lipofectamine 2000 reagent (Invitrogen, 11668-019) following the manufacturer's manuals. In each sample, R-HepG2 cells (3×10^5/well) were transfected with 4 μg pCMV6-XL4-CDH1 or empty vector (EV) DNA and named as R-HepG2-CDH1 or R-HepG2-EV cells. Cells were further incubated for 48 h before harvest.

2.5. FYN knockdown by siRNA

Single strand RNAs were synthesized (Invitrogen, Hong Kong) and annealed in Annealing buffer (10 mM Tris, 20 mM NaCl, pH 8.0) by incubating the RNAs at 90 °C for 1 min and continued by 37 °C incubation for 1 h to prepare siRNAs. siRNA-liposome complex was prepared by mixing siFYN or siCtl (sham control) with Lipofectamine 2000 Reagent in plain medium. The mixture was incubated for 20 min at room temperature before adding to the cell culture. R-HepG2 cells were transfected with FYN siRNA (siFYN) or control siRNA (siCtl) using the transient transfection protocol mentioned above. The optimal final concentration of FYN siRNA was determined by Western blotting. siFYN1 (100 nM) could effectively suppress 97% of FYN expression in R-HepG2-siFYN cells comparing to R-HepG2-siCtl cells (Fig. 4B). The cells were then incubated for 4 h. After that, the supernatant was replaced by fresh complete medium and the R-HepG2-siFYN cells and R-HepG2-siCtl cells were further incubated for 48 h before harvest. The siRNA sequences used were siFYN: GAUGCUGAGCGACAGCUAU, RsiFYN: AGCUGUC GCUCAGCAUCAU; siCtl: CUGGAGUUGUCCCAAUUCU, RsiCtl: AAUU GGGACAACUCCAGCU.

2.6. Doxorubicin uptake analysis

After 48 h of post-transfection with either plasmids or siRNAs, the medium was changed to 10 μM of doxorubicin solution and incubated for 3 h. The cells were then washed twice with PBS and trypsinized into a PBS suspension. The amount of doxorubicin uptake by the cells was analyzed by flow cytometry (FACSCanto™ Flow Cytometer, BD Biosciences) with signals collected at PE-A channel and data was analyzed by WinMDI v2.6 software.

2.7. Apoptosis analysis

Annexin V–FITC Apoptosis Detection kit (Bender MedSystems, BMS500F1/100) was used for the apoptotic detection. After 48 h of post-transfection, the medium was replaced by various concentrations of doxorubicin (50, 100, 150 μM) and the cells were further incubated for 24 h at 37 °C. After that, the cells were harvested and resuspended in 1X binding buffer at a density of $2–5 \times 10^5$ cells/mL. Then, Annexin V/FITC and propidium iodide were applied to each sample and incubated at room temperature for 15 min. Finally, another 250 μL of 1X binding buffer was applied to each sample and the samples were subjected to analysis by flow cytometry with signals collected at PE-A and FITC channel. Data was analyzed by WinMDI v2.6 software.

2.8. Statistical analysis

Experimental results were expressed as mean ± standard deviation (SD). One-way ANOVA was used for comparison between untreated R-HepG2 cells and transfected R-HepG2-CDH1, R-HepG2-siFYN cells. $^{**}p < 0.01$, $^{***}p < 0.001$ indicated significant difference between two samples compared.

3. Results

3.1. CDH1 expression was silenced by promoter hypermethylation in R-HepG2 cells

CDH1 gene sequence flanking exon1 of *CDH1* was acquired from Ensembl database (ENSG00000039068). The results in Fig. 1A showed that the *CDH1* promoter was hypomethylated in HepG2 cells and hypermethylated in R-HepG2 cells. Promoter CpG methylation is usually associated with gene silencing so we examined the expression of *CDH1* by RT-PCR and Western blotting. The results in Fig. 1B showed that *CDH1* gene was expressed in HepG2 cells but the expression was lost in R-HepG2 cells, both on mRNA and protein levels.

3.2. CDH1 expression was correlated with increased doxorubicin (Dox) uptake and decreased P-glycoprotein expression in R-HepG2-CDH1 cells

To examine the significance of the epigenetic silencing of *CDH1* and its relationship with MDR, full length *CDH1* cDNA containing pCMV-XL4-CDH1 was transfected into R-HepG2 cells. The expression of CDH1 was assessed by RT-PCR and Western Blotting. As indicated in Fig. 1B, CDH1 was expressed in the transfected R-HepG2-CDH1 cells.

Dox uptake was measured by flow cytometry to determine the intracellular Dox amount in HepG2, R-HepG2 and R-HepG2-CDH1 cells. It was shown in Fig. 2A that the amount of Dox uptake in R-HepG2 was much lower when compared to the uptake amount of HepG2 cells (17.5% of HepG2). The percentage of Dox uptake was 33.4% ($p < 0.001$) in R-HepG2-CDH1 cells comparing to HepG2 cells.

On the other hand, we investigated the expression level of P-glycoprotein in HepG2, R-HepG2 and R-HepG2-CDH1 cells by Western blotting. As indicated in Fig. 2B, P-glycoprotein expression was null in HepG2 cells but highly expressed in R-HepG2 cells.

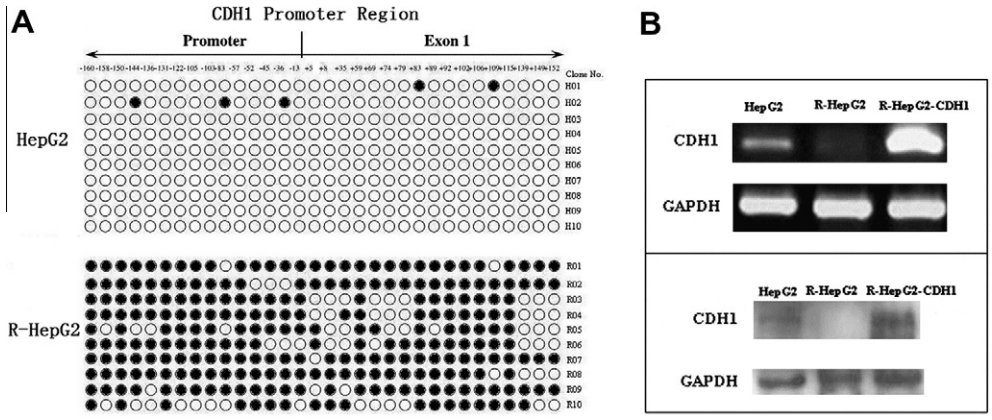

Fig. 1. *CDH1* promoter methylation and CDH1 expression analysis. (A) The bisulfite sequencing analysis of CDH1 promoter methylation in HepG2 and R-HepG2 cells. The locus of each CpG dinucleotides was shown with a number upstream (−) or downstream (+) of the transcription start nucleotide of CDH1 exon 1. Each circle represented a CpG dinucleotides unit in the CDH1 promoter region. The black circle represented a methylated CpG and the white one represents an unmethylated CpG. Data was representative of two independent experiments. (B) mRNA transcription levels of CDH1 (upper); Protein expression levels of CDH1 (lower) in HepG2, R-HepG2 and R-HepG2-CDH1 cells. GAPDH was used as internal control. Data was representative of three independent experiments.

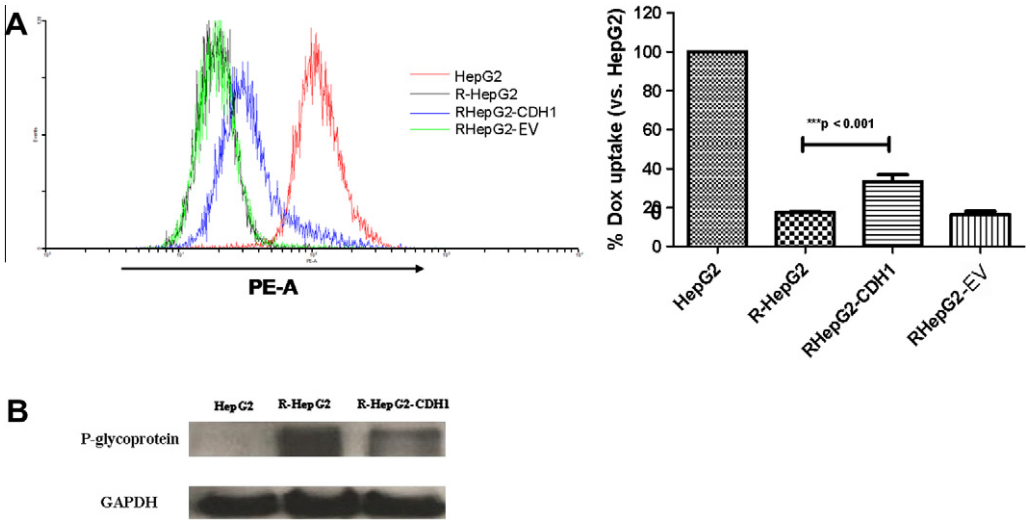

Fig. 2. Flow cytometry analysis of doxorubicin uptake and immunoblotting analysis of P-glycoprotein in CDH1 over-expression R-HepG2-CDH1 cells. (A) Dox uptake was quantified by PE-A channel signal after incubating the cells with 10 μM of doxorubicin for 3 h. The amount of doxorubicin uptake in each sample was calculated as percentage uptake against HepG2 (set as 100%). Data was expressed as mean ± SD for 3 independent trials. $^{***}p < 0.001$ indicated significant difference between R-HepG2 and R-HepG2-CDH1 cells. (B) Protein expression levels of P-glycoprotein in HepG2, R-HepG2 and R-HepG2-CDH1 cells. GAPDH was used as internal control. Data was representative of three independent experiments.

However, the expression was significantly downregualted in R-HepG2-CDH1 cells comparing to R-HepG2 cells. The results indicated that, upon *CDH1* cDNA transfection, P-glycoprotein expression was partially suppressed.

3.3. CDH1 over-expression induced apoptosis with attenuated cell viability in R-HepG2-CDH1 cells

The percentage of apoptotic populations induced by Dox (50, 100 or 150 μM) for 24 h were compared in R-HepG2, R-HepG2-CDH1 and R-HepG2-EV cells using Annexin V–PI co-staining flow cytometry. As shown in Fig. 3A, a dose-dependent increase ($p < 0.001$) in apoptotic population was found in R-HepG2-CDH1 cells treated with all three concentrations of Dox comparing to the sham control R-HepG2-EV and R-HepG2 cells. Trypan blue exclusion assay was carried out to compare the cell viability of HepG2, R-HepG2 and R-HepG2-CDH1 cells in the presence of Dox. The results in Fig. 3B showed that after incubation with 10 μM Dox for 48 h, the cell viability decreased obviously in Dox-treated HepG2 cells ($p < 0.001$) and R-HepG2-CDH1 cells ($p < 0.01$) comparing to the control groups without Dox treatment. This indicated that re-expression of CDH1 increased Dox sensitivity in R-HepG2 cells to undergo apoptosis.

3.4. CDH1 silencing correlated with FYN over-expression and MDR in R-HepG2 cells

Transcriptional differences of MDR-related genes between HepG2 and R-HepG2 cells had been compared by microarray. [15]. Among the genes of significant transcriptional differences, we found that the expression of FYN was significantly higher in R-HepG2 than in HepG2 cells. Notably in R-HepG2-CDH1 cells, the expression of FYN was significantly lower than those in R-HepG2 cells (Fig. 4A). FYN siRNA was transfected into R-HepG2 cells to construct FYN-knockdown R-HepG2-siFYN cells. Cell viability, Dox uptake and P-glycoprotein expression in HepG2, R-HepG2 and R-HepG2-siFYN cells were then examined. As indicated in Fig. 4C, the cell viability decreased obviously in Dox-treated

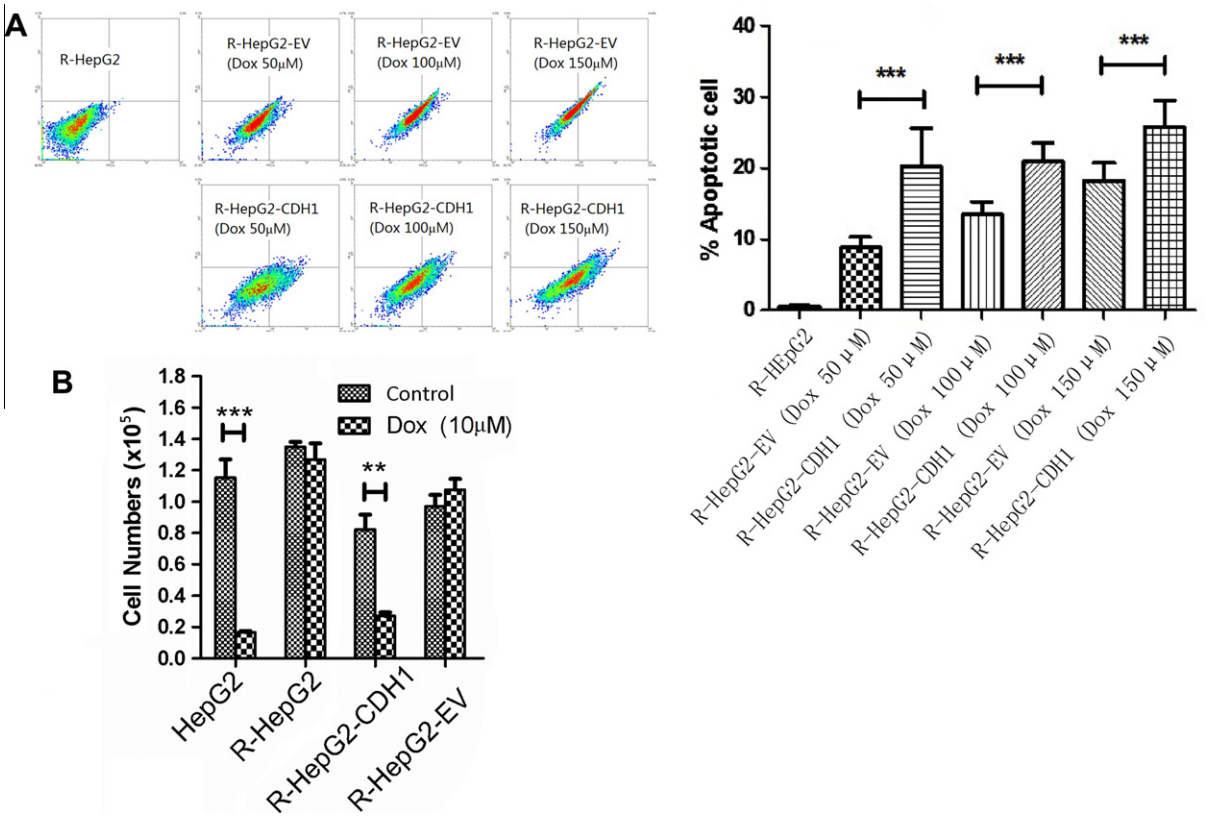

Fig. 3. Apoptosis and cell viability analysis in CDH1 over-expressed R-HepG2-CDH1 cells. (A) Flow cytometry analysis on apoptosis was carried out by Annexin V/FITC and propidium iodide double stain. The percentage of apoptotic cells in each sample was summarized. Data was expressed as mean ± SD for 3 independent trials. $^{***}p < 0.001$ indicated significant difference between untreated and CDH1-overexpressed R-HepG2 cells. (B) Cell viability analysis was done by trypan blue dye exclusion assay with a hemocytometer. Data was expressed as mean ± SD for 3 independent trials. $^{***}p < 0.001$ and $^{**}p < 0.01$ indicated significant cell viability difference between Dox treated HepG2 cells and CDH1 over-expressed R-HepG2-CDH1 cells.

R-HepG2-siFYN cells ($p < 0.01$) comparing to the control groups. Moreover, as shown in Fig. 4D, the relative Dox uptake in R-HepG2-siFYN cells was increased to 29.5% ($p < 0.01$) comparing to 17.5% of R-HepG2. The expression of P-glycoprotein shown in Fig. 4B revealed that P-glycoprotein expression level was lower in R-HepG2-siFYN cells than R-HepG2 cells. The results suggested that suppressed expression of FYN in R-HepG2-CDH1 cells by siRNA resulted in enhanced Dox uptake, suppressed cell viability and suppressed P-glycoprotein expression in R-HepG2-siFYN cells which indicated that FYN expression in CDH1 promoter hypermethylated R-HepG2 cells was related to MDR.

4. Discussion

In this study, it was found that hypermethylation in *CDH1* promoter epigenetically silenced *CDH1* gene in multidrug resistant human hepatocellular carcinoma cells (R-HepG2) by using bisulfite sequencing method. The relationship was further verified by expression level of CDH1 which was highly expressed in HepG2 cells but greatly suppressed in R-HepG2 cells. Our results suggested a potential role of *CDH1* in drug resistance development. We investigated the percentage of apoptotic cells in R-HepG2-CDH1 cells induced by doxorubicin. There was approximately 2-fold more apoptotic cells in R-HepG2-CDH1 than control cells at all of three doxorubicin concentrations. CDH1 is known to contribute to anoikis, an apoptotic response to the absence of cell-matrix interactions, which prevents cancer metastasis due to its interaction with the apoptosis inhibiting β-catenin of the Wnt pathway [16,17]. It is also reported that CDH1 expression sensitizes anoikis through promoting p14ARF activity [18]. This suggested that loss of CDH1 helped the progressed multidrug resistant cells to evade apoptosis.

The involvement of CDH1 in cancer invasion and metastasis is well known but its involvement in multidrug resistance was not widely reported. Research on a multidrug resistant breast cancer cell line demostrated that CDH1 was not detected by immunolabeling at the cell junctions of the drug resistant cells [19]. Another research reported that the transcriptional suppressor of *CDH1/Zeb-1* might be responsible for maintaining drug resistance on transcription level in pancreatic cancer cells [20]. Our findings elaborated that loss of CDH1 through promoter hypermethylation was an important mechanism involved in multidrug resistance. Suppression of CDH1 through promoter methylation in multidrug resistant cells contributed to multidrug resistance by increasing P-glycoprotein expression, increasing drug efflux and exhibiting higher cell viability, while re-expression of CDH1 in multidrug resistant cells suppressed cell viability, suppressed P-glycoprotein expression, prevented drug efflux and increased apoptosis.

FYN is a member of the Src family tyrosine kinases. It has important roles in mitogenic signaling and regulation of cell cycle entry, growth and proliferation. FYN over-expression has been found in many cancers including glioblastoma, head & neck squamous cell carcinoma, prostate cancer and melanoma [21]. FYN over-expression has also been reported to be an important determinant in chronic myeloid leukemia cell lines associated with BCR-ABL inhibitor (Imatinib and PD166326) resistance [22]. In this study, we found FYN was expressed in R-HepG2 and its expression was suppressed in the non-resistant/less-resistant HepG2 and R-HepG2-CDH1 cells, suggesting that FYN over-expression was related to MDR. In FYN-suppressed cells, by CDH1 transfection or

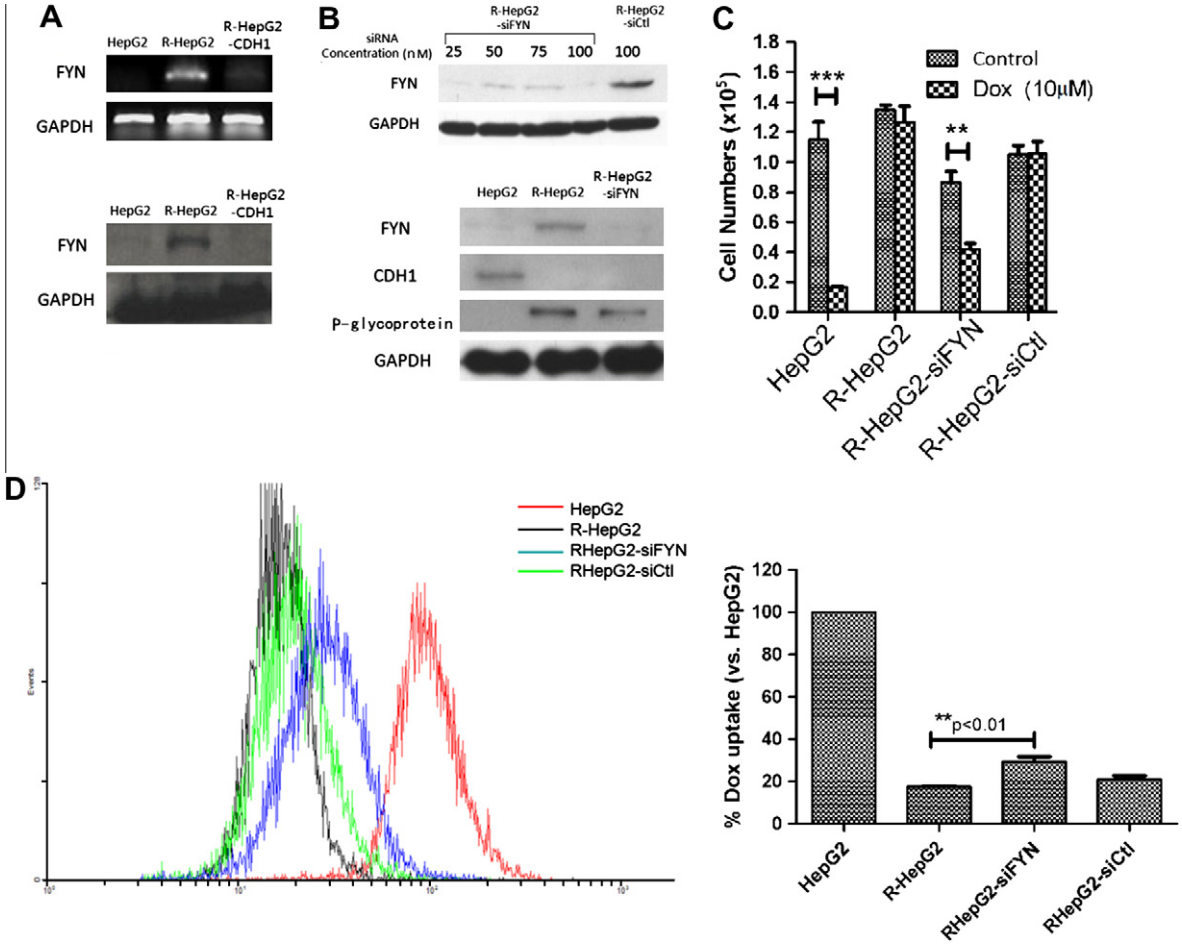

Fig. 4. FYN related analysis on gene expression, cell viability and Dox uptake. (A) FYN expression was determined by RT-PCR (upper) and Western blotting (lower) assays in HepG2, R-HepG2 and R-HepG2-CDH1. (B) The optimal final concentration of FYN siRNA was determined by Western blotting in R-HepG2 cells (upper). Expression of P-glycoprotein and CDH1 in R-HepG2-siFYN cells was determined by Western blotting (lower). GAPDH was used as internal control. Data was representative of three independent experiments. (C) Cell viability analysis was done by trypan blue dye exclusion assay with a hemocytometer. Data was expressed as mean ± SD for 3 independent trials. $^{***}p < 0.001$ and $^{**}p < 0.01$ indicated significant cell viability difference between Dox treated HepG2 cells and FYN knockdown R-HepG2-siFYN cells. (D) Dox uptake was quantified as described in Fig. 2. Data was expressed as mean ± SD for 3 independent trials. $^{**}p < 0.01$ indicated significant difference between R-HepG2 and R-HepG2-siFYN cells.

FYN knockdown, showed decreased MDR by suppressing cell viability, P-glycoprotein expression and drug efflux. Interestingly, we observed that upon FYN knockdown, CDH1 remained silenced in the R-HepG2-siFYN cells. So it was postulated that epigenetic silencing of CDH1 may function as an upstream regulator of FYN, which causes the suppressed drug sensitivity in multidrug resistant cancer cells.

Collectively, the promoter methylation of *CDH1* is an important molecular event in the MDR R-HepG2 cells. This event is involved in the regulation of the sensitivity of cancer cells toward chemotherapy. Search and application of agents which would modulate *CDH1* promoter methylation to increase the expression of CDH1 or agents which would inhibit the expression of genes such as *FYN* would provide a novel therapeutic strategy in cancer chemotherapy.

References

[1] M.M. Gottesman, T. Fojo, S.E. Bates, Multidrug resistance in cancer: role of ATP-dependent transporters, Nat. Rev. Cancer 2 (1) (2002) 48–58.
[2] H. Lage, An overview of cancer multidrug resistance. a still unsolved problem, Cell Mol. Life Sci. 65 (20) (2008) 3145–3167.
[3] M.M. Gottesman, Mechanisms of cancer drug resistance, Annu. Rev. Med. 53 (2002) 615–627.
[4] L.A. Doyle, D.D. Ross, Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2), Oncogene 22 (47) (2003) 7340–7358.
[5] N. Ibrahim, L. He, C.O. Leong, et al., BRCA1-associated epigenetic regulation of p73 mediates an effector pathway for chemosensitivity in ovarian carcinoma, Cancer Res. 70 (18) (2010) 7155–7165.
[6] A.A. Stavrovskaya, Cellular mechanisms of multidrug resistance of tumor cells, Biochemistry (Mosc) 65 (1) (2000) 95–106.
[7] G. Gifford, J. Paul, P.A. Vasey, et al., The acquisition of hMLH1 methylation in plasma DNA after chemotherapy predicts poor survival for ovarian cancer patients, Clin. Cancer Res. 10 (13) (2004) 4420–4426.
[8] R.M. Glasspool, J.M. Teodoridis, R. Brown, Epigenetics as a mechanism driving polygenic clinical drug resistance, Br. J. Cancer 94 (8) (2006) 1087–1092.
[9] F. Crea, S. Nobili, E. Paolicchi, et al., Epigenetics and chemoresistance in colorectal cancer: An opportunity for treatment tailoring and novel therapeutic strategies, Drug Resist Updat 14 (6) (2011) 280–296.
[10] H. Huo, P.G. Magro, E.C. Pietsch, et al., Histone methyltransferase MLL1 regulates MDR1 transcription and chemoresistance, Cancer Res. 70 (21) (2010) 8726–8735.
[11] O.G. McDonald, H. Wu, W. Timp, et al., Genome-scale epigenetic reprogramming during epithelial-to-mesenchymal transition, Nat. Struct. Mol. Biol. 18 (8) (2011) 867–874.
[12] B. Tsuchiya, Y. Sato, T. Kameya, et al., Differential expression of N-cadherin and E-cadherin in normal human tissues, Arch. Histol. Cytol. 69 (2) (2006) 135–145.
[13] J.Y. Chan, A.C. Chu, K.P. Fung, Inhibition of P-glycoprotein expression and reversal of drug resistance of human hepatoma HepG2 cells by multidrug resistance gene (mdr1) antisense RNA, Life Sci. 67 (17) (2000) 2117–2124.
[14] N.H. Bui-Xuan, P.M. Tang, C.K. Wong, et al., Pheophorbide a: a photosensitizer with immunostimulating activities on mouse macrophage RAW 264.7 cells in the absence of irradiation, Cell Immunol. 269 (1) (2011) 60–67.
[15] J. Wang, J.Y. Chan, C.C. Fong, et al., Transcriptional analysis of doxorubicin-induced cytotoxicity and resistance in human hepatocellular carcinoma cell lines, Liver Int. 29 (9) (2009) 1338–1347.

[16] Z. Weng, M. Xin, L. Pablo, et al., Protection against anoikis and down-regulation of cadherin expression by a regulatable beta-catenin protein, J. Biol. Chem. 277 (21) (2002) 18677–18686.
[17] H.G. Kang, J.M. Jenabi, J. Zhang, et al., E-cadherin cell-cell adhesion in ewing tumor cells mediates suppression of anoikis through activation of the ErbB4 tyrosine kinase, Cancer Res. 67 (7) (2007) 3094–3105.
[18] S. Kumar, S.H. Park, B. Cieply, et al., A pathway for the control of anoikis sensitivity by E-cadherin and epithelial-to-mesenchymal transition, Mol. Cell Biol. 31 (19) (2011) 4036–4051.
[19] M.A. dit Faute, L. Laurent, D. Ploton, et al., Distinctive alterations of invasiveness, drug resistance and cell-cell organization in 3D-cultures of MCF-7 a human breast cancer cell line, and its multidrug resistant variant, Clin Exp Metastasis 19 (2) (2002) 161–168.
[20] T. Arumugam, V. Ramachandran, K.F. Fournier, et al., Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer, Cancer Res. 69 (14) (2009) 5820–5828.
[21] Y.D. Saito, A.R. Jensen, R. Salgia, et al., Fyn: a novel molecular target in cancer, Cancer 116 (7) (2010) 1629–1637.
[22] S. Grosso, A. Puissant, M. Dufies, et al., Gene expression profiling of imatinib and PD166326-resistant CML cell lines identifies Fyn as a gene associated with resistance to BCR-ABL inhibitors, Mol. Cancer Ther. 8 (7) (2009) 1924–1933.