CHD1L contributes to cisplatin resistance by upregulating the ABCB1–NF-κB axis in human non-small-cell lung cancer

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Abstract
Chromodomain helicase/ATPase DNA binding protein 1-like gene (CHD1L) is a recently identified gene associated with malignant tumor progression and patient chemotherapy resistance in human hepatocellular carcinoma (HCC). Previously, we found an association between CHD1L overexpression and poor patient survival in non-small-cell lung cancer (NSCLC). However, little is known about the relationship between CHD1L expression and chemotherapy resistance of NSCLC. By employing immunohistochemistry, we analyzed the expression of CHD1L in NSCLC samples and elucidated the roles and mechanism of CHD1L in NSCLC chemoresistance. We found that the increased expression of CHD1L is positively correlated with a shorter survival time of patients who had received chemotherapy after surgery. We also found that the expression of CHD1L was increased after cisplatin treatment in A549 cells. Conversely, the depletion of CHD1L in cisplatin-resistance cells increased the cell sensitivity to cisplatin, indicating that CHD1L plays a critical role in cisplatin resistance of NSCLC cells. Importantly, we identified the ATP-Binding Cassette Sub-Family B Member (ABCB1) gene as a potential downstream target of CHD1L in NSCLC cells. Knocking down ABCB1 coupled with ectopic expression of CHD1L enhanced the effect of cisplatin on NSCLC cells apoptosis. In addition, overexpressed CHD1L increase the transcription of c-Jun which targeted directly to the promoter of ABCB1. Our data demonstrate that CHD1L could induce cisplatin resistance in NSCLC via c-Jun-ABCB1–NF-κB axis, and may serve as a novel predictive marker and the potential therapeutic target for cisplatin resistance in NSCLC.

Introduction
Lung cancer is the leading cause of cancer-related mortality worldwide1. Non-small-cell lung cancer (NSCLC) consists of a largely heterogeneous group of malignancies and is estimated to account for nearly 85% of all diagnosed lung cancers2. Cisplatin (DDP)-based chemotherapy is considered as one of the primary standard systematic treatment choices for advanced NSCLC2–4. Unfortunately, following cisplatin treatment a high rate of relapse occurs despite so many efforts on overcoming drug resistance5. Therefore, a better understanding of the molecular mechanisms underlying cisplatin resistance is crucial for optimizing the individual therapies and achieve better survival outcomes for NSCLC patients6.

Chromodomain helicase/ATPase DNA binding protein 1-like gene (CHD1L) is a newly identified oncogene isolated from a region of chromosome 1q that is frequently amplified in human hepatocellular carcinoma (HCC)7. Our previous work has demonstrated that CHD1L
contributes to HCC cell migration, invasion, and metastasis. We also found that CHD1L expression is positively associated with tumor progression in HCC patients8–11. Recently, CHD1L has also been reported as a novel biomarker for patients’ prognosis in several types of malignant tumors including breast cancer12, gastric cancer13, colorectal cancer14, bladder cancer15, as well as ovarian cancer16. Our previous study showed that overexpression of CHD1L associated with the advanced diagnostic stage and poorer survival rate of NSCLC patients17. However, little is known about the relationship between CHD1L expression and chemotherapy resistance of NSCLC.

In this study, high expression of CHD1L was detected in NSCLC patients tissues and the overexpression of CHD1L was associated with poorer survival rates in NSCLC patients who were treated with cisplatin-based chemotherapy. We also investigated the role of CHD1L in NSCLC cell-cisplatin resistance by analyzing CHD1L function both in vitro and in vivo. Here we demonstrate for the first time that CHD1L can contribute to cisplatin resistance by upregulating the ATP-Binding Cassette Sub-Family B Member (ABCB1) gene in lung cancer cells. Upregulation of ABCB1 by CHD1L is dependent on c-Jun transcription in NSCLC cells. Moreover, NF-κB pathway is closely correlated with ABCB1 expression. Our results provide the functional and mechanistic links between CHD1L expression and cisplatin resistance, and therefore indicated a potential therapeutic target for NSCLC.

Materials and methods

Patients and tissue specimens

Paraffin-embedded tissue samples from 248 NSCLC patients who had undergone surgery were obtained from the Pathology Department of Cancer Center, Sun Yat-sen University, Guangzhou, China, between February 1994 and January 1998. Adjuvant chemotherapy become a standard approach only after 2004, therefore adjuvant chemotherapy using cisplatin-based combinations was only administered to 89 of the patients with stage III NSCLCs. In addition to above, paraffin-embedded biopsy specimens from 30 locally advanced NSCLC patients who had received neoadjuvant cisplatin-based chemotherapy before surgery were also obtained from the archives of the Department of Pathology of Sun Yat-sen University between July 2006 and June 2012.

Data regarding cancer stage was determined according to the pathology Tumor-Node-Metastasis (pTNM) system (AJCC/UICC 2015). Tumor differentiation and histotype were determined according to the World Health Organization classification for NSCLC. To evaluate the patients’ response to neoadjuvant chemotherapy, we used the RECIST Criteria (v.1.1). This study was approved by the medical ethics committee of Cancer Center, Sun Yat-Sen University and was performed in accordance with the Declaration of Helsinki.

Construction of tissue microarrays

The tissue microarray (TMA) was constructed according to a method described previously. Briefly, formalin-fixed, paraffin-embedded tissue blocks and their corresponding histological H&E stained slides were overlaid for tissue TMA sampling. Tissues (248 surgical resected NSCLCs) were sampled using a tissue arraying instrument (Beecher Instruments, Silver Spring, MD); and a 0.6-mm-diameter cylinder of tissue was removed from each sample. Subsequently, tissue cylinders were re-embedded into a predetermined position in a recipient paraffin block. Three cores of tissue sample were selected from each primary NSCLC and normal lung tissue, and multiple sections (5 µm thick) were cut from the TMA block and mounted on to microscope slides.

Immunohistochemistry

The immunohistochemistry (IHC) staining of CHD1L was accomplished using a standard streptavidin-peroxidase method as previously described. The TMA sections and tissue slides were first deparaffinized and rehydrated. Any endogenous peroxidase activity was blocked with 3% H2O2 for 10 min. Next, slides were immersed in 10 mM citrate buffer (pH 6.0) and boiled in a microwave oven for 15 min for antigen retrieval. Non-specific antibody binding was blocked with 5% normal goat serum for 10 min. Slides were incubated in monoclonal antibody against CHD1L (Abcam; 1:100) at 4 °C overnight in a moist chamber. The slides were then sequentially incubated with biotinylated goat anti-mouse IgG (Santa Cruz Biotechnology; 1:100) and streptavidin-peroxidase conjugate, each for 30 min at room temperature. For a negative control an isotope-matched human IgG was used. Finally, the 3, 5-diaminobenzidine (DAB) Substrate Kit (Dako) was used for color development followed by Mayer hematoxylin counterstaining.

Expression of CHD1L primarily appeared in NSCLC nuclear. For the evaluation of CHD1L staining, a semi-quantitative scoring criterion was used in which both staining intensity and the percentage of positive cells was recorded. A staining index (ranging from 0 to 12) was determined by the intensity of CHD1L staining (0 = negative, 1 = weakly positive, 2 = moderate positive, 3 = strongly positive) multiplied by the proportion of immunopositive tumor cells (0% = 0, <10% = 1, 10% to <50% = 2, 50% to <75% = 3, ≥75% = 4). A score of 3 or higher was classified as overexpression of CHD1L. A minimum of 300 epithelial cells were counted for each sample. For scoring, two independent pathologists (Dr. Xie D and Chen JW) were blinded to the clinicopathologic information. Any inter-observer disagreements (about 5% of
the total informative cases) were reviewed a second time, followed by a conclusive judgment by both pathologists.

**Cell culture and reagents**

This study used human lung cancer cell lines GCL-82, PC9, A549, SPA-A1, H322 and L-78, which were purchased from China Center for Type Culture Collection (CCTCC, Shanghai, China), where all cell lines are authenticated by STR profiling before distribution. All cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 100 units/mL penicillin, and 100 mg/mL streptomycin and kept in a humidified atmosphere containing 5% CO₂ at 37 °C. To generate cisplatin-resistant PC9 cell lines, PC9 cells were first treated with 0.25 μM of cisplatin (DDP, Sigma), and then with increased concentrations of cisplatin in a stepwise manner during each subsequent treatment. To maintain the drug-resistant phenotype, DDP (with final concentration of 2 μM) was added to the culture media for A549/DDP cells and PC9/DDP cells. All DMSO was purchased from Sigma-Aldrich (St. Louis, MO, USA) and Opti-MEM from Gibco (Thermo Fisher Scientific, Grand Island, NY, USA).

**Western blot analysis**

Whole cell lysates of lung cancer samples were prepared with a proteinase and phosphatase inhibitor cocktail (Roche, CA, USA). Protein concentrations of lysates were measured using the BCA method (Thermo Fisher Scientific, USA). 20 μg of extracted protein was loaded on to a 10% SDS-polyacrylamide gels and then transferred onto PVDF membranes (Millipore, USA). Non-specific binding was blocked on the PVDF membranes with PBS buffer containing 0.1% Tween-20, 2% BSA, and 5% nonfat dry milk. Blots were then incubated with anti-rabbit primary antibodies overnight at 4 °C. The next day, membranes were extensively washed, and then incubated with horseradish peroxidase-conjugated anti-goat (ProteinTech) or anti-rabbit (Proteintech) IgG at room temperature for 1 h. Protein blots were probed with primary antibodies against CHD1L (Abcam#ab197019), p65 (Cell Signaling Technology #8242), p65-pSer536 (Cell Signaling Technology #3033), IkBa (Cell Signaling Technology #4814), and IkBo-pSer32 (Cell Signaling Technology #2859). Signals were visualized by chemiluminescence (Bio-rad, Hercules, California) and quantitated using a Quantity One system (Bio-Rad, Hercules, CA, USA).

**Construction of the recombinant lentiviral vector**

The CHD1L shRNA control vector (HS022909LVRH6GP) was constructed by and purchased from the GeneCopoeia Company (Rockville, MD, USA). The pEZ-Lv208-CHD1L and Lv208CT control vectors were also purchased from GeneCopoeia (Rockville, MD, USA). Vectors were packaged in 293FT cells using ViraPower Mix. After culturing period of 48 h, supernatant lentiviral particles were filtered by centrifugation at 500–600 × g for 10 min and then transfected into lung cancer cells. Stably infected cells were selected using puromycin (Gibco, USA). The scramble small-interfering RNA (NC) and the siRNAs targeting CHD1L(#1, 5'-CAACCTACA TATACTACCT-3'; #2, 5'-GTTTACCTCCAGAATTG-3') were synthesized and purchased from Gene-Pharma (Suzhou, Jiangsu, China).

**Cell counting Kit-8 (CCK8) assay**

Between 1000-2000 cells were seeded in 96-well plates, and CCK8 (Beyotime Technology, Shanghai, China) was added to each well and incubated for 2 h at 37 °C. The optical density (OD) of the cultures was measured at wavelengths of 450 nm using a V Max kinetic microplate reader at different time periods. Each experiment was performed in triplicate.

**Cell apoptosis analysis**

Annexin-V/PI was applied to quantify the amount of apoptotic cell. For the Annexin-V/PI binding assay, A549, A549/DDP and PC9/DDP cells were exposed to 10 μM cisplatin for 24 h and transiently transfected with siRNAs. Both floating and adherent cells were collected by centrifugation and trypsinization, respectively. Cells were then re-suspended in binding buffer and subsequently stained with FITC-Annexin-V and propidium iodide (PI) (BestBio, Shanghai, China). Stained cells were quantified by using FACS under the flow cytometer (BD Biosciences, Franklin lakes, NJ, USA) according to the manufacturer's directions.

**Animal experiments**

All animal experiments were conducted according to standards regarding the use of laboratory animals and all experiments were approved by the Sun Yat-sen University Cancer Center Institutional Animal Care and Usage Committee. Female BALB/c nude mice (4–5 weeks old) were randomly divided into three groups and subcutaneously injected with A549 cells stably expressing CHD1L shRNA1, shRNA2 or a scramble shRNA. Mice were subsequently monitored for xenograft development every 3 days. When the tumors reached a diameter of 5 mm in size, the mice in each group were randomly further divided into two subgroups and treated with intraperitoneal injections of cisplatin (3 mg/kg) or an equal volume (100 μl) of normal saline (NS) every 2 days for approximately 2 weeks. Mice were sacrificed 4 weeks after cell injection, and all tumors were removed and weighed.

**Statistical analysis**

Experiments were repeated at least three times for all in vitro experiments and twice for all animal experiments.
All data were analyzed with SPSS 16.0 (SPSS, Chicago, IL, USA). The association of CHD1L expression with NSCLC patients’ response to chemotherapy was assessed by the Chi-square test. The overall survival rate of patients was defined as the time from the day of diagnosis to patient death. Survival curves were assessed by Kaplan–Meier method and compared by the log-rank test. P value <0.05 was considered significant.

**Results**

**CHD1L overexpression correlates with worse outcome in cisplatin-treated advanced NSCLC patients**

We examined the expression of CHD1L using IHC in 233/248 (93.9%) of our NSCLC samples. The samples that were not analyzed and therefore not included in our data compilation included unrepresentative samples, samples with too few tumor cells (<300 cells per case) and lost samples. Overexpression of CHD1L was observed in 58 (38.4%) of the 151 NSCLC patients who went without cisplatin-based adjuvant chemotherapy and 40 (48.8%) of the 82 patients who had underwent with adjuvant chemotherapy (Fig. 1a). The median survival rate was significantly shorter in patients who demonstrated CHD1L overexpression than those with normal CHD1L expression in the chemotherapy group ($P = 0.001$ log-rank test), but not in the non-chemotherapy group ($P = 0.087$, log-rank test) (Fig. 1b). In order to evaluate the association between CHD1L overexpression and the response to cisplatin-based chemotherapy in NSCLC patients, we further tested CHD1L expression by IHC in a restricted cohort of locally advanced NSCLC treated with cisplatin-based neoadjuvant chemotherapy ($n = 30$). Of the 30 patients, 12 achieved a partial response (PR), whereas the other 18 patients were evaluated as a non-response (as either no change (NC) or as progressive disease (PD)). Our data indicate a significant association between CHD1L overexpression and chemotherapy response ($P = 0.011$, Table 1).

**CHD1L suppresses cisplatin-induced apoptosis in NSCLC cells**

CHD1L expression was examined in six different lung cancer cell lines by immunoblotting. The endogenous expression of CHD1L was detected in three of the cell lines (i.e., A549, PC9 and L-78), whereas the other three lines (i.e., GLC-82, SPA-A1 and H322) showed undetectable or very low levels of endogenous CHD1L (Fig. 1c). To further explore the roles of CHD1L in NSCLC, we established CHD1L downregulated NSCLC cell lines by using CHD1L shRNA transfection (the cells indicated as A549-shCHD1L and PC9-shCHD1L) (Fig. 2a). We also constructed an ectopic CHD1L overexpression A549 cell line (Fig. 2b) as well as the cisplatin-treated A549- CHD1L cells (Supplementary Fig. 1a). The Annexin-V and propidium iodide (PI) staining based FlowCytometry analysis revealed that the downregulation of CHD1L significantly enhanced cisplatin-induced apoptosis in both A549 and PC9 cells ($P < 0.05$ or $P < 0.01$) (Fig. 2c). Poly (ADP-ribose) polymerase (PARP) cleavage is a common marker of cell apoptosis, we therefore verified the effect of CHD1L on cisplatin-induced PARP cleavage in NSCLC cells. The result showed that knocking down of CHD1L sensitized cisplatin-induced apoptosis in A549, whereas the enforced CHD1L expression reduced PARP cleavage in cisplatin-treated A549-CHD1L cells when compared with their respective control cells (Supplementary Fig. 1b). The rescue assay showed that the enforced expression of CHD1L in A549-shCHD1L or PC9-shCHD1L cells significantly rescued cisplatin-induced apoptosis (Fig. 2d). To further determine whether CHD1L affects the sensitivity of NSCLC cells to cisplatin, the A549 cells were stably transfected with either a scramble shRNA(as a control) or shRNA specifically targeting CHD1L., and the co-transfected GFP was used as a transfection indicator, in which the parental A549 cells could be labeled with DsRed. All the GFP-labeled, CHD1LshRNA-transfected cells or the DsRed-labeled parental cells were mixed together and treated with cisplatin (Fig. 2e). After treating with cisplatin, the mixed cells were analyzed by using fluorescent microscopy or flow cytometry to detect the cells’ sensitivity to cisplatin. The results indicated that DsRed$^+$ cells significantly increase after treating with cisplatin, the decrease of GFP$^+$ cells was seen in shCHD1L-transfected group. As expected, neither the DsRed$^+$ nor GFP$^+$ cell number changed in the control shRNA-transfected group (Fig. 2f, g) ($P < 0.05$ or $P < 0.01$).

**Cisplatin resistance is associated with CHD1L activation**

In order to validate the correlation between CHD1L and drug resistance in NSCLC cells, we constructed cisplatin-resistant A549 and PC9 cell lines that displayed distinctive epithelial morphology. The A549/DDP and PC9/DDP cells exhibited fibroblastic morphology (Fig. 3a). The CCK8 assay showed that both A549/DDP and PC9/DDP cells exhibited significantly higher resistance to cisplatin than non-DDP-resistant cells (Fig. 3b). QPCR and western blotting revealed that DDP-resistant cells had higher levels of CHD1L expression than their non-DDP-resistant cells (Fig. 3c). To investigate the correlation between CHD1L expression and cisplatin sensitivity, we treated PC9 cells with stepwise cisplatin to generate the cells bearing different extents of resistance. After treatment with the indicated concentration of cisplatin, the surviving cells were harvested at every 10 days to evaluate CHD1L expression. As shown in Fig. 3d, the expression of CHD1L in viable cells that sustained the escalating dosage of cisplatin was increased clearly at the second time point,
and maintained its higher expression level in the following time point, which supporting our hypothesis that CHD1L is associated with cisplatin resistance (Fig. 3d).

**CHD1L suppresses cisplatin-induced apoptosis in cisplatin-resistant cells**

To assess the contribution of CHD1L in cisplatin resistance, two short hairpin RNAs (shRNAs) were generated and used to suppress CHD1L expression in two DDP-resistant cells with endogenous expression of CHD1L. The efficiency of CHD1L downregulation at the

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**Table 1** Correlation between the expression of CHD1L and therapy response in NSCLC patients ($N = 30$)

| CHD1L expression level | Cases | Normal expression | Overexpression | $P$ value |
|------------------------|-------|-------------------|----------------|-----------|
| **Chemotherapy response** |       |                   |                |           |
| NC + PD                | 18    | 5 (27.8%)          | 13 (72.2%)     | $P < 0.05$|
| PR                     | 12    | 9 (75.0%)          | 3 (25.0%)      |           |

NC no change, PD progressive disease, PR partial response
Fig. 2 (See legend on next page.)
Depletion of CHD1L enhances the sensitivity of xenograft tumors to cisplatin

To determine whether or not ectopic expression of CHD1L could decrease oncogenic function of NSCLC cells, we used an A549/DDP cell line, which stably knocks down expression of CHD1L. In clonogenic assays, we found that silencing of CHD1L in combination with cisplatin treatment caused a marked inhibition of proliferation in A549/DDP cells (Fig. 5a). We next wanted to know if silencing of CHD1L can also tumors to cisplatin in vivo. To test this, we stably knocked down the expression of CHD1L in A549/DDP cells (A549/DDP-sh1 and A549/DDP-sh2; A549/DDP-shNC as negative control cells) and established subcutaneous xenografts in nude mice, which were then treated with cisplatin. Transfection of CHD1L shRNAs effectively inhibited the growth of NSCLC xenografts in nude mice. With the treatment of cisplatin, both the tumor volume and tumor weight were found to be reduced significantly (Fig. 5b–d). All these data indicate that CHD1L knockdown enhances the sensitivity of xenograft tumors to cisplatin treatment.

ABCB1 is responsible for CHD1L-induced NSCLC cell cisplatin resistance

In order to determine any possible downstream targets of CHD1L in NSCLC cell cisplatin resistance, we analyzed mRNA expression of A549-CHD1L cells and its vector control, using Cancer Drug Resistance Real-time PCR Array containing 84 cell drug resistance-related genes. As shown in Fig. 6a, three upregulated genes (ABCB1, CYP2C19, and SULT1E1) and two downregulated genes (ERCC3 and GSTP1), were identified by more than two-fold change in mRNA levels between the two groups (Fig. 6a, Table 2). All candidate genes were further validated using immunoblotting. Only the blot of ATP-Binding Cassette Sub-Family B Member (ABC21) was matched with the results from RT-PCR Array, therefore we pursued this candidate further (Fig. 6b). ABC21 encoding P-glycoprotein (P-gp), is one of pharmaceutical carriers that can decrease the effective intracellular concentration of the drug, leading to drug resistance. In addition, a significant positive correlation between the overexpression of CHD1L and ABC21 was evaluated in our large cohort of NSCLC tissues (Fig. 6c, P = 0.03, Supplementary Table 1). To clarify its role in CHD1L-induced cisplatin resistance, RNAi was used to knocking down ABC21 in A549-CHD1L cells. In clonogenic assays, we also found that silencing of ABC21 in combination with cisplatin caused a marked inhibition of proliferation in A549-CHD1L cells (Fig. 6d). Annexin-V and PI staining revealed that silencing ABC21 expression significantly enhanced cisplatin-induced apoptosis in A549-CHD1L cells (Fig. 6e). Histone H2AX phosphorylation at serine-139 (also known as γH2AX), is an established marker of DNA double-strand breaks. Our results showed that the cisplatin-induced γ-H2AX overexpression could be rescued by silencing ABC21 in CHD1L-overexpressing NSCLC cells (Fig. 6f). To investigate whether ABC21 is required for CHD1L-induced NSCLC cell cisplatin resistance, we established subcutaneous xenografts in female, adult, nude mice, which were then treated with cisplatin.
Fig. 3 Cisplatin resistance is associated with CHD1L activation. a A549 and PC9 cells displayed epithelial morphology, and A549/DDP and PC9/DDP cells exhibited fibroblastic morphology (original magnification, ×200). b Two DDP-resistant cells and their parental cells were treated with indicated concentrations of cisplatin for 48 h and then were subjected to CCK assay (n = 5). The results show that A549/DDP and PC9/DDP cells are more resistant to cisplatin than their parental cells in vitro. c qRT-PCR and Western blotting illustrate increased expression of CHD1L in A549/DDP and PC9/DDP cells. d Schema of step-wise cisplatin treatment on PC9 cells; CHD1L expression during cisplatin treatment was measured using western blotting. Statistics were generated from three independent experiments. *P<0.05, **P<0.01, ***P<0.001, Student’s t-test; error bar: ±S.D.
Fig. 4 (See legend on next page.)
Fig. 4 CHD1L suppresses cisplatin-induced apoptosis in cisplatin-resistance cells. (a) Western blotting reveals that CHD1L was efficiently knocked down by the treatment of CHD1L-shRNA-1 or CHD1L-shRNA-2 in A549/DDP and PC9/DDP cells. (b) A549/DDP and PC9/DDP cells were transfected with CHD1L-shRNA-1 or CHD1L-shRNA-2 and seeded in 96-well cell culture plates. The next day, cells were incubated with or without the indicated concentration of cisplatin for 48 h and subsequently subjected to a CCK assay. (c) WB assays (c-PARP, cleaved PARP). (d) Annexin-V-FITC/PI dual staining assay (left, representative plots for flow cytometry; right, bar charts indicating the average percentages of apoptotic cells). NC, negative control siRNA; si1, si2, CHD1L siRNAs; ns, normal saline. *P<0.05; **P<0.01; NS, no significance.

Fig. 5 Depletion of CHD1L expression enhances the sensitivity of xenograft tumors to cisplatin. (a) A549/DDP cells transfected with CHD1L-shRNA-1 or CHD1L-shRNA-2 were treated with cisplatin at the indicated concentration for 14 days. Colonies were stained with crystal violet (Left). The number of colonies were taken from three independent experiments (Right). (b) CHD1L knockdown reverses cisplatin resistance of cisplatin-resistant cells in xenograft tumors implanted onto adult female nude mice. A549/DDP cells transfected with CHD1L-shRNA-1 or CHD1L-shRNA-2 were subcutaneously injected to generate xenograft tumors in nude mice; cisplatin treatment was performed as described in Materials and Methods. Normal saline (ns) was used as a treatment control. Images of xenograft tumors harvested at the end of the experiment. (c) Growth curves of tumor xenografts. (d) The weights of tumors are presented as a Cleveland dot plot, and the average ±S.D is included (n=6/group; **P< 0.01, ***P <0.001; NS, no significance).
Transfection of ABCB1 shRNAs showed no obvious inhibitory effects of NSCLC xenografts in nude mice (Fig. 6g), however, both the volume and tumor weight of the subcutaneous transplanted tumor decreased significantly with cisplatin treatment (Fig. 6h, i). Taken together, these data provided evidence that ABCB1 a downstream target of CHD1L-induced cisplatin resistance in NSCLC cells.

**ABCB1 upregulation by CHD1L was partly dependent on c-Jun**

Several studies reported that c-Jun could bind to ABCB1 and promote its transcription. Our Western blot analysis showed that both c-Jun and ABCB1 were increased by enforced CHD1L expression in A549 cells (Fig. 7a). Moreover, the dual luciferase reporter assays demonstrated that the increased transcriptional activity...
Table 2  List of genes differentially expressed in A549 cells after CHD1L overexpression using a Cancer Drug Resistance Real-time PCR Array

| Gene    | Fold change | Location  | Function                                                                 |
|---------|-------------|-----------|--------------------------------------------------------------------------|
| **Upregulated genes**                                                                                      |
| ABCB1   | +3.63       | 7q21.12   | Decreases drug accumulation                                               |
| ABCC1   | +1.25       | 16p13.11  | Involves in multi-drug resistance                                         |
| ABCC2   | +1.00       | 10q24.2   | Involves in multi-drug resistance                                         |
| ABCC3   | +1.02       | 17q21.33  | Involves in multi-drug resistance                                         |
| ABCCS   | +1.01       | 3q27.1    | Provides resistance to thiopurine anticancer drugs                        |
| ABCG2   | +1.69       | 4q22.1    | Responses to mitoxantrone and anthracycline exposure                     |
| ACTB    | +1.16       | 7p22.1    | Involves in cell motility, structure, integrity, and intercellular signaling |
| AHR     | +1.45       | 7p21.1    | Regulate xenobiotic-metabolizing enzymes                                  |
| AR      | +2.03       | Xq12      | Encode polyglutamine and polyglycine tracts                              |
| ATM     | +1.01       | 11q22.3   | Cell response to DNA damage                                              |
| B2M     | +1.14       | 15q21.1   | Association with the MHC                                                 |
| BCL2L1  | +1.14       | 20q11.21  | Apoptotic inhibitor                                                      |
| BLMH    | +1.02       | 17q11.2   | Metabolic inactivation                                                   |
| BRCA1   | +1.13       | 17q21.31  | Maintains genomic stability                                              |
| BRCA2   | +1.62       | 13q13.1   | Involves in maintenance of genome stability                              |
| CCND1   | +1.12       | 11q13.3   | Required for cell cycle G1/S transition                                  |
| CCNE1   | +1.13       | 19q12     | Required for cell cycle G1/S transition                                  |
| CDK2    | +1.29       | 12q13.2   | Regulates progression through the cell cycle                             |
| CDK4    | +1.02       | 12q14.1   | Regulates progression through the cell cycle                             |
| CLPTM1L | +1.67       | 5p15.33   | Increases susceptibility to cancers                                       |
| CYP2C19 | +2.24       | 10q23.33  | Variable ability to metabolize mephenytoin                              |
| CYP2CB  | +1.53       | 10q23.33  | Anticonvulsive drug mephenytoin                                          |
| CYP2E1  | +2.02       | 10q26.3   | Involves in drug metabolism                                              |
| CYP3A5  | +1.46       | 18q21.1   | Involves in drug metabolism                                              |
| DHFR    | +1.00       | 5q14.1    | Identified on separate chromosomes                                       |
| EGFR    | +1.10       | 7p11.2    | Promotes cell proliferation                                              |
| EK1     | +1.25       | Xp11.23   | Promotes cell proliferation                                              |
| ESR1    | +1.07       | 6q25.1-q25.2 | A ligand-activated transcription                                    |
| ESR2    | +1.49       | 14q23.2-q23.3 | Inhibits cell proliferation                      |
| FGF2    | +1.27       | 4q28.1    | Nervous system development, wound healing, and tumor growth              |
| FOS     | +1.26       | 14q24.3   | Cell proliferation and transformation                                     |
| GSTP1   | +1.02       | 11q13.2   | Reduces glutathione and detoxification                                   |
| HPR1    | +1.05       | Xq26.2-q26.3 | Plays a central role in the generation of purine nucleotides           |
| MET     | +1.40       | 7q31.2    | Induces dimerization and activation of the receptor,                     |
| MSH2    | +1.18       | 2p21-p16.3 | Consistent with the characteristic alterations                           |
| MYC     | +1.31       | 8q24.21   | Participates in cell cycle progression, apoptosis and cellular transformation |
| NAT2    | +1.19       | 8p22.2    | Associated with higher incidences of cancer and drug toxicity            |
| NFKB2   | +1.03       | 10q24.32  | Involves in inflammation and immune function                             |
Table 2 continued

| Gene      | Fold change | Location | Function                                           |
|-----------|-------------|----------|----------------------------------------------------|
| PPARA     | +1.27       | 22q13.31 | Promotes proliferation                            |
| PPARG     | +1.74       | 3p25.2   | A regulator of adipocyte differentiation           |
| RARB      | +1.64       | 3p24.2   | Mediates signaling in cell growth and differentiation |
| SULT1E1   | +2.11       | 4q13.3   | Controls levels of estrogen receptors              |
| TNFRSF11A | +1.00       | 18q21.33 | Promotes proliferation                            |
| UCGC      | +1.41       | 9q31.3   | The core structure of many glycosphingolipids      |

Downregulated genes

| Gene      | Fold change | Location | Function                                           |
|-----------|-------------|----------|----------------------------------------------------|
| XPA       | −1.00       | 9q22.33  | Plays a central role in nucleotide excision repair |
| ABCC1     | −1.27       | 16p13.11 | Involves in multi-drug resistance                  |
| AP1S1     | −1.09       | 7q22.1   | Involves in endocytosis and Golgi processing       |
| APC       | −1.19       | 5q22.2   | Cell migration and adhesion                        |
| ARNT      | −1.05       | 1q21.3   | Involves in xenobiotic metabolism                  |
| BAX       | −1.43       | 19q13.33 | Involves in P53-mediated apoptosis                  |
| BCL2      | −1.14       | 18q21.33 | In multiple transcript variants                    |
| CDKN1A    | −1.10       | 6p21.2   | Interact with proliferating cell nuclear antigen,  |
| CDKN1B    | −1.15       | 12p13.1  | Controls the cell cycle progression                |
| CDKN2A    | −1.13       | 9p21.3   | Inhibits proliferation                            |
| CDKN2D    | −1.09       | 19p13.2  | Participates in proliferation                      |
| CYP1A1    | −1.13       | 15q24.1  | Involves in drug metabolism                        |
| CYP1A2    | −1.02       | 15q24.1  | Involves in drug metabolism                        |
| CYP2B6    | −1.02       | 19q13.2  | Involves in drug metabolism                        |
| CYP2C9    | −1.23       | 10q23.33 | Involves in drug metabolism                        |
| CYP2D6    | −1.06       | 22q13.2  | Involves in drug metabolism                        |
| CYP3A4    | −1.04       | 7q22.1   | Involves in drug metabolism                        |
| EPHX1     | −1.25       | 1q42.12  | Activation and detoxification of epoxides           |
| ERBB2     | −1.27       | 17q12    | Promotes cell proliferation                        |
| ERBB3     | −1.30       | 12q13.2  | Promotes cell proliferation                        |
| ERBB4     | −1.23       | 2q34     | Promotes cell proliferation                        |
| ERCC3     | −2.10       | 2q14.3   | Nucleotide excision repair                         |
| GAPDH     | −1.08       | Xq26.2-q26.3 | Identified as a moonlighting protein             |
| GSK3A     | −1.07       | 19q13.2  | Regulates glycogen synthase and transcription factors |
| GSTP1     | −2.86       | 11q13.2  | Reduces glutathione and detoxification             |
| HIF1A     | −1.24       | 14q23.2  | Involves in energy metabolism, angiogenesis, apoptosis |
| IGF1R     | −1.21       | 15q26.3  | Enhances cell survival                            |
| IGF2R     | −1.12       | 6q25.3   | Enhances cell survival                            |
| MVP       | −1.47       | 16p11.2  | participates in multiple cellular processes       |
| NFKB1     | −1.02       | 4q24     | Leads to cell development or delayed cell growth   |
| NFKB1B    | −1.07       | 19q13.2  | A transcription factor                            |
| NFKB1E    | −1.09       | 6p21.1   | Promotes proliferation                            |
and expression of ABCB1 by CHD1L were largely blocked after silencing c-Jun in CHD1L-overexpressing NSCLC cells (Fig. 7b). Meanwhile, silencing ABCB1 in CHD1L-overexpressed NSCLC cells could increase the phosphorylation of p65 and IκBα. (Fig. 7c). Through the concentration gradient experiment, we proved that the expression level of ABCB1 in PC9 cells is consistent with cisplatin gradient, and the NFκB downstream IκBα and p65 was phosphoried with the upregulated ABCB1 (Fig. 7d). Schematic diagram depicting a proposed model for a major mechanism of CHD1L and its upregulation in the promotion of NSCLC cell cisplatin-resistance. Cisplatin generates DNA damage, which eventually leads to cell apoptosis. Meanwhile, CHD1L is upregulated after cisplatin treatment, possibly through a direct or indirect induction by DNA damage response. Therefore, CHD1L functions through activating NF-κB signaling to mediate resistance to cisplatin (Fig. 7e).

Discussion

Over amplification of chromosome 1q has been found to play an important role in tumor pathogenesis and disease progression in several human cancers; including NSCLC. Several candidate oncogenes including CHD1L and MUC1 have been found on chromosome region 1q21. These candidate oncogenes have been found to be associated with a poor chemotherapy response and poor prognosis in HCC and ovarian cancer, respectively, implying that putative oncogenes within this region may contribute to not only tumor progression but also to chemotherapy resistance in human cancers. Previously we found patients with NSCLC had a significantly increased frequency of overexpression and amplification of CHD1L compared to healthy lung tissue. Overexpression of CHD1L was associated with advanced disease stage and overall poorer outcomes in NSCLC patients that were treated with radical surgery. In the present study, we further showed that overexpression of CHD1L was associated with a poor chemotherapy response and worse outcome in cisplatin-treated NSCLC patients. These data, taking together, suggest that CHD1L might have an important role in cisplatin resistance and might serve as a predictor of chemotherapy response in NSCLC.

Consistent with our clinical observations, we found that overexpression of CHD1L desensitized NSCLC cells to cisplatin, and conversely, downregulation of CHD1L increased the sensitivity to cisplatin. These results indicate that CHD1L may cause the development of cisplatin resistance known to acquire malignant phenotypes after repeated exposure to cisplatin. The primary anticancer mechanism of cisplatin is to bind DNA covalently to form platinum-DNA adducts and induce DNA damage in the proliferating cancer cells. In recent years, CHD1L has been defined as a DNA damage–response protein, and could be recruited to the damaged sites by associated factor PARP-1. Cisplatin-DNA platinum adducts may function as an activator of some unknown molecules which is effective to upregulate CHD1L, or there may be some other complicated mechanisms which need to be further investigate. Interestingly, we also found that

| Gene   | Fold change | Location | Function                                                                 |
|--------|-------------|----------|--------------------------------------------------------------------------|
| PPARD  | −1.89       | 6p21.31  | Inhibits the ligand-induced transcriptional activity                     |
| RARA   | −2.78       | 17q21.2  | Implicated in regulation of development, differentiation, apoptosis      |
| RARG   | −1.23       | 12q13.13 | Involves in various biological processes                                  |
| RB1    | −1.40       | 13q14.2  | A negative regulator of the cell cycle                                   |
| RELB   | −1.02       | 19q13.32 | Activation in anti-inflammatory decidual endothelial cells               |
| RPLP0  | −1.27       | 12q24.23 | The functional equivalent of the E. coli L10 ribosomal protein           |
| RXRA   | −1.71       | 9q34.2   | Mediates the biological effects of retinoids                             |
| RXRB   | −1.19       | 6p21.32  | Increases DNA binding                                                    |
| SOC1   | −1.10       | 21q22.11 | A homodimer to convert naturally occurring                               |
| TOP1   | −1.28       | 20q12    | Controls the topologic states of DNA during transcription.               |
| TOP2A  | −1.11       | 17q21.2  | Controls the topologic states of DNA during transcription.               |
| TOP2B  | −1.32       | 3p24.2   | Controls the topologic states of DNA during transcription.               |
| TP53   | −1.08       | 17p13.1  | Participates in cell cycle arrest, apoptosis, senescence, DNA repair     |
| TPMT   | −1.19       | 6p22.3   | Correlated with variations in sensitivity and toxicity                   |
| XPC    | −1.02       | 3p25.1   | Plays an important role in the early steps of global genome nucleotide excision repair |
cisplatin treatment increased the expression level of CHD1L in PC9 cells, and it is possible this increased expression of CHD1L contributes to the resistant cell subtype transformation. We called this process of cisplatin treatment demonstrating an initially favorable outcome, then later on developing chemoresistance “acquired resistance”. Our results suggest that silencing CHD1L expression may have therapeutic potential, especially in enhancing the chemosensitivity of NSCLC to cisplatin. Our results provide us with new insights into the role CHD1L in drug resistance, and importantly, CHD1L may act as a potential target to overcome cisplatin resistance in NSCLC.

The molecular mechanism by which CHD1L regulates cancer cell cisplatin resistance remains unclear. In our recent study, we found that CHD1L might confer chemoresistance by inhibiting the Nur77/ Cyto c/caspase 9 pathway in HCC10. However, in this study we did not look for altered levels of active Nur77, Cyto c and caspase9 before and after CHD1L overexpression or knockdown. Therefore, we concluded that in our NSCLC cells, CHD1L regulated cancer cell cisplatin resistance by the regulation of targets and/or pathways other than the activation of Nur77/Cyto c/caspase 9, suggesting that the mechanism(s) by which CHD1L regulates cancer cisplatin resistance may be tumor-type specific.

To better understand the potential downstream molecular mechanism of CHD1L in drug resistance, a Cancer Drug Resistance Real-time PCR Array containing 84 well-known drug resistance-related genes was used to compare mRNA expression profiles between A549-CHD1L cells and those of control A549-vector cells. Of the 84 well-known resistance genes, five were found to be differentially expressed (mRNA levels altered by two fold or more). ABCB1, CYP2C19, and SULT1E1 were all upregulated and ERCC3 and GSTP1 were downregulated.
Western blot data indicate both ABCB1 and ERCC3 are consistent to the result, however, downregulated CHD1L in A549-DDP cells decrease the ABCB1 and ERCC3, indicating that ERCC3 may be regulated more complicated than ABCB1 do. As a result, we just focus on ABCB1 in present study. (supplementary Fig. 2a). ABCB1, initially isolated in drug-resistant Chinese hamster ovary cancer cells, was hypothesized to be the most obvious choice for a downstream target gene of CHD1L in NSCLC cells. And indeed, we did observe a significant positive correlation between the overexpression of CHD1L and ABCB1 in our large cohort of NSCLC tissues. These results, collectively, suggest that in NSCLC cells, CHD1L might regulate cell cisplatin resistance by the regulation of ABCB1.

In recent years, numerous studies have shown that ABCB1 is widely expressed in human tumor cells at different stages. The patients who suffer from tumors with high levels of ABCB1, including patients with colorectal cancer, pancreatic cancer, liver cancer, adrenal cortex carcinoma, acute leukemia, and ovarian cancer, are usually found to also have a poorer prognosis. It is also reported that ABCB1 has an important effect on absorption, distribution, metabolism, and excretion of its substrate drugs. Inhibition of ABCB1 efflux activity increases the accumulation of chemotherapeutic drugs in tumor cells with high expression of ABCB1, thereby enhancing the inhibitory effect of chemotherapeutic drugs on tumor cells. The results of our rescue experiment indicate that CHD1L-mediated cisplatin-resistance can be dramatically prevented by knockdown of ABCB1. These data suggest that ABCB1 might be a critical downstream target of CHD1L and may be responsible for the CHD1L-induced cisplatin-resistance in NSCLC cells.

To date, however, the mechanisms by which CHD1L regulates ABCB1 expression have not been elucidated. Our previous study found no evidence to support a direct binding of CHD1L on the promoter region of ABCB1, indicating that an indirect regulatory mechanism might exist between CHD1L and ABCB1 in NSCLC cells. Because it has been improved that c-Jun could bind to ABCB1 promoter, we therefore verified whether c-Jun expression could be affected by CHD1L. The Western blot analysis showed that both c-Jun and ABCB1 were increased by enforced CHD1L expression in A549 cells. Moreover, ABCB1 expression depends on c-Jun level. The dual luciferase reporter assay for ABCB1 showed that overexpressed CHD1L increase the luciferase activity of ABCB1, while, blocking c-Jun by siRNA approach significantly attenuates the luciferase activity, indicating that c-Jun is involved in the promoting effect of CHD1L on ABCB1 expression. Furthermore, the functional studies also showed that CHD1L-induced cisplatin-resistance could be attenuated by c-Jun down-regulation in A549 cells. All together, our results indicated that the c-Jun mediated the promoting effect of CHD1L on ABCB1 expression.

NF-κB signaling is a well-known survival-related pathway that upregulates anti-apoptotic genes, and has recently been assessed to inhibit apoptosis and mediate cisplatin resistance and tumor development in cancer. A recent study reported that NF-κB pathways was involved in the development of multiple drugs resistance (MDR) in MCF-7/ADR cells through the upregulation of the ABCB1 gene expression. We thus verified the correlation between ABCB1 expression and NF-κB signaling activation in CHD1L overexpressed lung cancer cell lines. Through the concentration gradient experiment, we proved that the expression level of ABCB1 in A549 cells is consistent with cisplatin gradient, and the NFκB downstream IκBα and p65 was phosphoryled with the upregulated ABCB1. Besides, silencing ABCB1 in CHD1L ectopic A549 cells will decrease the phosphorylation of p65 and IκBα. However, we failed to prove the direct binding between ABCB1 and NFκB promoter region, but the results could indicate that the NF-κB pathway is closely associated with ABCB1 expression.

In summary, our study describes, for the first time, that CHD1L contributes to cisplatin resistance in NSCLC. Furthermore, we demonstrated that CHD1L plays a critical role in inducing cisplatin-resistance of NSCLC cells via upregulation of ABCB1 through c-Jun. Our results suggest that CHD1L and ABCB1 may serve as potential therapeutic targets to overcome cisplatin-resistance in NSCLC.

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Conflict of interest
The authors declare that they have no conflict of interest.

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