Am J Transl Res 2019;11(3):1581-1592
www.ajtr.org /ISSN:1943-8141/AJTR0085895

Original Article

CHD1L promotes cell cycle progression and cell motility by up-regulating MDM2 in breast cancer

Wei Wang[1], Jiayi Wu[1], Xiaochun Fei[2], Weiguo Chen[1], Yafen Li[1], Kunwei Shen[1], Li Zhu[1]

[1]Comprehensive Breast Health Center, [2]Pathology Department, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, 197 Ruijin Second Road, Shanghai 200025, P. R. China

Received September 19, 2018; Accepted January 14, 2019; Epub March 15, 2019; Published March 30, 2019

Abstract: Chromodomain helicase/ATPase DNA-binding protein 1-like (CHD1L) gene is a novel oncogene amplified in many solid tumors. We investigated its role in breast cancer. CHD1L was over-expressed in 49.1% (57/116) breast cancer patients. Overexpression of CHD1L was significantly associated with younger age at diagnosis (P = 0.016), lymph node involvement (P = 0.040), higher tumor grade (P = 0.027), higher proliferation rate (P = 0.007) and shorter disease-free survival rate (77.2% vs. 91.5%, P = 0.037). A cDNA microarray analysis identified MDM2 as an important downstream target of CHD1L. And MDM2/p53 signaling pathway was showed to be significantly modulated by CHD1L. Further in vitro study showed that overexpression of CHD1L can promote tumorigenesis, metastasis, invasion and cell cycle progress. In vivo study confirmed the tumorigenesis ability of CHD1L. shRNA-mediated CHD1L silencing could abolishes the tumor-promotion effect of CHD1L in vitro and in vivo. In conclusion, CHD1L may promote the progress of breast cancer cells via the MDM2/p53 signaling pathway. This study identified CHD1L as a prognostic factor for breast cancer and MDM2 might be used as a potential target for therapeutic intervention in CHD1L overexpression breast cancer.

Keywords: Breast neoplasm, CHD1L, gene regulation, MDM2, tumor progression

Introduction

Breast cancer is the most frequently diagnosed cancer among Chinese women, with an incidence of 41.73/105 in 2013 [1]. Although adjuvant chemotherapy improves survival in early breast cancer for some patients, the mortality rate of breast cancer is in rise [2]. Therefore, to explore the pathogenesis of breast cancer and to find new therapeutic target is still of vital importance to improve the prognosis of breast cancer patients.

It has been reported that the occurrence of breast cancer is closely related to the instability of the genome, such as gene amplification, deletion, mutation [3, 4]. Gains in 1q were one of the most frequent genetic alterations in breast cancer and Comparative Genomic Hybridization (CGH) analysis indicated that more than 30% of breast tumors had regional (.10 Mb) gains in 1q [5-7]. The chromodomain helicase/ATPase DNA binding protein 1-like gene (CHD1L) was recently identified as a target oncogene within the 1q21 amplicon in hepatocellular carcinoma (HCC), which was closely related to the proliferation and invasion of liver cancer, and can independently predict the survival of HCC patients [8-10].

CHD1L exhibits an oncogenic role during malignant transformation and was identified as a novel oncogene [11]. In a CHD1L transgenic mouse model, transgenic CHD1L expression induced the spontaneous formation of tumors [12]. Overexpression of CHD1L protein is considered to be a biomarker of poor prognosis in many solid tumors such as ovarian cancer, pancreatic cancer, colorectal cancer and gastric cancer [13-16]. CHDIL gene was also overexpressed in 40%-50% breast cancer patients. The overexpression of CHD1L was proved to be related to more aggressive tumor behavior and worse prognosis in breast cancer [17, 18]. While the mechanism of CHD1L promoting breast cancer progressing has not been fully understood.

As a member of SNF2-like family, CHD1L contains a conserved SNF2_N domain, a helicase superfamily domain, which may play important roles in transcriptional regulation, maintenance of chromosome integrity and DNA repair [11]. Therefore, investigating CHD1L transcriptionally regulated network would aid in the elucidation of oncogenetic molecular mechanism of CHD1L and may further provide new therapeutic targets for cancer treatment.

In this study, we found that CHD1L could promote proliferation and invasion of breast cancer cells. Expression profile microarray result showed that CHD1L may act through MDM2/P53 signaling pathway in vitro and in vivo.

Materials and methods

Patients and clinical specimens

One hundred and sixteen patients who underwent surgery for breast cancer at our center were included in this study. None of these patients received preoperative chemotherapy or radiotherapy. The surgical specimens (both tumor and adjacent non-tumor tissue) were embedded in paraffin block. All patients gave written informed consent on the use of clinical specimens for medical research. Studies using human tissue were reviewed and approved by the Committees for Ethical Review of Research involving Human Subjects of our institute. The construction of tissue microarray (TMA) were performed as described previously [17].

Cell lines and culture conditions

The breast cancer cell lines MDA-MB-231, T47D, SKBR3, MCF7 and BT-474 were obtained from the cell bank of Chinese Academy of Sciences (Shanghai). MDA-MB-231, T47D and SKBR3 were maintained in PRIM-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. MCF7 and BT-474 were maintained in high-glucose DMEM supplemented with 10% fetal calf serum (Invitrogen). The cells were incubated at 37°C in a humidified chamber containing 5% CO_2.

Antibodies

CHD1L (rabbit, HPA028670, sigma), β-actin (rabbit monoclonal, 12620s, Cell Signaling Technology, CST), MDM2 (rabbit, AF1244, R&D), p53 (mouse monoclonal, sc-126, Santa Cruz). ER: clone 1D5 (rabbit monoclonal, Gene), PR: clone PR636 (mouse monoclonal, Dako), HER2: c-erbB-2 4B5 (2009-2012, rabbit monoclonal, Roche), Ki-67: clone MIB-1 (mouse monoclonal, Dako).

Immunohistochemistry (IHC)

IHC staining was performed with the standard streptavidin biotin-peroxidase complex method according to the manufacturer's instructions. The degree of immunostaining of sections was reviewed and scored independently by two observers. Staining status was decided as previously described [17]: CHD1L status was determined by staining intensity times the proportion score with a cut off value of median score (Staining Intensity was defined as 0 = negative, 1 = weak, 2 = moderate, and 3 = strong. Positive Cells was scored as 0 = negative, 1 = 1%-10%, 2 = 11%-30%; 3 = 31%-50%, 4 = 51%-80% and 5 ≥80% positive cells). Positive staining for ER/PR was defined as nuclear staining in ≥1% of tumor cells. HER2 positivity was considered as HER2 3+ by IHC or positive on FISH, whereas cases with 0 to 1+ or 2+ without FISH positivity were regarded as negative. Representative tumor specimens were stained for Ki-67 according to manufacturer's recommendations.

RNA extraction and quantitative real-time polymerase chain reaction (QRT-PCR)

Total RNA extraction and QRT-PCR were performed as described previously [17]. Cycle threshold was measured during the exponential amplification phase, and the amplification plots were analyzed using SDS 1.9.1 software (Life Technologies).

Western blot

Western blot analysis was performed using the standard method with antibodies. The densitometry data were analyzed using Scion Image software, version beta 3b (Scion, Frederick, MD).

Lentivirus-mediated gene overexpression and knockdown

The full-length CHD1L cDNA was amplified and cloned into the GV492 expression vector

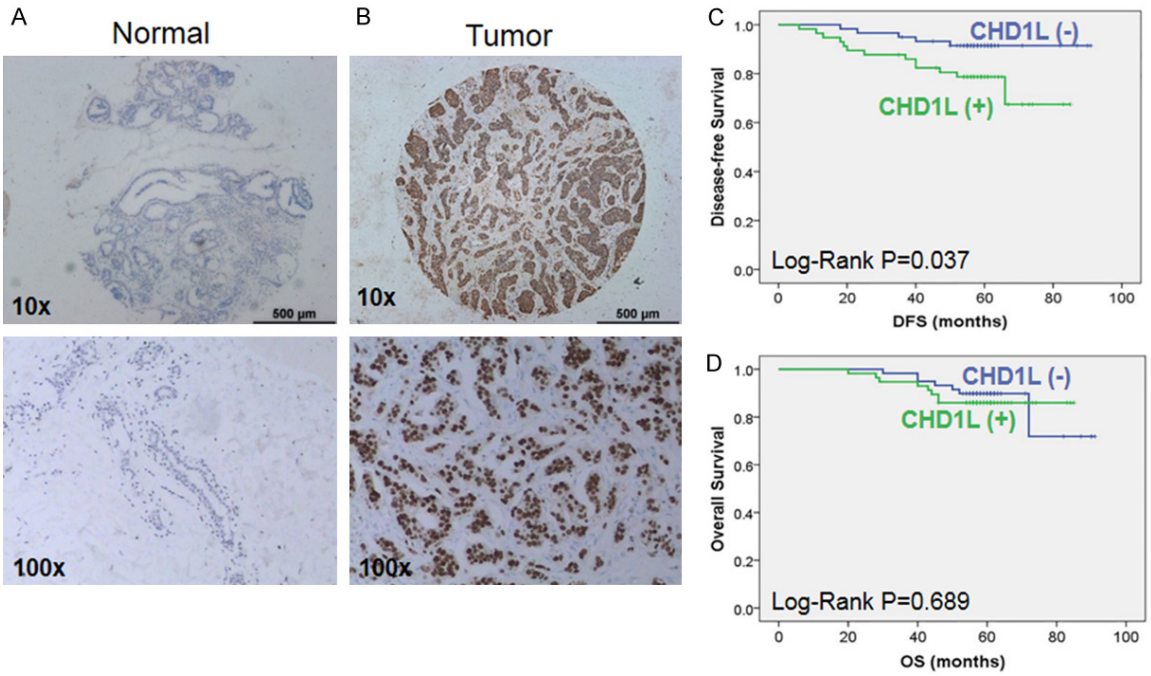

Figure 1. Expression of CHD1L in breast cancer tissue. (A) Normal expression of CHD1L in adjacent non-tumor tissue. (B) Overexpression of CHD1L in primary breast cancer tissue. (C) Kaplan-Meier disease-free survival curve and (D) overall survival curve of breast cancer patients correlated with CHD1L expression. CHD1L (+), patients with CHD1L overexpression; CHD1L (-), patients without CHD1L overexpression.

(Genechem) according to the manufacturer's instructions. Either the CHD1L expression construct or the GFP control plasmid was transfected into the 293FT cell line. Virus-containing supernatants were collected for subsequent transduction into BT-474 cells. For the knockdown assay, short hairpin RNA (shRNA) of CHD1L were designed (5'-GTATTGGACATGCCACGAAA-3') based on the gene sequence (NM_004284). The oligoduplexes were cloned into the GV248 (Genechem) vector or GFP control plasmid. Viruses were packaged and transduced into MDA-MB-231 cells. Puromycin (Sigma-Aldrich) was used to select stably transduced cells.

Cell viability

Cell viability was determined using the CCK-8 (Cell Counting Kit-8; Dojindo, Japan) assay. A total of 10000 per well cells were seeded in 96-well plates. After incubation for every 24 h, 10 ul of CCK-8 solution was added to each well. The absorbance at 450 nm was measured using multi-well scanning spectrophotometer (Thermo). All experiments were performed using triplicate wells per condition in each assay and were repeated at least 3 times.

Colony formation assay

Cells were cultured in 6-well plate at a density of 1,000 cells per well and incubated for 15 days to allow colony formation. Colonies containing more than 50 cells were counted and evaluated.

Wound healing and invasion assays

The cell migration status was determined by measuring the movement of cells into a scraped area created using a 200-μl pipette tip. The spread of wound closure was observed after 48 hours and photographed under a microscope. Invasion assays were performed with 24-well BioCoat Matrigel Invasion Chambers (BD) according to the manufacturer's instructions. The number of cells that invaded through the Matrigel was counted in 10 fields under a ×20 objective lens.

Cell cycle analysis

Cells were cultured in full medium containing 10% fetal bovine serum. Serum was withdrawn from the culture medium when the cells were 70% confluent. After 24 h, 10% fetal bovine

Table 1. Association of CHD1L overexpression with clinicopathologic features

	CHD1L expression		*P* value
	Overexpression	Normal expression	
Age			0.016
≤55	31 (54.4%)	19 (32.2%)	
>55	26 (45.6%)	40 (67.8%)	
T stage			0.305
1	28 (49.1%)	31 (52.5%)	
2	26 (45.6%)	28 (47.5%)	
3	3 (5.3%)	0 (0.0%)	
LN involvement			0.040
Yes	29 (51.8%)	19 (32.8%)	
No	27 (48.2%)	39 (67.2%)	
Grade			0.027
I-II	21 (41.2%)	35 (62.5%)	
III	30 (58.8%)	21 (37.5%)	
ER			0.129
Positive	21 (36.8%)	30 (50.8%)	
Negative	36 (63.2%)	29 (49.2%)	
PR			0.300
Positive	17 (29.8%)	23 (39.0%)	
Negative	40 (70.2%)	36 (61.0%)	
Her2			0.928
Positive	11 (19.3%)	11 (18.6%)	
Negative	46 (80.7%)	48 (81.4%)	
Ki67	45.3±24.5	33.7±20.6	0.007

serum was added to the medium for an additional 24 h. The cells were fixed in 70% ethanol, stained with propidium iodide, and analyzed using a flow cytometer. Triplicate independent experiments were performed.

Tumor xenograft mouse model

Female athymic nude mice (4-6 weeks old) were housed under standard conditions and cared for according to the institutional guidelines for animal care. All experimental animal procedures were conducted in compliance with institutional requirements and were approved by the Shanghai Jiao Tong University School of Medicine Committee for the Use and Care of Animals (Shanghai, China). For the xenograft tumor growth assay, control cells (Vec-474, and Con-231) were injected subcutaneously into the left dorsal flank of mice, and CHD1L-expressing or knockdown cells (CHD1L-474, and shCHD1L-231) were injected into the right dorsal flank of the same animal in a laminar flow cabinet. Tumor volume was measured weekly and calculated as $0.5 \times l \times w^2$ [9]. The mice were euthanized on the fifth or sixth week, and the tumors were excised and embedded in paraffin.

RNA microarray hybridization and signal scanning

The Affymetrix PrimeView™ Human Gene Expression Array (Affymetrix, 901838, Santa Clara, CA, USA) was used to profile differentially expressed genes in con-231 vs. shCHD1L-231 cells following the manufacturer's instructions. Briefly, extracted RNA template was reversely transcribed into cDNA and digested into fragments with endonucleases. These fragments were labeled with DNA labeling reagent and labeled cDNAs were hybridized to the microarray via incubation at 45°C and rotated at 60 rpm for 16 h. Following washing and staining, the arrays were scanned using a GeneChip Scanner3000 with GeneChip Operating Software.

Data analysis

The data were expressed as the mean ± standard deviation (SD) or mean ± Standard Error of Mean (SEM) (xenograft data). Statistical analyses were carried out in SPSS version 17.0 (SPSS, Inc., Chicago, IL). The clinicopathological features in CHD1L expression level of patients were compared using Pearson χ^2 test for categorical variables and independent Student's t test for continuous data. Kaplan-Meier plots and log-rank tests were used for survival analysis. Independent Student's t-test were used for statistics of cell cycle, cell growth rate, foci formation, cell migration distance, invasion cell numbers, and in vivo tumorigenesis volume. A *P* value less than 0.05 was considered statistically significant.

For the gene expression array results, the screening criteria for significant differently expressed gene was fold change (FC) >2. Pathway enrichment analysis were performed based on differently expressed genes.

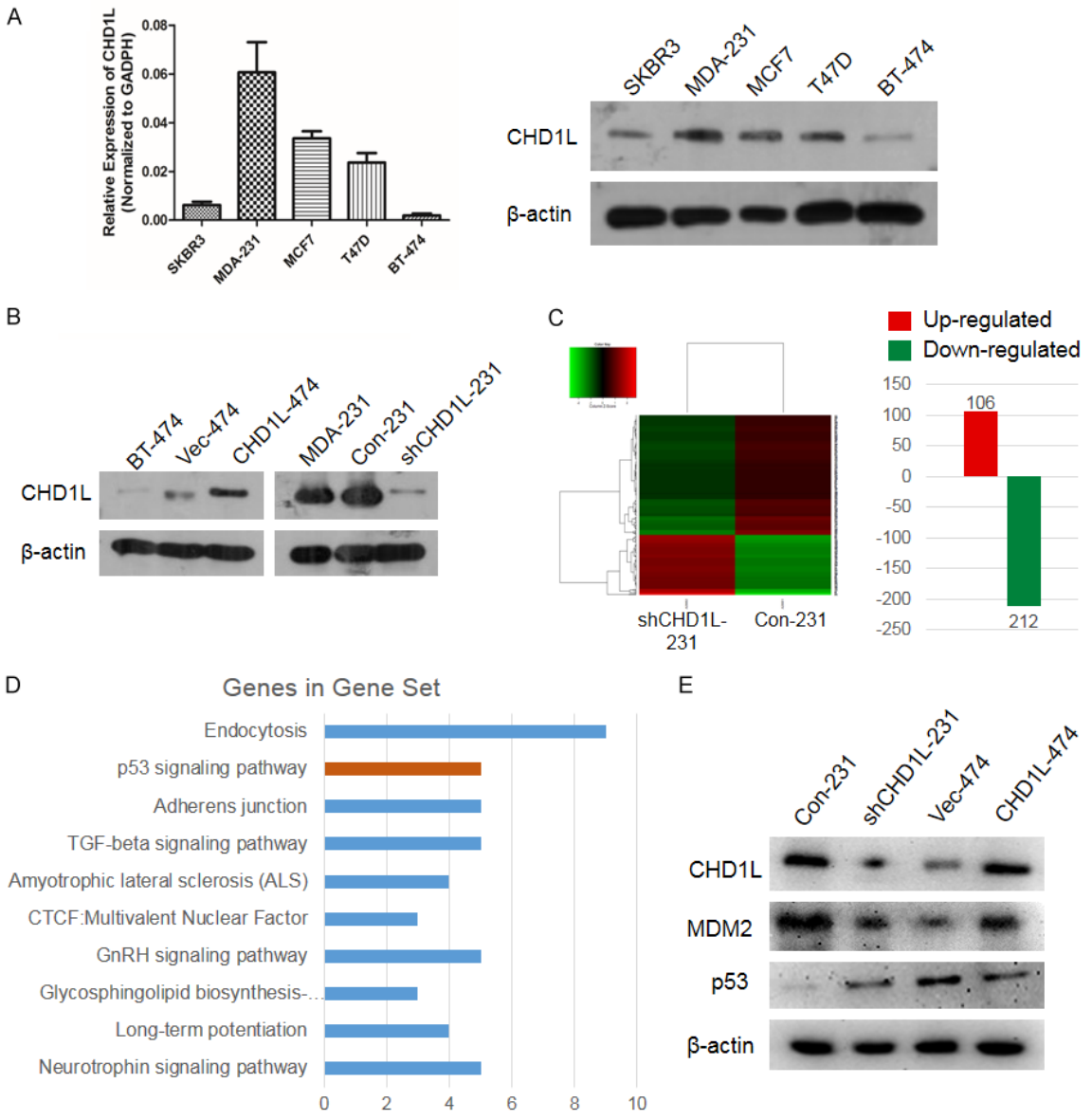

Figure 2. Identification of CHD1L target genes and network. A. The mRNA expression level and protein level of CHD1L in breast cancer cell lines were detected by RT-PCR (GAPDH was used as internal control) and western blot (β-actin was used as a loading control). B. Ectopic expression of CHD1L was detected in CHD1L-transfected cells by western blot (β-actin was used as a loading control). C. Left: Heatmap of the cDNA microarray analysis comparing the expression profiles between MDA-231 cells transfected with shCHD1L or control vector. Right: The up-regulated and down-regulated genes number in CHD1L-knockdown MDA-231 cells compared with control-231 cells. D. The top ten pathways regulated by CHD1L according to the *P* values of pathway enrichment analysis basing on differently expressed genes. E. The protein levels of CHD1L, MDM2, p53 were detected in Con-231, shCHD1L-231, Vec-474 and CHD1L-474 cells by Western blot analysis. β-actin was used as a loading control.

Results

Expression and clinical significance of CHD1L in breast cancer

IHC staining was used to study the expression pattern of CHD1L in paraffin sections from normal breast and paired breast cancer tissues. The expression of CHD1L was significantly higher in tumor tissues compared with adjacent non-tumor tissues (**Figure 1A**, **1B**).

With staining index of 5 as cut-off value, CHD1L was over-expressed in 49.1% breast cancer patients. The correlations between the expression of CHD1L and the clinicopathological parameters of breast cancer were analyzed. **Table 1** shows that the overexpression of

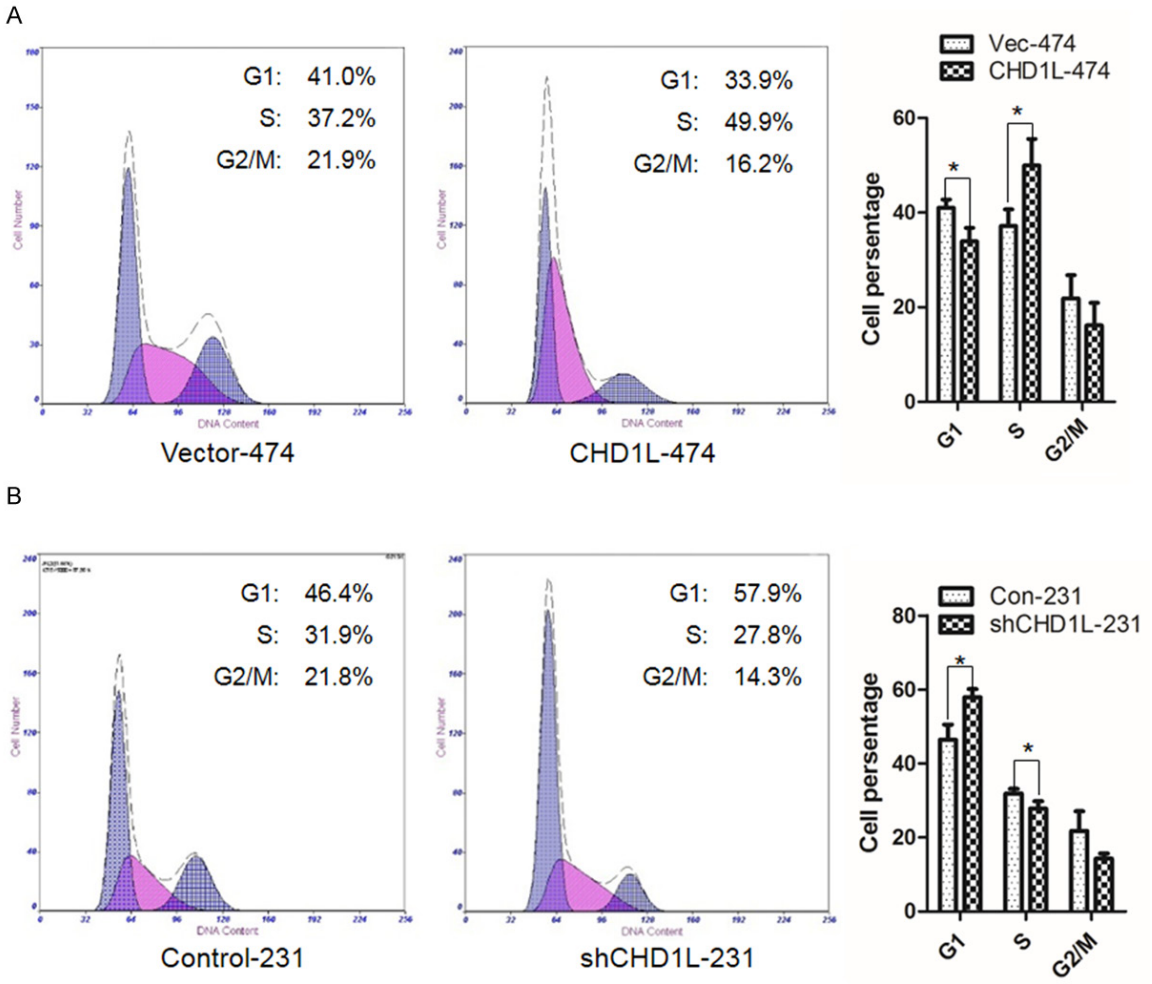

Figure 3. CHD1L promotes G1/S phase transition. A. Flow cytometry was used to compare cell cycle distribution between Vec-transfected and CHD1L-transfected BT474 cells. The percentages of cells at G0/G1, S and G2/M were summarized in bar charts. Data was shown as the mean ± standard deviation of three independent experiments (*P<0.05; independent Student's t-test). B. Depletion of CHD1L by shRNA in MDA-231 cells blocked cell cycle at G1 phase. Data were shown as the mean ± standard deviation of three independent experiments (*P<0.05; independent Student's t-test).

CHD1L was significantly associated with younger age at diagnosis (P = 0.016), lymph node involvement (P = 0.040), higher tumor grade (P = 0.027) and higher proliferation rate Ki67 (P = 0.007).

After a median follow-up of 57 months (range 20-91 months), patients with CHD1L overexpression had significantly poorer disease-free survival (DFS) (77.2% vs. 91.5%, P = 0.037, **Figure 1C**). However, no statistical significant differences could be found for overall survival between CHD1L overexpression and normal expression groups (86.0% vs. 88.1%, P = 0.689, **Figure 1D**)

Identification of CHD1L target genes

The expression levels of CHD1L in breast cancer cell lines were evaluated by RT-PCR and western blot (**Figure 2A**). To explore its role in tumorigenicity, CHD1L was cloned into an expression vector and stably transfected into the breast cancer cell lines BT-474 and was silenced with lentivirus-mediated shRNA in MDA-231 cell line (**Figure 2B**).

Like other SNF2-like family members, CHD1L may also be able to regulate gene expression at transcriptional level. To identify genes potentially regulated by CHD1L, a cDNA microarray

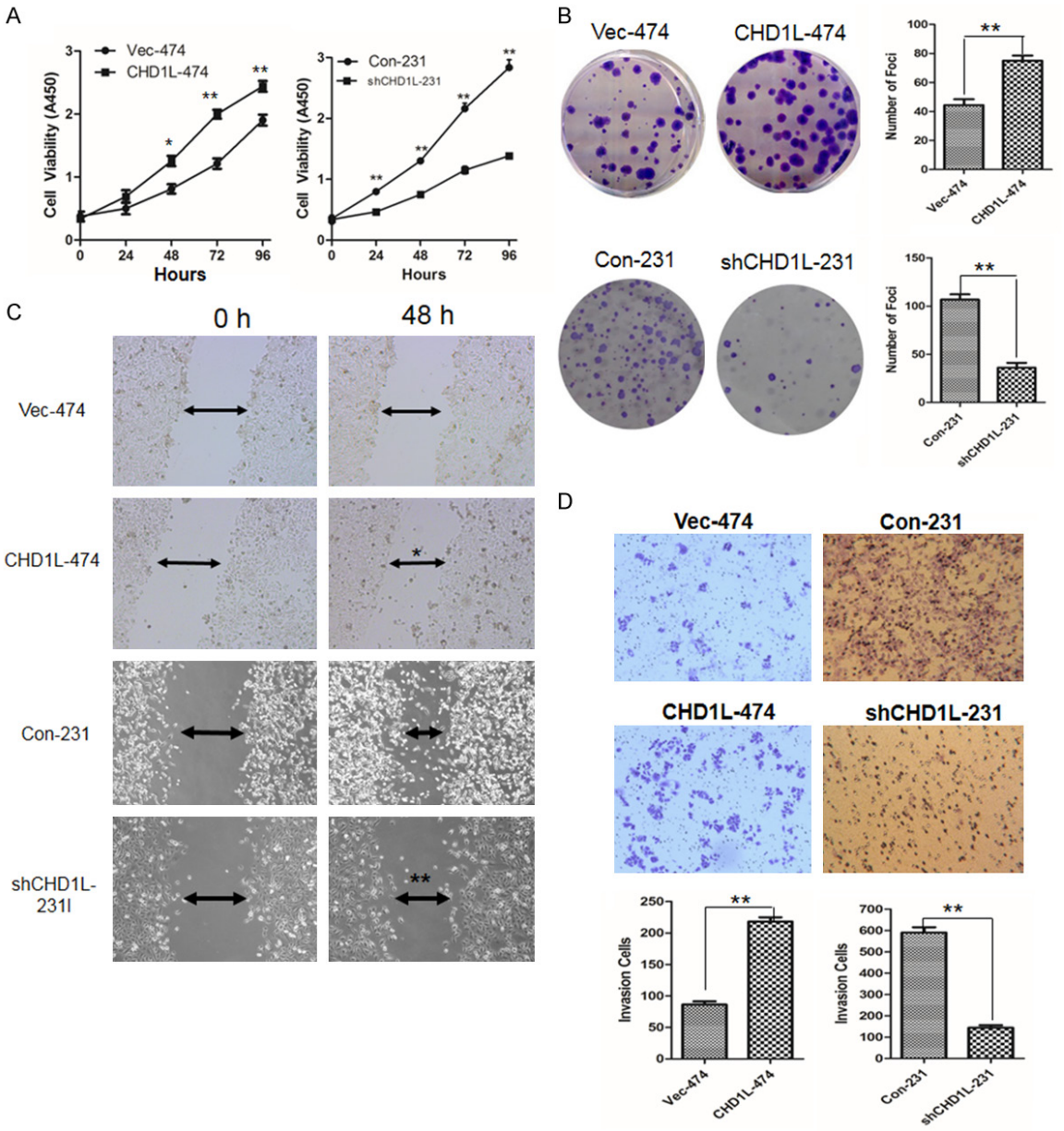

Figure 4. CHD1L promotes tumorigenesis, tumor metastasis and invasion. A. The cell growth rates were determined with CCK8 proliferation assay (*P<0.05; **P<0.001; independent Student t test). B. Representative images of foci formation induced by Vec-474, CHD1L-474 and Con-231, shCHD1L-231 cells. The numbers of foci were calculated and are depicted in the bar chart. The values indicate the mean ± standard deviation of 3 independent experiments (**P<0.001; independent Student t test). C. Cell migration rates of Vec-474, CHD1L-474 and Con-231, shCHD1L-231 cells were compared using wound healing assays. Microscopic observation was recorded at 0 and 48 h after scratching the surface of a confluent layer of cells. D. Matrigel invasion assay was performed to study the invasion ability of Vec-474, CHD1L-474 and Con-231, shCHD1L-231 cells. The number of invaded cells was calculated and is depicted in the bar chart. The values indicate the mean ± standard deviation of 3 independent experiments (**P<.001; independent Student t test).

was used to compare the gene expression profiles between MDA-231 cells transfected with shCHD1L or control vector. The results showed that 106 genes were up-regulated and 212 genes were down regulated in CHD1L-knockdown MDA-231 cells compared with control-231 cells (**Figure 2C**). The differential genes were enriched and analyzed based on the gene information in Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.kegg.jp/)

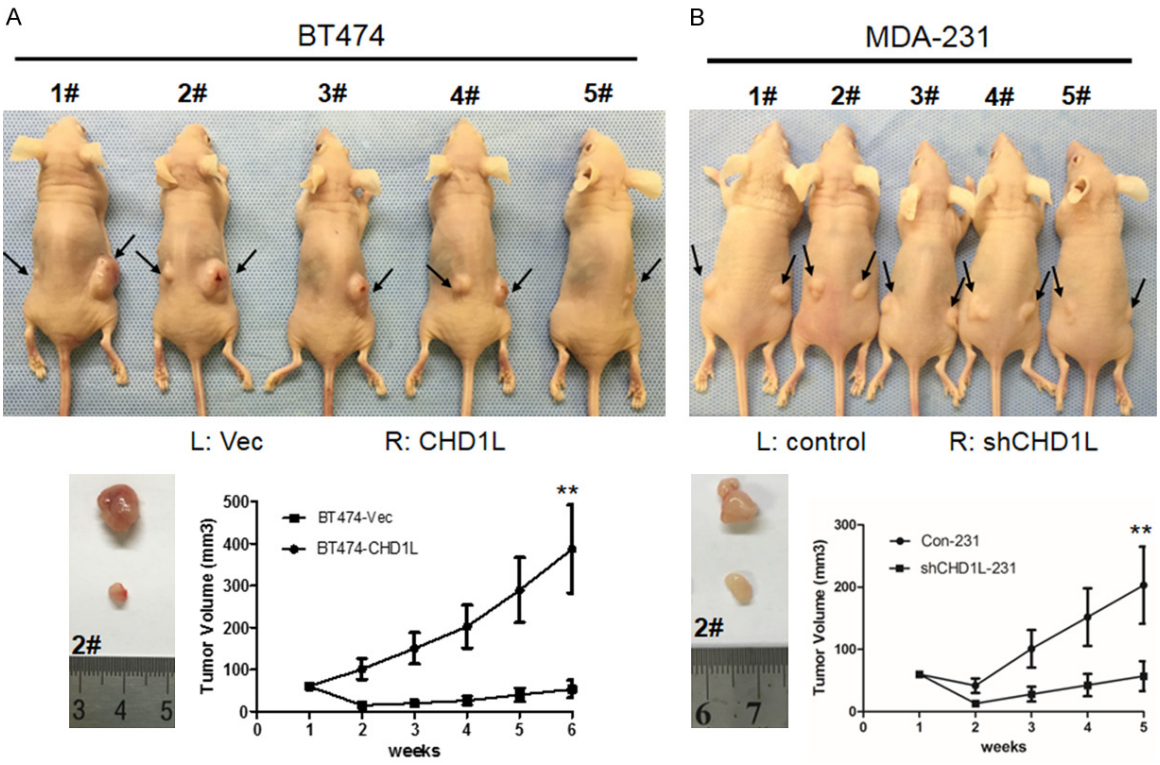

Figure 5. CHD1L promotes tumorigenesis in vivo. Representative examples of tumors (indicated by arrows) formed in nude mice injected with (A) Vec-474 and CHD1L-474 cells and (B) Con-231 and shCHD1L-231 cells. Tumor growth curves are summarized in the line chart. The average tumor volume was expressed as the mean ± standard error of mean of 5 mice. (***P*<0.001; independent Student t test).

pathway and BioCarta (https://cgap.nci.nih.gov/Pathways/BioCarta_Pathways) database, and the top ten pathways according to the *P* values were showed in **Figure 2D**. Among them, p53 signaling pathway was a key regulated pathway.

According to the microarray analysis results, one important negative regulator of the p53 tumor suppressor MDM2 was significantly down-regulated in shCHD1L-231 cells compared with con-231 (Tables S1 and S2). We then examined the correlation of expression level between MDM2 and CHD1L. Western Blot results showed that MDM2 was down-regulated in CHD1L-knockdown cells and was highly up-regulated in CHD1L-overexpression cells (**Figure 2E**).

CHD1L promotes G1/S phase transition in breast cancer cells

Since p53 signaling pathway was an important regulator of cell cycle, the role of CHD1L in the cell cycle was investigated. After synchronization of the cells at the G1 phase by serum starvation for a 24 h period, higher percentage of CHD1L-474 cell had G1/S phase transition stimulated by adding serum to the culture medium compared with Vec-474 (**Figure 3A**). By contrast, silencing CHD1L expression in MDA-231 inhibit the cell cycle at the G1/S checkpoint (**Figure 3B**). The percentage of cells arrested in the G1 phase was significantly higher in the shCHD1L-231 cells (57.9% vs. 46.4%) compared with the control-231 cells.

CHD1L promotes tumorigenesis, tumor metastasis and invasion

The tumorigenic ability of CHD1L was assessed by cell proliferation and foci formation assays. Compared with empty vector-transfected cells, CHD1L-transfected cells showed increased growth rates and higher foci formation frequencies. CHD1L depletion could reverse the tumorigenic phenotype in shCHD1L cells by inhibiting the cell growth rate and foci formation efficiencies. (**Figure 4A** and **4B**).

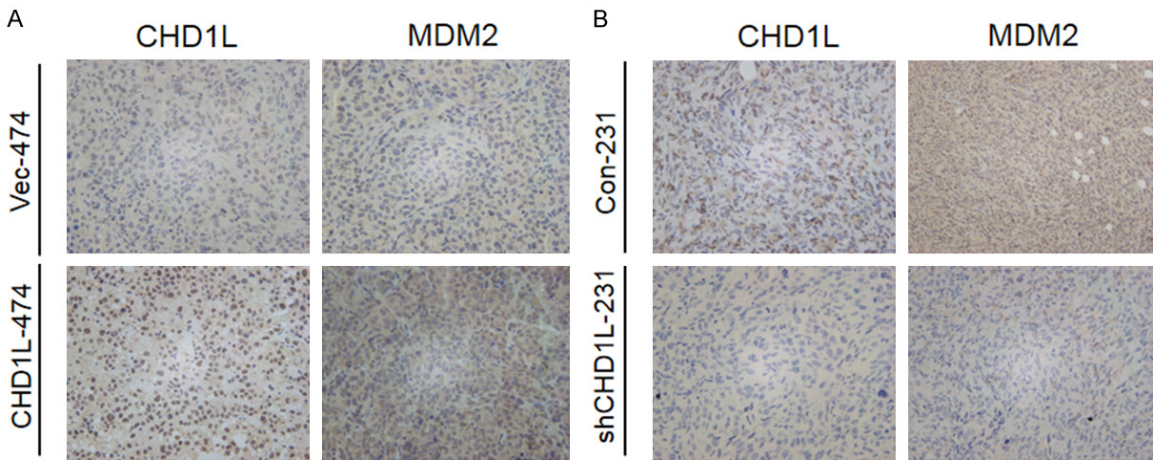

Figure 6. CHD1L promotes tumorigenesis in nude mice through regulation of MDM2. IHC staining for CHD1L and MDM2 on serial sections of tumors induced by (A) Vec-474 and CHD1L-474 and (B) Con-231 and shCHD1L-231 cells. (100× magnification).

To investigate the effects of CHD1L on metastasis and invasion, an in vitro wound healing and Matrigel invasion assay were performed. The wound healing assay demonstrated that overexpression of CHD1L increased cell motility in the CHD1L-474 cells and silencing CHD1L expression by shRNA in MDA-231 cells impaired the migration ability (**Figure 4C**). The Matrigel invasion assay showed that the invasive capability of CHD1L-overexpression BT-474 cells was greater than that of the empty vector transfected cells and was abolished in the shCHD1L-231 cells (**Figure 4D**).

CHD1L promotes tumorigenesis in vivo

To further investigate the in vivo tumorigenic ability of CHD1L, empty vector and CHD1L-transfected BT474 cells were injected subcutaneously into the left and right dorsal flank of nude mice (n = 5), respectively. Tumors induced by CHD1L-474 transfectants (tumor formation in 5 of 5 mice) showed significantly shorter latency and larger mean tumor volume than tumors induced by Vec-474 cells (tumor formation in 3 of 5 mice) (**Figure 5A**). While tumor induced by shCHD1L-231 showed a significantly smaller mean tumor volume compared with the control-231 cells (**Figure 5B**). Collectively, these data indicate that CHD1L possesses strong tumorigenicity in vivo.

To verify the regulation effect of CHD1L on MDM2 in vivo, IHC with antibodies against CHD1L and MDM2 was performed on serial sections of tumor induced by CHD1L transfectants in the nude mouse. Increased expression of MDM2 was observed in tumors induced by CHD1L-474 cells compared with tumors induced by Vec-474 (**Figure 6A**) and decreased expression of MDM2 was observed in tumors induced by shCHD1L-231 (**Figure 6B**). This indicated that CHD1L may also regulate the MDM2 expression and p53 signaling pathway in vivo.

Discussion

Amplification of 1q is an early event and is detected in more than 30% of breast tumors [5-7]. CHD1L was an oncogene isolated from this frequently amplified region and has been shown to exert profound effects on carcinoma progression and metastasis in HCC [8-10]. While its oncogenic effect in breast cancer has not been well investigated. In this study, CHD1L was overexpressed in breast cancer tissues compared with normal breast tissues and overexpression of CHD1L was significantly correlated with more aggressive tumor behavior and worse prognosis in breast cancer patients. In vitro study showed that overexpression of CHD1L can promote tumorigenesis, metastasis, invasion and cell cycle progress. In vivo study confirmed the tumorigenesis ability of CHD1L. Moreover, shRNA-mediated CHD1L silencing could abolish the tumor-promotion effect of CHD1L. These data indicate that CHD1L detection can provide useful information about CHD1L as a potential prognosis biomarker in breast cancer.

As a member of the SNF2-like family of transcription factors, CHD1L affects a broad spectrum of cellular processes. CHD1L can up-regulate ARHGEF9 and subsequently activate Rho small GTPases to promote tumor cell migration, invasion, and metastasis through CHD1L-ARHGEF9-Cdc42-EMT pathway [9]. CHD1L activates expression of SPOCK1, which activates Akt signaling to block apoptosis and invasion in HCC cells [19]. Another study found that NTKL was another downstream target of CHD1L and CHD1L-related NTKL overexpression could promote G1/S transition by decreasing P53 and increasing CyclinD1 expressions [20]. In the present study, a cDNA microarray was performed to unravel the intricate CHD1L-regulated network and identified a novel downstream target gene, MDM2. MDM2 is best characterized for its dynamic negative regulation of the major tumor suppressor p53 [21] and its protein levels have been identified as an independent prognostic biomarker in human breast cancer [22]. Some other identified CHD1L-regulated network by our research such as endocytosis and TGF-beta signaling pathway were also unveiled before. These data may help us better understand the action mechanism of CHD1L in cancer development and progress. While it is still hard to say whether CHD1L act through different network with tissue specificity and further studies were required.

Overexpression of MDM2 poses a risk for breast by multiple oncogenic capacities in addition to its capacity to destroy p53. Elevated MDM2 levels were identified to promote E-cadherin ubiquitination and degradation that in turn drove cancer cell invasiveness [23]. Besides, MDM2 overexpression affected the transcriptional activity of SMAD family of proteins and thereby blocking TGF-β mediated growth arrest, which in turn contributed to TGF-β resistance [24]. Pathway enrichment analysis in this study also showed modulation of TGF-β signaling pathway by CHD1L. But whether this modulation was mediated by MDM2 was not further explored. In addition, MDM2 promotes cell cycle transition in p53-dependent [25] and p53-independent [26] manner. The promotion effect of CHD1L on cell cycle in our study may also attribute to the CHD1L-MDM2 modulation.

Although curative surgery offers an opportunity for a cure in breast cancer, patients' life are threatened by relapsing, drug-resistant and metastatic disease. Identifying factors that contribute to aggressive cancer offers important leads for therapy. In the present study, the proliferative and invasive effect of CHD1L in clinical specimens was also addressed by IHC results of a breast cancer TMA containing 116 pairs of primary tumors and adjacent non-tumor tissue. We found that the overexpression of CHD1L was significantly correlated with younger age at diagnosis, lymph node metastasis, poorer differentiation and higher proliferation index. Furthermore, we also found that overexpression of CHD1L in tumor tissues could successfully distinguish a set of patients with increased risk of tumor recurrence or shorter DFS. These findings directly demonstrate the clinical significance of CHD1L overexpression and its oncogenic effect in breast cancer. Recent studies have investigated the anti-tumor effect of MDM2 inhibitors and the results were very promising [27-29]. Identification of the CHD1L-MDM2/p53 regulation network in breast cancer in this study may help provide a new method for the treatment of CHD1L overexpression breast cancers.

Acknowledgements

We thank Shuning Ding and Yidong Du for their efforts in data entry for this study. This work was supported by National Natural Science Foundation of China (81572581) and Medical Guidance Foundation of Shanghai Municipal Science and Technology Commission (1641-1966900).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Li Zhu, Comprehensive Breast Health Center, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, 197 Ruijin Second Road, Shanghai 200025, P. R. China. Tel: 86 21 6437 0045-602101; Fax: 86 21 6437 0045-602101; E-mail: zhuli8@yeah.net

References

[1] Chen W, Zheng R, Zhang S, Zeng H, Zuo T, Xia C, Yang Z, He J. Cancer incidence and mortality

in China in 2013: an analysis based on urbanization level. Chin J Cancer Res 2017; 29: 1-10.

[2] Goss PE, Strasser-Weippl K, Lee-Bychkovsky BL, Fan L, Li J, Chavarri-Guerra Y, Liedke PE, Pramesh CS, Badovinac-Crnjevic T, Sheikine Y, Chen Z, Qiao YL, Shao Z, Wu YL, Fan D, Chow LW, Wang J, Zhang Q, Yu S, Shen G, He J, Purushotham A, Sullivan R, Badwe R, Banavali SD, Nair R, Kumar L, Parikh P, Subramanian S, Chaturvedi P, Iyer S, Shastri SS, Digumarti R, Soto-Perez-de-Celis E, Adilbay D, Semiglazov V, Orlov S, Kaidarova D, Tsimafeyeu I, Tatishchev S, Danishevskiy KD, Hurlbert M, Vail C, St Louis J, Chan A. Challenges to effective cancer control in China, India, and Russia. Lancet Oncol 2014; 15: 489-538.

[3] Turnbull C, Ahmed S, Morrison J, Pernet D, Renwick A, Maranian M, Seal S, Ghoussaini M, Hines S, Healey CS, Hughes D, Warren-Perry M, Tapper W, Eccles D, Evans DG; Breast Cancer Susceptibility Collaboration (UK), Hooning M, Schutte M, van den Ouweland A, Houlston R, Ross G, Langford C, Pharoah PD, Stratton MR, Dunning AM, Rahman N, Easton DF. Genome-wide association study identifies five new breast cancer susceptibility loci. Nat Genet 2010; 42: 504-507.

[4] Goncalves R, Warner WA, Luo J, Ellis MJ. New concepts in breast cancer genomics and genetics. Breast Cancer Res 2014; 16: 460.

[5] Günther K, Merkelbach-Bruse S, Amo-Takyi BK, Handt S, Schröder W, Tietze L. Differences in genetic alterations between primary lobular and ductal breast cancers detected by comparative genomic hybridization. J Pathol 2001; 193: 40-47.

[6] Tsuji K, Kawauchi S, Saito S, Furuya T, Ikemoto K, Nakao M, Yamamoto S, Oka M, Hirano T, Sasaki K. Breast cancer cell lines carry cell line-specific genomic alterations that are distinct from aberrations in breast cancer tissues: comparison of the CGH profiles between cancer cell lines and primary cancer tissues. BMC Cancer 2010; 10: 15.

[7] Loo LW, Grove DI, Williams EM, Neal CL, Cousens LA, Schubert EL, Holcomb IN, Massa HF, Glogovac J, Li CI, Malone KE, Daling JR, Delrow JJ, Trask BJ, Hsu L, Porter PL. Array comparative genomic hybridization analysis of genomic alterations in breast cancer subtypes. Cancer Res 2004; 64: 8541-8549.

[8] Ma NF, Hu L, Fung JM, Xie D, Zheng BJ, Chen L, Tang DJ, Fu L, Wu Z, Chen M, Fang Y, Guan XY. Isolation and characterization of a novel oncogene, amplified in liver cancer 1, within a commonly amplified region at 1q21 in hepatocellular carcinoma. Hepatology 2008; 47: 503-510.

[9] Chen L, Chan TH, Yuan YF, Hu L, Huang J, Ma S, Wang J, Dong SS, Tang KH, Xie D, Li Y, Guan XY. CHD1L promotes hepatocellular carcinoma progression and metastasis in mice and is associated with these processes in human patients. J Clin Invest 2010; 120: 1178-1191.

[10] Chen L, Chen L, Yuan YF, Li Y, Chan TH, Zheng BJ, Huang J, Guan XY. Clinical significance of CHD1L in hepatocellular carcinoma and therapeutic potentials of virus-mediated CHD1L depletion. Gut 2011; 60: 534-543.

[11] Cheng W, Su Y, Xu F. CHD1L: a novel oncogene. Mol Cancer 2013; 12: 170.

[12] Chen M, Huang JD, Hu L, Zheng BJ, Chen L, Tsang SL, Guan XY. Transgenic CHD1L expression in mouse induces spontaneous tumors. PLoS One 2009; 4: e6727.

[13] He WP, Zhou J, Cai MY, Xiao XS, Liao YJ, Kung HF, Guan XY, Xie D, Yang GF. CHD1L protein is overexpressed in human ovarian carcinomas and is a novel predictive biomarker for patients survival. BMC Cancer 2012; 12: 437.

[14] Liu C, Fu X, Zhong Z, Zhang J, Mou H, Wu Q, Sheng T, Huang B, Zou Y. CHD1L expression increases tumor progression and acts as a predictive biomarker for poor prognosis in pancreatic cancer. Dig Dis Sci 2017; 62: 2376-2385.

[15] Su Z, Zhao J, Xian G, Geng W, Rong Z, Wu Y, Qin C. CHD1L is a novel independent prognostic factor for gastric cancer. Clin Transl Oncol 2014; 16: 702-707.

[16] Ji X, Li J, Zhu L, Cai J, Zhang J, Qu Y, Zhang H, Liu B, Zhao R, Zhu Z. CHD1L promotes tumor progression and predicts survival in colorectal carcinoma. J Surg Res 2013; 185: 84-91.

[17] Wu J, Zong Y, Fei X, Chen X, Huang O, He J, Chen W, Li Y, Shen K, Zhu L. Presence of CHD1L over-expression is associated with aggressive tumor biology and is a novel prognostic biomarker for patient survival in human breast cancer. PLoS One 2014; 9: e98673.

[18] Mu QJ, Li HL, Yao Y, Liu SC, Yin CG, Ma XZ. Chromodomain Helicase/ATPase DNA-Binding Protein 1-Like Gene (CHD1L) expression and implications for invasion and metastasis of breast cancer. PLoS One 2015; 10: e0143030.

[19] Li Y, Chen L, Chan TH, Liu M, Kong KL, Qiu JL, Li Y, Yuan YF, Guan XY. SPOCK1 is regulated by CHD1L and blocks apoptosis and promotes HCC cell invasiveness and metastasis in mice. Gastroenterology 2013; 144: 179-191, e4.

[20] Wang J, Liu M, Chen L, Chan TH, Jiang L, Yuan YF, Guan XY. Overexpression of N-terminal kinase like gene promotes tumorigenicity of hepatocellular carcinoma by regulating cell cycle progression and cell motility. Oncotarget 2015; 6: 1618-30.

[21] Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. Nature 1997; 387: 296-9.
[22] Park HS, Park JM, Park S, Cho J, Kim SI, Park BW. Subcellular localization of Mdm2 expression and prognosis of breast cancer. Int J Clin Oncol 2014; 19: 842-51.
[23] Yang JY, Zong CS, Xia W, Wei Y, Ali-Seyed M, Li Z, Broglio K, Berry DA, Hung MC. MDM2 promotes cell motility and invasiveness by regulating E-cadherin degradation. Mol Cell Biol 2006; 26: 7269-82.
[24] Sun P, Dong P, Dai K, Hannon GJ, Beach D. p53-independent role of MDM2 in TGF-beta1 resistance. Science 1998; 282: 2270-2.
[25] Deb SP. Cell cycle regulatory functions of the human oncoprotein MDM2. Mol Cancer Res 2003; 1: 1009-16.
[26] Girnita L, Shenoy SK, Sehat B, Vasilcanu R, Vasilcanu D, Girnita A, Lefkowitz RJ, Larsson O. Beta-arrestin and Mdm2 mediate IGF-1 receptor-stimulated ERK activation and cell cycle progression. J Biol Chem 2007; 282: 11329-38.
[27] Lu J, McEachern D, Li S, Ellis MJ, Wang S. Reactivation of p53 by MDM2 inhibitor MI-77301 for the treatment of endocrine-resistant breast cancer. Mol Cancer Ther 2016; 15: 2887-2893.
[28] Qin JJ, Wang W, Sarkar S, Zhang R. Oral delivery of anti-MDM2 inhibitor SP141-loaded FcRn-targeted nanoparticles to treat breast cancer and metastasis. J Control Release 2016; 237: 101-14.
[29] Singh AK, Chauhan SS, Singh SK, Verma VV, Singh A, Arya RK, Maheshwari S, Akhtar MS, Sarkar J, Rangnekar VM, Chauhan PMS, Datta D. Dual targeting of MDM2 with a novel small-molecule inhibitor overcomes TRAIL resistance in cancer. Carcinogenesis 2016; 37: 1027-1040.

Table S1. Pathway analysis of differential genes in CHD1L-knockdown cells

Gene Set Name	*P*-value	Gene symbol
KEGG_ENDOCYTOSIS	0.000628	MDM2, RAB5A, IL2RB, SH3GLB1, SH3KBP1, ARFGAP3, PARD6B, ADRBK2, CHMP2B
KEGG_P53 SIGNALING PATHWAY	0.0112	MDM2, RCHY1, EI24, PERP, SESN2
KEGG_ADHERENS JUNCTION	0.0112	TGFBR2, CSNK2A2, MLLT4, FER, LMO7
KEGG_TGF-BETA SIGNALING PATHWAY	0.0161	TGFBR2, SMAD5, BMPR2, INHBB, ID4
KEGG_AMYOTROPHIC LATERAL SCLEROSIS ALS	0.0194	RAB5A, PPP3CB, PPP3R1, CASP1
BIOCARTA_CTCF PATHWAY	0.0194	MDM2, TGFBR2, SMAD5
KEGG_GNRH SIGNALING PATHWAY	0.0194	NRAS, GNAQ, MAPK9, MAP3K2, HBEGF
KEGG_GLYCOSPHINGOLIPID BIOSYNTHESIS -LACTO AND NEOLACTO SERIES	0.0238	B3GNT5, GCNT2, ST3GAL6
KEGG_LONG TERM POTENTIATION	0.0353	PPP3CB, PPP3R1, NRAS, GNAQ
KEGG_NEUROTROPHIN SIGNALING PATHWAY	0.0365	NRAS, MAPK9, MAP2K5, ARHGDIB, IRAK2

Table S2. Gene symbols and fold changes of differential genes from pathway analysis

Gene symbol	Fold change
MDM2	-2.150386
RAB5A	2.20257
IL2RB	-2.168064
SH3GLB1	-3.0789459
SH3KBP1	-2.1663184
ARFGAP3	2.2219067
PARD6B	-2.127054
ADRBK2	-2.045534
CHMP2B	2.3225563
RCHY1	-2.036728
EI24	-4.036784
PERP	-2.8190122
SESN2	-2.1081407
TGFBR2	2.1529
CSNK2A2	2.9088726
MLLT4	2.053146
FER	-2.3064106
LMO7	-2.025733
SMAD5	-2.171897
BMPR2	-2.1004963
INHBB	-2.1706445
ID4	-2.0064933
PPP3CB	2.2663465
PPP3R1	-2.011478
CASP1	-2.3410146
NRAS	-2.069724
GNAQ	-2.4953043
MAPK9	-2.8616924
MAP3K2	2.0362465
HBEGF	-2.0292392
B3GNT5	-2.625445
GCNT2	2.0998924
ST3GAL6	-2.37331
MAP2K5	2.1273732
ARHGDIB	4.21353
IRAK2	-2.1438763