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CHCHD2 decreases docetaxel sensitivity in breast cancer *via* activating MMP2

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Abstract. – OBJECTIVE: This study aims to clarify the influences of CHCHD2 and MMP2 on docetaxel resistance in breast cancer.

PATIENTS AND METHODS: Differential levels of CHCHD2 in breast cancer and para-tumor tissues were detected. The relationship between CHCHD2 and prognosis in breast cancer patients was analyzed. After generating Docetaxel-resistant breast cancer cell lines (MCF-7/DTX and SKBR3/DTX), the regulatory effects of CHCHD2 on proliferative and migratory potentials were assessed by Cell Counting Kit-8 (CCK-8) and transwell assay, respectively. The interaction between CHCHD2 and MMP2 was tested by Western blot and Pearson correlation test. At last, the involvement of MMP2 in CHCHD2-regulated proliferation and migration in Docetaxel-resistant breast cancer cells was analyzed.

RESULTS: CHCHD2 was upregulated in breast cancer tissues. It predicted high incidence of distant metastasis and poor prognosis in breast cancer patients. Proliferation inhibition rate was lower in MCF-7/DTX and SKBR3/DTX cells compared with their parental cells. After knockdown of CHCHD2 in MCF-7/DTX and SKBR3/DTX cells, their proliferative and migratory potentials were markedly reduced. MMP2 was upregulated in breast cancer tissues, and its level was positively regulated by CHCHD2. Overexpression of MMP2 could reverse the regulatory effects of CHCHD2 on proliferative and migratory potentials in MCF-7/DTX and SKBR3/DTX cells.

CONCLUSIONS: Upregulated CHCHD2 in breast cancer is related to distant metastasis rate and poor prognosis. CHCHD2 and MMP2 are positively correlated to each other. CHCHD2 stimulates proliferative and migratory potentials in Docetaxel-resistant breast cancer cells by upregulating MMP2.

Key Words:
CHCHD2, MMP2, Breast cancer, Docetaxel.

Introduction

Breast cancer is the major malignant tumor that threats health and lives in women. Its incidence is on the rise and ranks the first in female malignancies. Effective prevention and treatment of breast cancer have been well concerned[1,2]. Oncogenes and tumor suppressors involved in the carcinogenesis of breast cancer are of great significance[3,4]. Although therapeutic efficacy of breast cancer has largely improved, drug resistance and metastasis in advanced patients seriously restrict the prognosis[5-8]. Docetaxel is a cell-cycle-specific agent acting on the M phase. By promoting the microtubule assembly during mitosis and inhibiting their dissolution, as well as inhibiting spindle formation, docetaxel suppresses cell proliferation. Docetaxel can also destroy the microtubular structure, which is necessary for the cell mitosis process. Therefore, it exerts the anti-cancer effect[9-11]. At present, docetaxel is a chemotherapy medication commonly applied for breast cancer treatment[12,13]. However, docetaxel resistance cannot be ignored[13,14].

CHCHD2 is located on human chromosome 7p11.25[15]. It contains a conserved CHCH domain at the C terminal and a mitochondrial localization sequence at the N terminal[15,16]. It is reported that CHCHD2 is upregulated in many types of tumors and may be utilized as a prognostic factor[16,17].

Tumor metastasis is the major reason for cancer treatment failure and death, involving multiple steps and gene mutations. Degradation of extracellular matrix (ECM) and basement membrane is the initial link that is essentially involved in the process of tumor metastasis[18]. Matrix metalloproteinases (MMPs) are important proteases relevant to metastasis. MMP2 can efficiently degrade collagen type IV, which is the main component of the basement membrane. Hence, MMP2 is considered to be linked to malignant phenotypes of tumor cells and prognosis in tumor patients[19-21]. Highly expressed MMP2 can be detected in the blood or ascites of breast cancer patients, and its level increases with the malignant aggravation. This study aims to clarify the influences of CH-

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CHD2 and MMP2 on docetaxel sensitivity in breast cancer, which provides new ideas for clinical treatment.

Patients and Methods

Breast Cancer Samples

Forty-two breast cancer and para-tumor tissues were surgically resected and placed at 4°C overnight. Samples were stored at -80°C for later use. In this study, each patient was diagnosed by pathological examination and none of recruited patients were treated with preoperative chemotherapy or radiotherapy before. Tumor node metastasis (TNM) staging and histological subtypes were evaluated based on the criteria proposed by The Union for International Cancer Control (UICC). Exclusion criteria: patients complicated with other malignancies, those with mental disease, those complicated with heart failure or other chronic diseases. In addition, this research was in line with the declaration of Helsinki clinical practice guidelines. This study was approved by the Ethics Committee of the First Hospital of Jilin University. Signed written informed consents were obtained from all participants before the study.

Cell Lines and Reagents

Human breast cancer cell lines (MCF-7, SKBR3, MDA-MB-435S and MDA-MB-231) and the mammary epithelial cell line (MCF-10A) were purchased from Cell Bank of Type Culture Collection (Shanghai, China). They were cultivated in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) in a 5% CO_2 incubator at 37°C.

Transfection

RNAi MAX (Invitrogen, Carlsbad, CA, USA) was used for cell transfection. Cells were cultivated in antibiotics-free DMEM for 50-70% confluence and transfected for 48 h. Transfected cells were used for the subsequent functional experiments.

Cell Proliferation Assay

5×10^3 cells were implanted in each well of a 6-well plate, where 10 μL of Cell Counting Kit-8 (CCK-8) solution was added (TaKaRa, Dalian, China). After 1-h culturing in the dark, 450 nm absorbance was measured using a microplate reader at day 1, 2, 3 and 4. Viability curves were plotted.

Transwell Migration Assay

Transwell chambers (Millipore, Billerica, MA, USA) were inserted in each well of a 24-well plate. 100 μL of serum-free suspension (3×10^4 mL) was applied in the upper layer of the chamber, and 500 μL of medium containing 10% FBS was applied in the bottom. After 48-h incubation, migratory cells in the bottom were reacted with 15-min methanol, 20-min crystal violet and captured using a microscope. Migratory cells were counted in 5 randomly selected fields per sample.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used for isolating total cellular RNAs, followed by incubation with chloroform. After 10-min centrifugation at 4°C, the upper layer was collected and incubated with 700 μL of isopropanol. The mixture was centrifuged at 4°C, 12,000 rpm for 10 min, and the precipitant was washed in 75% ethanol. The collected RNAs were reversely transcribed into cDNAs (PrimeScript RT Reagent; TaKaRa, Dalian, China). Using the SYBR® Premix Ex Taq™ kit (TaKaRa, Dalian, China) and StepOne Plus Real-time PCR system (Applied Biosystems, Foster City, CA, USA), qRT-PCR was carried out. Relative level was calculated by $2^{-\Delta\Delta Ct}$ and normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). CHCHD2 forward: 5'-CTTACCAGGAGCCTCAGGGA-3'; reverse: 5'-CAAGTCGGCACTGTTTCAGC-3'. MMP2 forward: 5'-GGACTTAGACCGCTTGGCTT-3'; reverse: 5'-GTGTTCAGGTATTGCATGTGCT-3'. GAPDH forward: 5'-CGCTCTCTGCTCCTCCTGTTC-3'; reverse: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Western Blot

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China) on ice for 30 min. Cell lysate was centrifuged at 4°C, 14000×g for 15 min. Extracted protein samples were quantified by bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). Protein samples were electrophoresed in 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and loaded on polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, non-specific anti-

gens were blocked in 5% skim milk for 2 hours. The membranes were reacted with primary and secondary antibodies for indicated time. Band exposure and analyses of grey values were finally conducted.

Statistical Analysis

Data were expressed as mean ± standard deviation and analyzed by Statistical Product and Service Solutions (SPSS) 22.0 (IBM Corp., Armonk, NY, USA). Differences between groups were analyzed by the *t*-test. The relationship between CHCHD2 and clinical factors in breast cancer patients was assessed by Chi-square test. Overall survival was assessed by Kaplan-Meier method and log-rank test. A significant difference was set at $p < 0.05$.

Results

High Expression of CHCHD2 in Breast Cancer

CHCHD2 was upregulated in 39/42 breast cancer tissues, and its expression was weakly positive or negative in 3/42 cancer tissues (Figure 1A). Similarly, CHCHD2 was highly expressed in breast cancer cell lines (Figure 1B). By analyzing clinical data of recruited patients, CHCHD2 was found to be correlated to the incidence of distant metastasis in breast cancer ($p<0.05$) (Table I). Kaplan-Meier method uncovered worse prognosis in breast cancer patients expressing high level of CHCHD2 than those with low level (Figure 1C).

CHCHD2 was closely related to Docetaxel-Resistant Breast Cancer Cells

CCK-8 assay showed lower proliferation inhibition rate in MCF-7/DTX and SKBR3/DTX cells than their parental cells, indicating the resistant characteristics in breast cancer cells (Figure 2A). Protein level of CHCHD2 was markedly downregulated in MCF-7/DTX and SKBR3/DTX cells (Figure 2B). It is suggested that CHCHD2 was closely related to docetaxel resistance in breast cancer.

Knockdown of CHCHD2 Inhibited Proliferative and Migratory Potentials in Docetaxel-Resistant Breast Cancer Cells

CHCHD2 knockdown model was generated in MCF-7/DTX and SKBR3/DTX cells by transfection of sh-CHCHD2 (Figure 2C). The knockdown of CHCHD2 decreased viability and migratory cell number in MCF-7/DTX and SKBR3/DTX cells, suggesting the inhibited proliferative and migratory potentials (Figure 3A, 3B).

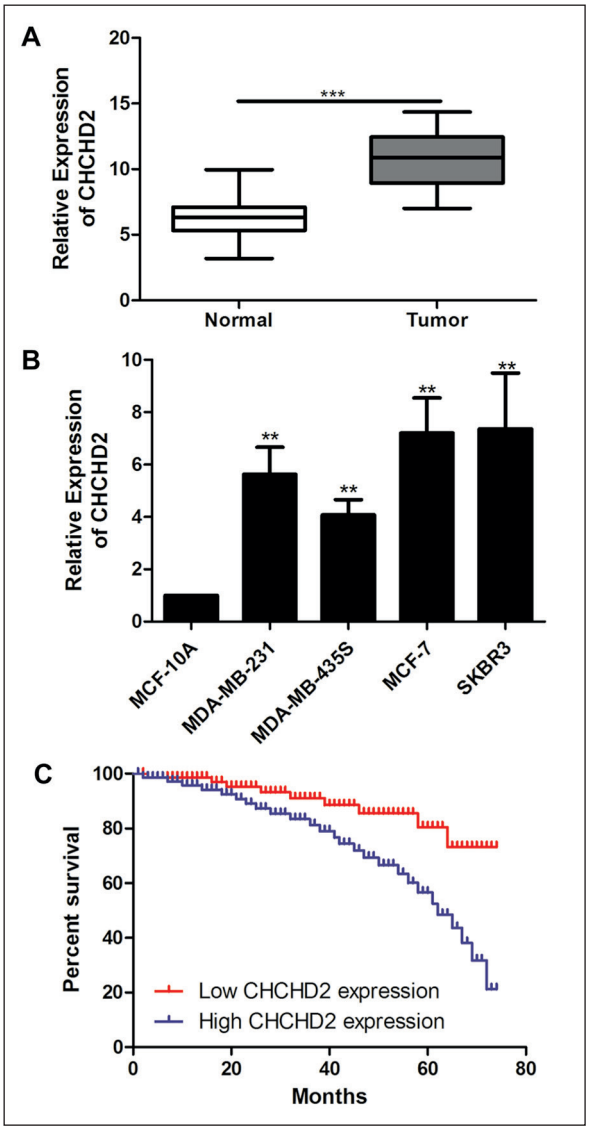

Figure 1. High expression of CHCHD2 in breast cancer. **A,** Differential expressions of CHCHD2 in breast cancer and normal tissues; **B,** CHCHD2 level in breast cancer cell lines; **C,** Overall survival in breast cancer patients based on CHCHD2 expressions. Data were expressed as mean±SD. $^{**}p < 0.01$, $^{***}p < 0.001$.

CHCHD2 Positively Regulated MMP2 Level

Bioinformatics analysis uncovered a potential interaction between CHCHD2 and MMP2. Western blot analysis showed that protein level of MMP2 was downregulated in MCF-7/DTX and SKBR3/DTX cells transfected with sh-CHCHD2 (Figure 4A). In breast cancer tissues, MMP2 was

Table I. Association of CHCHD2 expression with clinicopathologic characteristics of breast cancer.

Parameters	Number of cases	CHCHD2 expression		*p*-value
		Low (%)	High (%)	
Age (years)				0.629
< 60	16	9	5	
≥ 60	26	18	10	
Tumor size (cm)				0.024
< 3	21	17	4	
≥ 3	21	10	11	
T stage				0.222
T1-T2	30	21	9	
T3-T4	12	6	6	
Lymph node metastasis				0.270
No	27	19	8	
Yes	15	8	7	
Distance metastasis				0.010
No	25	20	5	
Yes	17	7	10	

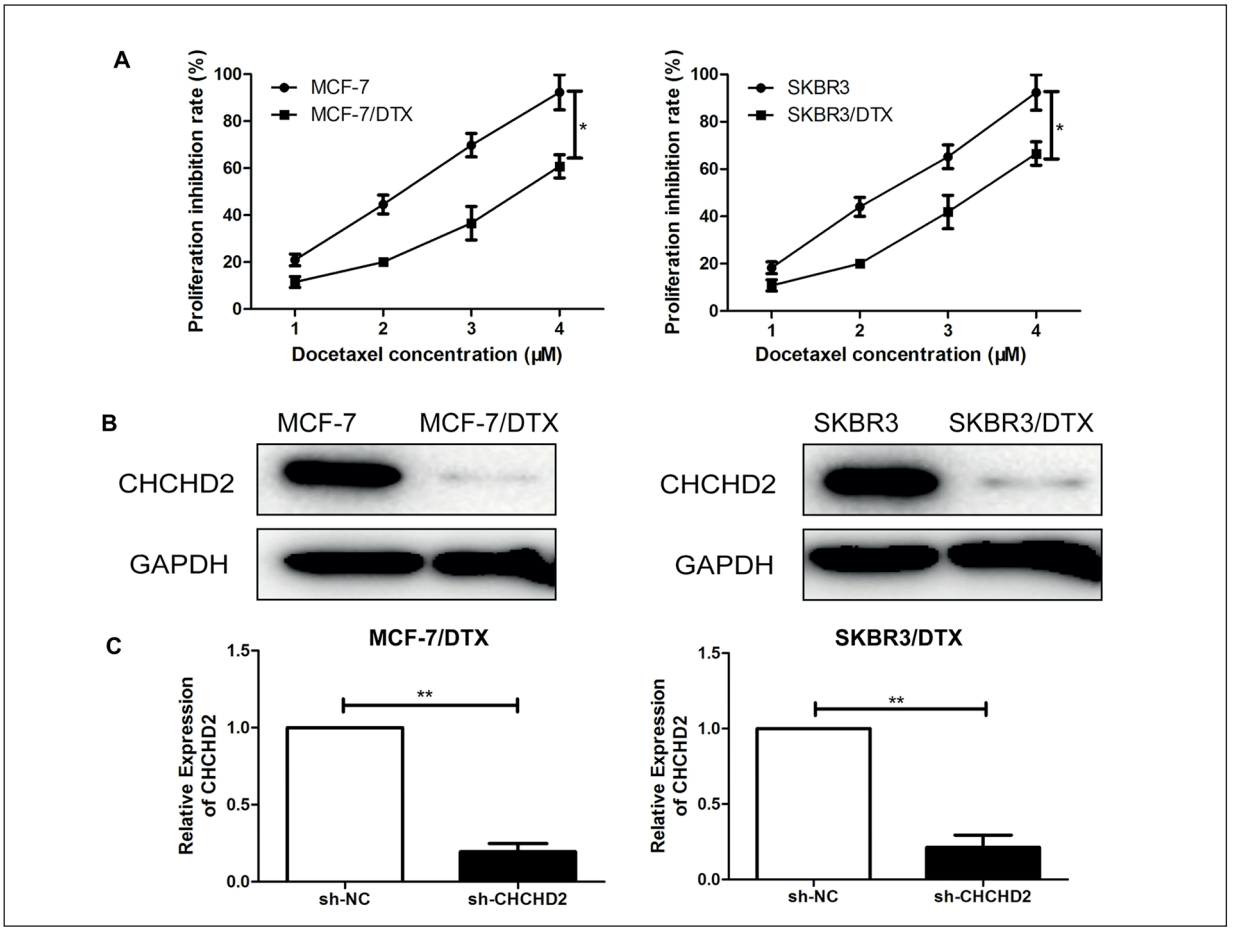

Figure 2. CHCHD2 was downregulated in Docetaxel-resistant breast cancer cells. **A,** Proliferation inhibition rate in MCF-7/DTX and SKBR3/DTX cells, and their parental cells; **B,** Protein level of CHCHD2 in MCF-7/DTX and SKBR3/DTX cells, and their parental cells; **C,** Transfection efficacy of sh-CHCHD2 in MCF-7/DTX and SKBR3/DTX cells. Data were expressed as mean±SD. **p* < 0.05, ***p* < 0.01.

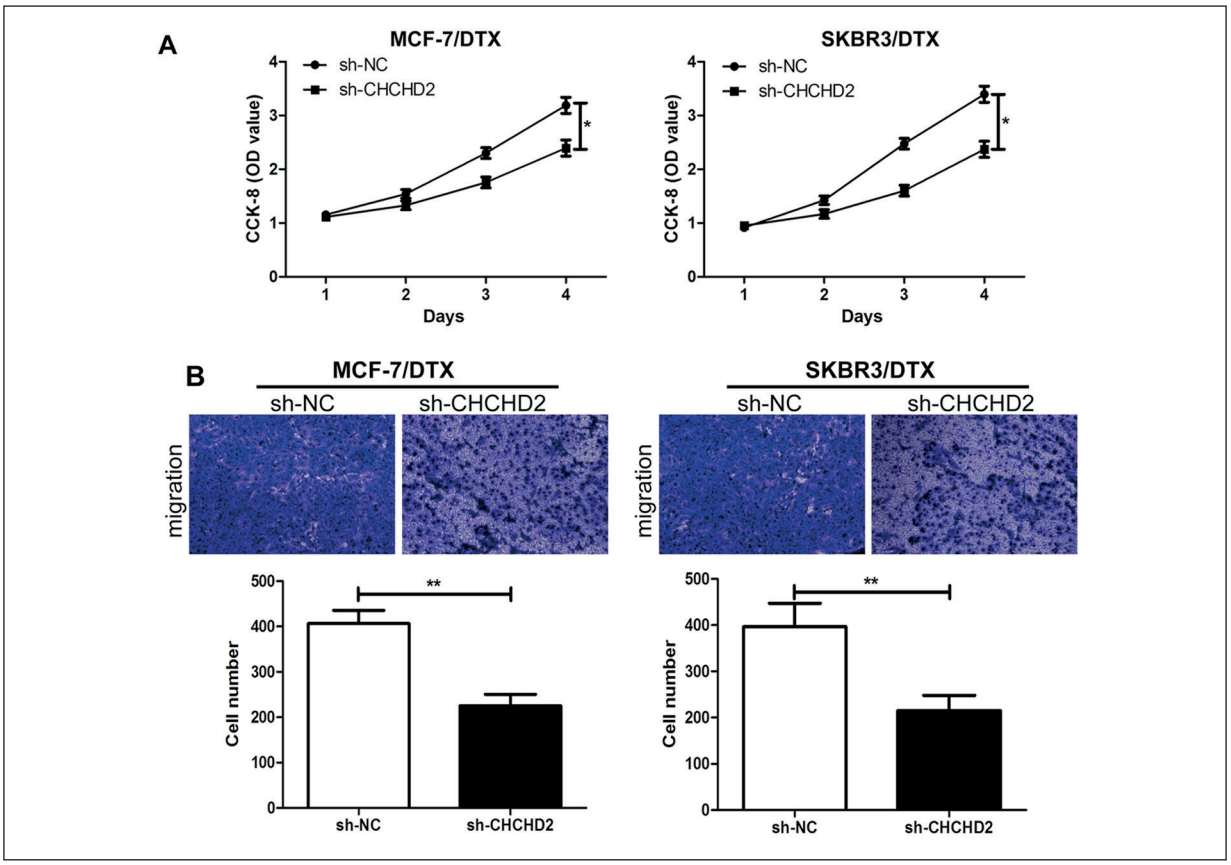

Figure 3. Knockdown of CHCHD2 inhibited proliferative and migratory potentials in Docetaxel-resistant breast cancer cells. **A,** Viability in MCF-7/DTX and SKBR3/DTX cells transfected with sh-NC or sh-CHCHD2; **B,** Migration in MCF-7/DTX and SKBR3/DTX cells transfected with sh-NC or sh-CHCHD2 (magnification: 20×). Data were expressed as mean±SD. *$p < 0.05$, **$p < 0.01$.

upregulated and positively correlated to CHCHD2 level (Figure 4B, 4C).

MMP2 Was Regulated by CHCHD2 in Docetaxel-Resistant Breast Cancer Cells

We thereafter explored potential influences of CHCHD2 and MMP2 on docetaxel-resistant breast cancer cell lines. Overexpression of MMP2 was able to enhance CHCHD2 level in MCF-7/DTX and SKBR3/DTX cells with CHCHD2 knockdown (Figure 5A). Compared with those co-transfected with sh-CHCHD2 and pcDNA-NC, the viability and migratory cell number were higher in MCF-7/DTX and SKBR3/DTX cells co-transfected with sh-CHCHD2 and pcDNA-MMP2 (Figure 5B, 5C). The above data demonstrated that overexpression of MMP2 could reverse the inhibited proliferative and migratory potentials in Docetaxel-resistant breast cancer cell lines with CHCHD2 knockdown.

Discussion

Breast cancer has been well studied because of its high-level invasiveness and malignancy[1-3]. Currently, effective evidence-based targeted therapy for breast cancer is lacked[5,6]. It is necessary to uncover potential targets for breast cancer[5-7]. Docetaxel is an agent targeting microtubules that is extensively applied for chemotherapy[7,8]. Nevertheless, docetaxel resistance remarkably limits its therapeutic efficacy[8-10]. Previous studies[12-14] have reported the development of docetaxel resistance in breast cancer patients. The potential involvement of CHCHD2 in docetaxel resistance remains unclear.

Through literature review, CHCHD2 is closely linked to tumor diseases[16,17]. However, the mechanism of CHCHD2 in breast cancer is not clear. Therefore, the objective of this study was firstly to elucidate the oncogenic role of CHCHD2 in

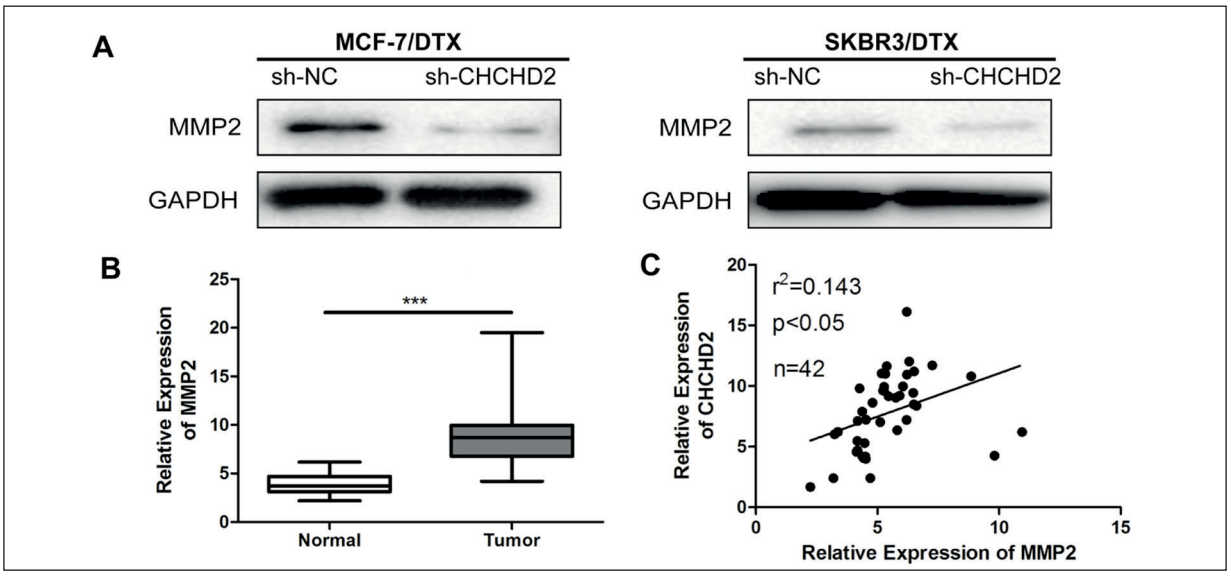

Figure 4. CHCHD2 positively regulated MMP2 level. **A,** Protein level of MMP2 in MCF-7/DTX and SKBR3/DTX cells transfected with sh-NC or sh-CHCHD2; **B,** Differential expressions of MMP2 in breast cancer and normal tissues; **C,** Positive correlation between levels of CHCHD2 and MMP2 in breast cancer tissues. Data were expressed as mean±SD. ***$p < 0.001$.

the progression of breast cancer. In this paper, we collected 42 pairs of breast cancer tissues and para-tumor ones. CHCHD2 was markedly upregulated in cancer tissues, as well as breast cancer cell lines. By analyzing clinical data of recruited patients, it is shown that CHCHD2 was linked to distant metastasis and overall survival in breast cancer. We speculated that CHCHD2 exerted a carcinogenic role in the progression of breast cancer. To elucidate the role of CHCHD2 in Docetaxel resistance in breast cancer, we generated MCF-7/DTX and SKBR3/DTX cell lines. Compared with their parental cell lines, proliferation inhibition rate was lower in MCF-7/DTX and SKBR3/DTX cells, confirming the successful modeling of Docetaxel-resistant breast cancer cells. CHCHD2 was markedly downregulated in MCF-7/DTX and SKBR3/DTX cells. Notably, both proliferative and migratory potentials in MCF-7/DTX and SKBR3/DTX were inhibited by knockdown of CHCHD2. It is suggested that CHCHD2 was closely related to proliferation and migration in Docetaxel-resistant breast cancer.

Compared with normal tissues, MMP2 was upregulated in breast cancer tissues. MMP2 displayed a positive correlation to CHCHD2. We thereafter speculated that MMP2 was involved in CHCHD2-regulated Docetaxel resistance in breast cancer. Notably, overexpression of MMP2 markedly enhanced CHCHD2 in MCF-7/DTX and SKBR3/DTX cells with CHCHD2 knockdown. It is indicated that MMP2 activation may be parallel to that of CHCHD2 during the development of Docetaxel resistance. Subsequently, higher viability and migratory cell number were detected in MCF-7/DTX and SKBR3/DTX cells co-transfected with sh-CHCHD2 and pcDNA-MMP2 compared with those co-transfected with sh-CHCHD2 and pcDNA-NC. To sum up, MMP2 was responsible for CHCHD2-regulated proliferative and migratory potentials in Docetaxel-resistant breast cancer cells. Our findings proposed a novel idea in explaining Docetaxel resistance in breast cancer, which may lead to the improvement of Docetaxel chemotherapy efficacy for the patients with breast cancer.

Conclusions

These data showed that upregulated CHCHD2 is related to distant metastasis and poor prognosis of breast cancer patients. CHCHD2 and MMP2 are positively correlated to each other, and CHCHD2 can stimulate the proliferative and migratory potentials in Docetaxel-resistant breast cancer cell lines by upregulating MMP2.

Conflict of Interest

The Authors declare that they have no conflict of interests.

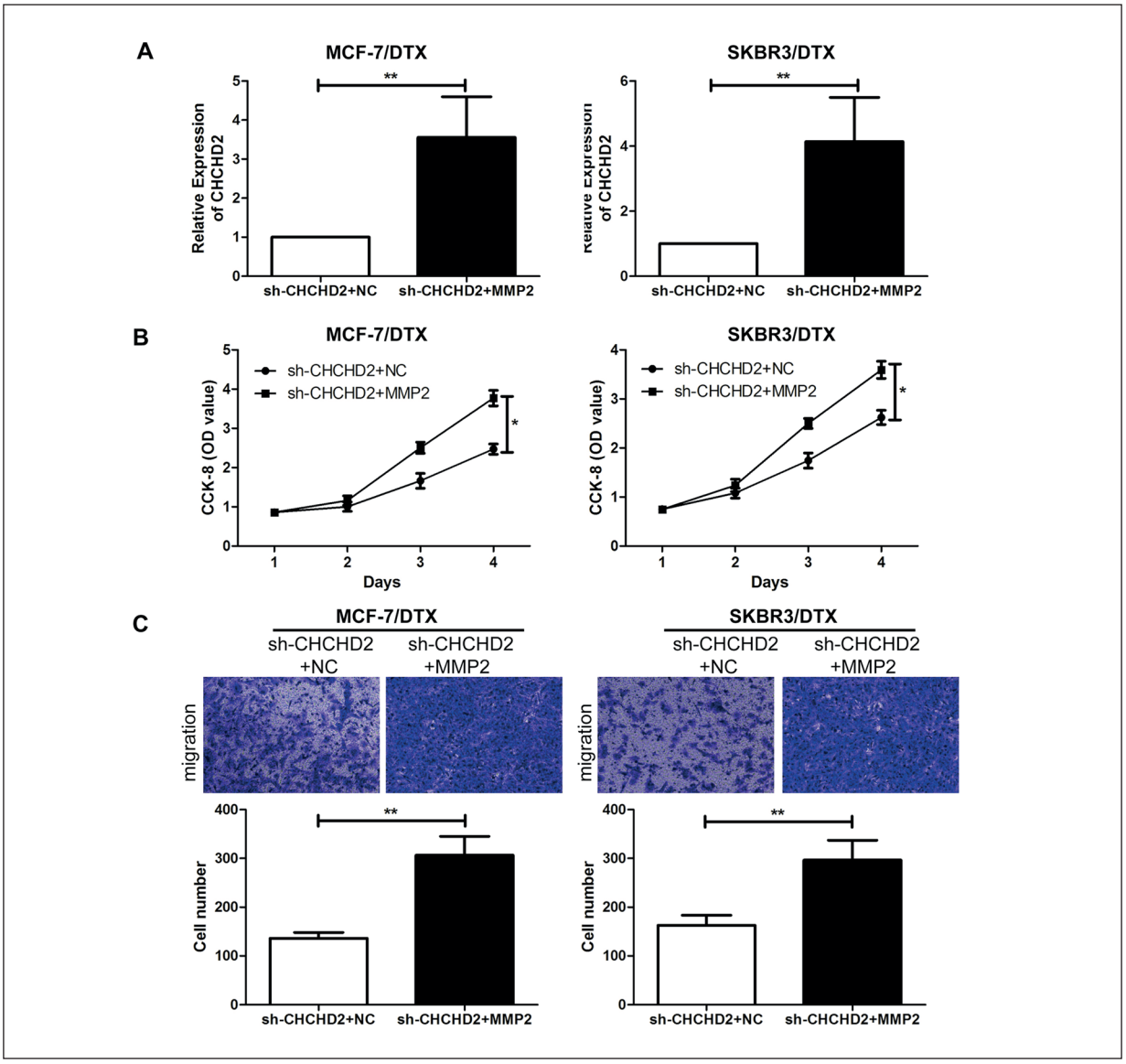

Figure 5. MMP2 was involved in CHCHD2-induced regulation in Docetaxel-resistant breast cancer cells. **A,** CHCHD2 level in MCF-7/DTX and SKBR3/DTX cells co-transfected with sh-CHCHD2 and pcDNA-NC or sh-CHCHD2 and pcDNA-MMP2; **B,** Viability in MCF-7/DTX and SKBR3/DTX cells co-transfected with sh-CHCHD2 and pcDNA-NC or sh-CHCHD2 and pcDNA-MMP2; **C,** Migration in MCF-7/DTX and SKBR3/DTX cells co-transfected with sh-CHCHD2 and pcDNA-NC or sh-CHCHD2 and pcDNA-MMP2 (magnification: 20×). Data were expressed as mean±SD. *$p < 0.05$, **$p < 0.01$.

References

1) Woolston C. Breast cancer. Nature 2015; 527: S101.
2) Ito H, Matsuo K. Molecular epidemiology, and possible real-world applications in breast cancer. Breast Cancer-Tokyo 2016; 23: 33-38.
3) Sotiriou C, Pusztai L. Gene-expression signatures in breast cancer. N Engl J Med 2009; 360: 790-800.
4) Narod SA. Cancer risk estimates for study of multiple-gene testing after diagnosis of breast cancer. JAMA Oncol 2018; 4: 1787-1788.
5) Peart O. Breast intervention and breast cancer treatment options. Radiol Technol 2015; 86: 535M-558M, 559-562.
6) Rockson SG. Lymphedema after breast cancer treatment. N Engl J Med 2018; 379: 1937-1944.
7) de Melo GD, Chavez-MacGregor M. Delays in adjuvant chemotherapy among breast cancer patients: an unintended consequence of breast surgery? Ann Surg Oncol 2018; 25: 1786-1787.
8) Leveque D, Becker G. Generic docetaxel. J Clin Pharmacol 2017; 57: 935.
9) Stathopoulos GP, Zoublios C, Stathopoulos J. Metastatic lung disease treated with peme-

trexed-docetaxel combination chemotherapy. J BUON 2011; 16: 166-169.

10) Cetin B, Ozet A. The potential for chemotherapy-free strategies in advanced prostate cancer. Curr Urol 2019; 13: 57-63.

11) Varnai R, Koskinen LM, Mantyla LE, Szabo I, Fitz-Gerald LM, Sipeky C. Pharmacogenomic biomarkers in docetaxel treatment of prostate cancer: from discovery to implementation. genes (Basel) 2019; 10: 599.

12) Fernandes R, Mazzarello S, Stober C, Vandermeer L, Dudani S, Ibrahim MF, Majeed H, Perdrizet K, Shorr R, Hutton B, Fergusson D, Clemons M. Optimal primary febrile neutropenia prophylaxis for patients receiving docetaxel-cyclophosphamide chemotherapy for breast cancer: a systematic review. Breast Cancer Res Treat 2017; 161: 1-10.

13) Decoster L, Fontaine C, Vanacker L, Schallier D, De Greve J. Docetaxel/cyclophosphamide chemotherapy in older patients with breast cancer. Breast J 2018; 24: 695-697.

14) Huang P, Li F, Li L, You Y, Luo S, Dong Z, Gao Q, Wu S, Brunner N, Stenvang J. LncRNA profile study reveals the mRNAs and lncRNAs associated with docetaxel resistance in breast cancer cells. Sci Rep 2018; 8: 17970.

15) Imai Y, Meng H, Shiba-Fukushima K, Hattori N. Twin CHCH proteins, CHCHD2, and CHCHD10: key molecules of Parkinson's disease, amyotrophic lateral sclerosis, and frontotemporal dementia. Int J Mol Sci 2019; 20: 908.

16) Cornelissen T, Spinazzi M, Martin S, Imberechts D, Vangheluwe P, Bird M, De Strooper B, Vandenberghe W. CHCHD2 harboring the Parkinson's disease-linked T61I mutation precipitates inside mitochondria and induces precipitation of wild-type CHCHD2. Hum Mol Genet 2020: (DOI: 10.1093/hmg/ddaa028).

17) Yin X, Xia J, Sun Y, Zhang Z. CHCHD2 is a potential prognostic factor for NSCLC and is associated with HIF-1a expression. BMC Pulm Med 2020; 20: 40.

18) Yuzhalin AE, Lim SY, Kutikhin AG, Gordon-Weeks AN. Dynamic matrisome: ECM remodeling factors licensing cancer progression and metastasis. Biochim Biophys Acta Rev Cancer 2018; 1870: 207-228.

19) Kapoor C, Vaidya S, Wadhwan V, Kaur G, Pathak A. Seesaw of matrix metalloproteinases (MMPs). J Cancer Res Ther 2016; 12: 28-35.

20) Shay G, Lynch CC, Fingleton B. Moving targets: emerging roles for MMPs in cancer progression and metastasis. Matrix Biol 2015; 44-46: 200-206.

21) Zhao MY, Zhao T, Meng QY, Zhao L, Li XC. Estrogen and estrogen receptor affects MMP2 and MMP9 expression through classical ER pathway and promotes migration of lower venous vascular smooth muscle cells. Eur Rev Med Pharmacol Sci 2020; 24: 1460-1467.