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Identification of CHD4-β1 integrin axis as a prognostic marker in triple-negative breast cancer using next-generation sequencing and bioinformatics

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ABSTRACT

Aims: Triple-negative breast cancer (TNBC) is a special subtype of breast cancer that lacks receptor expression and is difficult to cure. Epigenetic regulators have been suggested as targets for cancer therapy in recent years. Our previous study indicated that the chromodomain-helicase-DNA-binding protein 4 (CHD4) is a prognostic biomarker of TNBC and therapeutic target in patients with TNBC. However, the exact mechanisms regulated by CHD4 are still unclear.

Methods: In this study, we compared differences in gene expression in parental and CHD4-deficient cells by next-generation sequencing and Ingenuity Pathway Analysis.

Key findings: We found that β1 integrin is a downstream target gene of CHD4, which could be transcriptionally regulated by CHD4 in TNBC cells. Consistent with *in vitro* data, immunohistochemistry revealed that co-expression of β1 integrin and CHD4 was significantly associated with metastatic state, recurrence, and survival status in TNBC patients. It also showed a positive correlation between β1 integrin and CHD4 in vivo.

Significance: This is the first study to suggest that CHD4 regulates β1 integrin in TNBC. Overall, CHD4-β1 integrin axis could potentially be a predictive marker in patients with TNBC and the use of β1 integrin inhibitors may be a therapeutic option for TNBC patients with high CHD4 expression.

1. Introduction

Breast cancer is a heterogeneous disease and one of the leading causes of death among women worldwide [1]. Diverse biological characteristics lead to different responses to treatments in patients with breast cancer [2]. In clinical diagnosis, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 are conventionally used biomarkers for classifying breast cancer types and drug selection [2,3]. Most breast cancer subtypes express these biomarkers; however, one subtype, triple-negative breast cancer (TNBC), shows a lack of biomarker expression and accounts for 15–20% of all breast cancer cases [4,5]. Previous studies indicated that TNBC is more aggressive, more resistant to chemo-/radio-therapies, has higher metastatic and recurrent rates, and has shorter overall survival than other subtypes [6]. In addition to surgery, chemotherapies, such as cell proliferation inhibitors, DNA alkylating agents, and p53 inhibition drugs, are the most common treatments for patients with TNBC [7]. However, chemotherapy typically fails after 3–5 years of treatment because of drug resistance and recurrence. Therefore, new treatment strategies for patients with TNBC are needed.

Several recent studies have suggested relationships between genomic and epigenetic factors and the pathogenesis of various diseases, including cancer (Wee et al., 2014). Additionally, epigenetic factors were also suggested to participate in regulating the tumorigenesis, plasticity, and heterogeneity of cancer cells by mediating reversible changes at the chromatin level [8,9]. Therefore, investigating these epigenetic regulators or identifying the relative molecules and signaling pathways mediated by these epigenetic regulators may reveal new candidates for developing next-generation anti-cancer drugs. Chromodomain helicase DNA-binding protein 4 (CHD4), the largest subunit of the nucleosome remodeling and histone deacetylase complex, is indispensable in controlling homologous recombination repair

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to maintain genome stability (Pan et al., 2012). Previous studies suggested that CHD4 is also associated with several oncogenic effects, including inducing abnormal stem cell renewal, blunting differentiation, and altering cell-cycle control (D'Alesio et al., 2016, Xia et al., 2017). Thus, deficiencies in CHD4 may have therapeutic implications for cancer treatment. Our previous study also indicated that CHD4 could mediate the metastatic ability of TNBC cells and it also could be a prognostic biomarker in TNBC patients (Hou et al., 2017, Luo et al., 2018). However, no drugs that directly target CHD4 have been developed and the downstream genes and relative signaling pathways mediated by CHD4 remain unclear.

Advancements in technology have enabled rapid sequencing of DNA or RNA by next-generation sequencing (NGS). This method has been used for gene expression profiling, chromosome counting, detection of epigenetic changes, molecular analysis, and identification of new biomarkers, among other applications, which may lead to the development of personalized medicine [10]. Therefore, the aim of this study was to explore the downstream genes and novel signaling pathways involved in CHD4 activation using NGS and bioinformatics tools. The results of our study may lead to the development of drugs for treating TNBC patients with high levels of CHD4 expression.

2. Materials and methods

2.1. Cell culture

MDA-MB-231, MDA-MB-468, Hs578T, and 4T-1 TNBC cell lines (ATCC, Manassas, VA, USA) were used in this study. Cells were cultured in Dulbecco's modified Eagle medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (Hyclone, Logan, UT, USA) at 37 °C in 95% humidified air with 5% CO_2 and antibiotics.

RNA extraction, genome-wide transcriptome characterization, and expression profiling by RNA-sequencing.

Total RNA from parental and stable knockdown CHD4 (shCHD4) 4T-1 cells were extracted by Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The quality of the extracted RNA was evaluated by OD260/OD280 absorbance ratio and RNA integrity was evaluated by 18S/28S quality using BioTek's Epoch Multi-Volume Spectrophotometer System (Epoch Biosciences Inc.) and nucleic acid agarose gels, respectively. After extracting total RNA, subsequent analyses were performed by Genewiz Company (AllBio Science, Inc., Taipei, Taiwan). mRNA from parental and shCHD4 cells were considered as differentially expressed when a fold-change > 2.0 was observed and fragments per kilobase of transcript per million (FPKM) > 0.2 for mRNA was observed. Using FPKM > 0.2 as the threshold for RNA-seq decreased the numbers of false-positive results/negative detection and increased the confidence in the measured expression level.

2.2. Ingenuity Pathway Analysis (IPA)

IPA (Ingenuity Systems, Inc., Redwood City, CA, USA) is a web-software based on numerous types of literature reviewed and updated by experts. This software can be applied for the analysis, integration, and interpretation of data derived from “omics experiments” [11]. Functions analysis, canonical pathways, upstream analysis, and networks are the main results obtained when using the IPA tool. IPA also helps users to generate network graphics and further analyze a specific network. Additionally, IPA software provides tools for causal analysis, which helps users to generate hypothetical signaling pathways from the results of gene datasets and comparison of data.

2.3. RNA interference transfection

Short interfering RNA (siRNA) for human CHD4 (J-009774-05), β1 integrin (L-004506-00), and negative control siRNA were purchased from Dharmacon (Lafayette, CO, USA). Cells were transfected with nontargeting or specific siRNA using Lipofectamine 2000 and Opti-MEM (Invitrogen) according to the manufacturer's instructions. Specific RNA interference expression plasmids for human and mouse CHD4 and control shRNA were purchased from the National RNAi Core Facility (Academia Sinica, Taiwan). The sequences of CHD4 and luciferase were used as described in our previous study [12].

Table 1
Specific primers used in real-time PCR analysis (mouse and human).

Name	Primer	Sequence(5′-3′)
mouse-CHD4	FW	TCCTCTGTCCACCATCATCA
	RV	ACCCAAGATGGCCATATCAA
mouse-ITGB1	FW	CTGCTCTAAAATTGAGATCAGGA
	RV	TCCATAAGGTAGTAGAGATCAATAGGG
mouse-GAPDH	FW	TGCCCCCATGTTTGTGATG
	RV	TGTGGTCATGAGCCCTTCC
human-CHD4	FW	GGTTTTGGTTCCAAGCGTAA
	RV	CTCCTCCTCGCCTTTCTTTT
human-ITGB1	FW	GAAGGGTTGCCCTCCAGA
	RV	GCTTGAGCTTCTCTGCTGTT
human-GAPDH	FW	AAGGCTGGGGCTCATTTGC
	RV	GCTGATGATCTTGAGGCT

2.4. Quantitative real-time polymerase chain reaction (q-PCR)

Total RNA extraction and q-PCR protocols were performed as previously described [13]. Briefly, total RNA was extracted using Trizol reagent (Invitrogen) and reverse-transcribed using SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's protocol. Synthesized cDNA was used as a template for PCR amplification with primers for human CHD4, human β1 integrin, human GAPDH, mouse CHD4, mouse β1 integrin, and mouse GAPDH (Table 1). Quantitative real-time PCR was performed in a 20-μL reaction volume using the standard protocols provided with the Roche LightCycler 480 II system (Basel, Switzerland). CHD4 and β1 integrin gene expression were determined as follows: ΔCT = CT (target gene) − CT (GAPDH) and ΔΔCT = ΔCT (experimental group) − ΔCT (control group).

2.5. Antibodies

β1 integrin (#9699) and GAPDH (#2118) were purchased from Cell Signaling Technology (Danvers, MA, USA). CHD4 (GTX124186) and β1 integrin (GTX128839) was purchased from GeneTex International Corporation (Irvine, CA, USA).

2.6. Western blotting

Protein extraction and immunoblotting were performed as previously described [14]. Briefly, the cells were lysed by M-PERTM mammalian protein extraction buffer (Thermo Fisher Scientific) and centrifuged at 15,000 g, and then cellular debris was removed. Extracted proteins were quantified, denatured, separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and immunoblotted with the indicated antibodies.

2.7. Human specimens

Formalin-fixed, paraffin-embedded blocks of tissues from 51 TNBC patients were collected from the Department of Pathology, Kaohsiung Medical University Hospital, Kaohsiung city, Taiwan. Institutional Review Board approval for the use of these tissues was given by the Research Ethics Committee of the Kaohsiung Medical University Hospital (IRB: KMUHIRB-E(I)-20150181 and KMUHIRB-E(II)-

20150086). Data were analyzed anonymously, and therefore no additional informed consent was required. All methods were performed in accordance with approved guidelines of the Kaohsiung Medical University Hospital.

2.8. Immunohistochemistry (IHC) staining

IHC staining was performed as previously described (Yin et al., 2016). Briefly, blocks of tissue embedded in paraffin were cut into 4-μm-thick sections, de-paraffinized and rehydrated, immersed in a pH 6.0 retrieval solution (DAKO, Carpinteria, CA, USA), and autoclaved at 121 °C for 10 min for antigen retrieval. After incubation in 3% hydrogen peroxide (Sigma, St. Louis, MO, USA) for 10 min to block endogenous peroxidase activity, the samples were incubated with primary antibodies at room temperature for 1 h, followed by application of the DAKO REAL™ EnVision™ Detection System for 1 h. Finally, the sections were incubated with 3′,3-diaminobenzidine for 5 min, counterstained with Mayer's hematoxylin, mounted, and analyzed.

2.9. Scoring

CHD4 and β1 integrin expression was scored as described previously [15,16]. Briefly, the samples were scored based on the intensity of the signal (0, 1+, 2+, and 3+) and proportion of positive cells (0: ≤10%, 1: 10–25%, 2: 25–50%, 3: > 50%). The staining index was calculated as the product of the signal intensity and positive cell proportion. Scores ≤4 and ≥ 6 were defined as low and high expression, respectively.

2.10. Statistical analysis

CHD4 and β1 integrin expression in patients with TNBC as determined by IHC staining and compared using the Chi-square test. To evaluate the feasibility of using the expression of these proteins as prognostic markers for patients with TNBC, survival curves were analyzed using the Kaplan-Meier method. Cox proportional hazards model was used to evaluate univariate and multivariate comparisons of overall survival with the clinicopathological variables. Two-tailed Student's *t*-test was used to compare differences between groups. A P-value < 0.05 was considered to indicate statistically significant differences between groups. All statistical analyses were performed by using SAS 9.3 software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Identification of differentially expressed genes between normal and shCHD4 4T-1 tumor cells

Differentially expressed genes in the parental and shCHD4 4T-1 cells are displayed as a volcano plot in Fig. 1A. The results of cuff-diff analysis revealed 540 significant differentially expressed genes. There were 283 genes up-regulated and 257 genes down-regulated in shCHD4 4T-1 cells (Fig. 1B). Clustering analysis was conducted to calculate and classify the data according to similarity so that samples or genes with similar expression patterns could be grouped. This can assist in predicting the function of unknown genes and whether they participate in the same cellular pathway. Fig. 1C shows the results of cluster analysis of differentially expressed genes in parental and shCHD4 4T-1 cells. Log10 (FPKM + 1) values were used for clustering. Highly expressed genes are shown in red, while genes expressed at low levels are shown in blue. The clustering analysis performed in shCHD4 4T-1 cells identifies a series of differentially expressed genes were ranked in Supplemental Table 1.

3.2. Dysregulated signaling pathway in CHD4 depression is associated with integrin signaling pathway

Previous studies suggested that CHD4 is an oncogene involved in epigenetic suppression of multiple tumor suppressor genes [17]. To identify novel signaling pathways involved in CHD4 activation, the IPA approach was applied to analyze the 257 down-regulated genes in shCHD4 4T-1 cells. Our results showed that more than 500 signaling pathways participated in CHD4-related pathways. According to the P-value and Z-score from the IPA data, Table 2 shows the top 10 signaling pathways affected by CHD4 depression. Previous studies suggested that integrin signaling pathways mediate tumor cell survival, progression, motility, and drug resistance [18]. Therefore, we focused on the role of CHD4 in the integrin signaling pathway.

3.3. Knockdown of CHD4 decreases gene and protein expression of β1 integrin in mouse and human TNBC cells

To investigate if the β1 integrin protein is modulated by CHD4 in TNBC cells, we used mouse 4T-1 and human MDA-MB-231 TNBC cells to investigate gene and protein expression of CHD4 and β1 integrin. q-PCR showed that β1 integrin gene expression decreased after knockdown of CHD4 in both TNBC cells (Fig. 2A). However, knockdown of β1 integrin did not affect CHD4 gene expression (Fig. 2B). Western blotting revealed that β1 integrin protein expression decreased after knockdown of CHD4 in both TNBC cells (Fig. 2C). However, knockdown of β1 integrin did not affect CHD4 protein expression (Fig. 2D). In addition, this decrease in β1 integrin protein expression as a result of CHD4 knockdown was also observed in other human TNBC (MDA-MB-231 and HS578T) cells (Fig. 2E). Therefore, CHD4 may be the upstream mediator of β1 integrin. Our results also indicated that knockdown of CHD4 decreased vimentin, β-catenin, and Snail expression (Fig. 2C), similar to the results of our previous study showing that CHD4 mediates the epithelial-mesenchymal transition (EMT) in human and mouse TNBC cells. Overall, downregulation of β1 integrin protein expression may be as a result of gene regulation by CHD4.

3.4. β1 integrin is an important molecule regulated by CHD4 in mouse TNBC cells

Integrins are glycoprotein receptors that sense and respond to cell-microenvironment stress. In mammalian cells, 18 α and 8 β subunits can assemble into 24 different types of integrin [16]. According to several previous studies, β1 integrin plays important roles in regulating cell proliferation, motility, mortality, and drug resistance in several different types of cancer. Therefore, it is important to identify the upstream regulator mediating β1 integrin. In our NGS data, β1 integrin is not only involved in integrin signaling but also in several other pathways, which are modulated by CHD4 (Table 2). In addition, systemic analysis suggested that CHD4 upregulates the expression of β1 integrin by interacting with candidate proteins including Snail1, Notch1, SMARCA4, JUN, VCAM1, BRD4, CD4, IL-4, MYC, and Snail2 (Fig. 3). These data indicate that CHD4 functions as a transcription activator in regulating the expression of the β1 integrin gene.

3.5. Relationships among CHD4, β1 integrin, and several clinicopathological parameters in patients with TNBC

Previous studies indicated that CHD4 and β1 integrin can be used as prognostic markers in patients with TNBC [15,16]. In this study, NGS and *in vitro* data indicated that β1 integrin is mediated by CHD4. However, whether the CHD4-β1 integrin axis is important in patients with TNBC remains unclear. We investigated the relationships among CHD4, β1 integrin, and co-expression of CHD4 and β1 integrin with other clinicopathological parameters from patients with TNBC by IHC staining. Fig. 4A shows high expression [(a) and (b) respectively] and

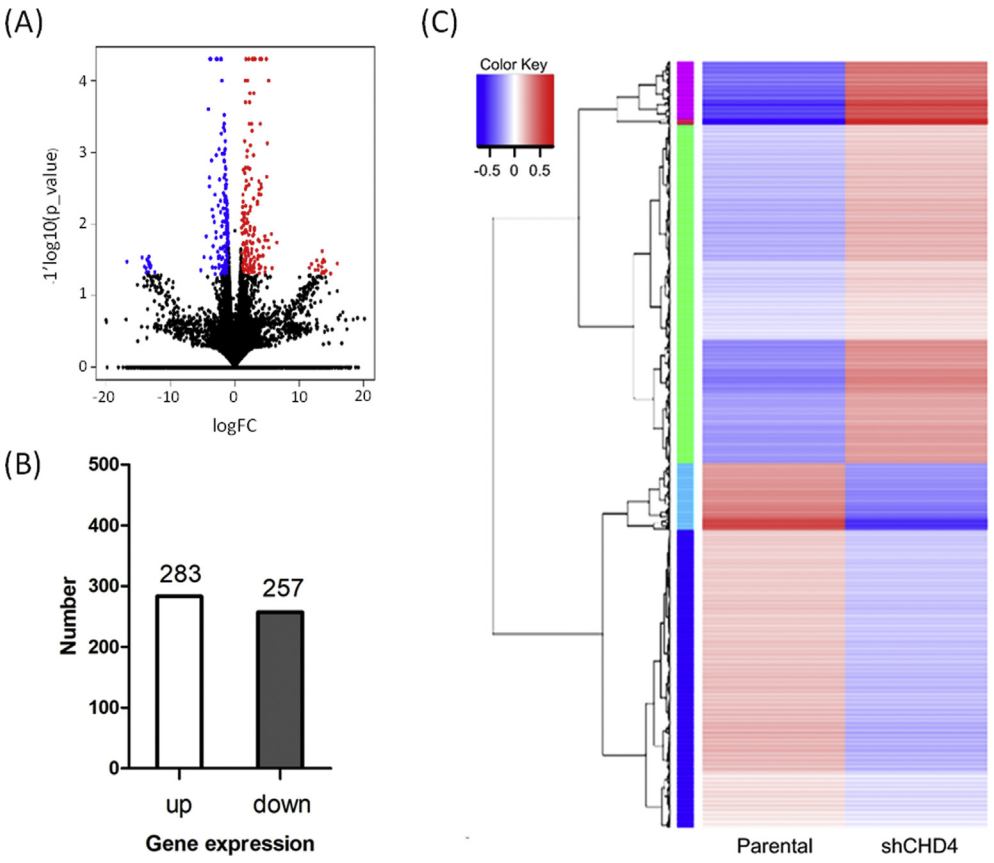


Fig. 1. Differentially expressed genes in parental and shCHD4 4T-1 cells. (A) The different gene expression in parental and shCHD4 cells from RNA sequencing displayed as a volcano plot. Red marks represent up-regulated genes and blue marks represent down-regulated genes in shCHD4 4T-1 cells. (B) A total of 540 significant differentially expressed genes were obtained from Cuff-diff analysis. Among these, 283 significantly up-regulated and 257 significantly down-regulated genes were identified in shCHD4 cells. (C) The total number of genes (540) is presented as a heat map. Red column shows high levels of gene expression while blue column shows low levels of gene expression through cluster analysis. The regions of different colors (purple, green, light and dark blue) represent different clusters. Genes with similar expression patterns are within the same cluster and close to each other, and they may have similar functions or participate in the same biological processes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

low expression [(c) and (d) respectively] of CHD4 and β1 integrin in the same patients. Consistent with the NGS and *in vitro* data, CHD4 and β1 integrin ($P = 0.049$) were positively correlated in 51 patients with TNBC (Table 3). Table 4 shows that CHD4, β1 integrin, and co-expression of CHD4 and β1 integrin were significantly associated with metastasis, recurrence, and death. Kaplan-Meier analysis also showed the average overall survival duration was 27.8 ± 13.6 months in patients with co-expression of CHD4 and β1 integrin (Fig. 4B). This value was significantly lower than other groups which had an average survival duration of 47.2 ± 30.1 months ($p = 0.0016$). Fig. 4C shows that co-expression of CHD4 and β1 integrin (27.8 ± 13.6 months) resulted in the shortest survival duration amongst the groups (β1 integrin + CHD4-: 35.7 ± 15.1 months; β1 integrin- CHD4+: 36.9 ± 23.0 months; β1 integrin- CHD4-: 58.5 ± 31.5 months). Univariate and multivariate Cox regression analyses (Table 5) revealed that the overall survival ($p = 0.0038$, $p = 0.006$) and nodal stage ($p = 0.0007$, $p = 0.0015$) of patients was significantly associated with co-expression of CHD4 and β1 integrin. Overall, these data indicate that co-expression of CHD4 and β1 integrin are important independent predictors of overall survival in patients with TNBC.

3.6. β1 integrin expression accompanies with CHD4 expression in TNBC patients

To further identify the relationship between CHD4 and β1 integrin in TNBC patients, the Ualcan and Oncomine databases were used. Data from Ualcan database indicated that CHD4 was positive correlated with β1 integrin (Pearson: 0.37, $p < 0.05$) (Fig. 5A). From Oncomine database, one dataset indicated that CHD4 and β1 integrin expression was significant higher in TNBC patients and basal-like subtype breast patients, and CHD4 is highly correlated with β1 integrin ($r = 0.69$) (Fig. 5B). Another dataset indicated that CHD4 is highly correlated with β1 integrin especially in TNBC patients ($r = 0.398$) (Fig. 5C). These above-mentioned results were similar with our results. Therefore, β1 integrin appears to be an important molecule regulated by CHD4 in patients with TNBC.

4. Discussion

Although TNBC accounts for a minority of all breast cancer cases, it is the most difficult to treat. The lack of specific biomarkers, few therapeutic options, and slow development of new anti-cancer drugs may explain why TNBC patients have low survival rates. Therefore, identifying new targets for diagnosis, prognosis, and drug development are important issues in treating TNBC. Previous studies demonstrated that CHD4 is involved in several oncogenic effects of many cancers [17,19–23]. Additionally, CHD4 interacts physically and functionally with other core subunits of nucleosome remodeling and histone deacetylase complexes to maintain the silencing of tumor suppressor gene expression [24]. Our previous studies also indicated that CHD4-mediated p21 and E-cadherin suppression can induce chemoresistance and metastasis in BRCA-proficient breast cancer [12,15]. However, to date no anti-cancer drugs have been developed that target CHD4. Thus, it is necessary to search for other downstream molecules or preclinical drugs, which may cause similar effects to CHD4 deficiency.

In the search for molecules or pathways mediated by CHD4, we found that the integrin signaling pathway was affected by CHD4 knockdown. Interestingly, β1 integrin was not only involved in integrin signaling but also in many other pathways among several genes regulated by CHD4, indicating that β1 integrin could be an important molecule mediated by this epigenetic regulator. Knockdown of CHD4 inhibited gene and protein expression of β1 integrin, while knockdown of β1 integrin gene did not affect the protein expression of CHD4, suggesting that CHD4 is an upstream mediator of β1 integrin (Fig. 2). Ten possible mediators were found to be involved in modulating β1 integrin by CHD4 through IPA data analysis (Fig. 3). Previous studies indicated

Table 2
Top 10 signaling pathways could be affected by CHD4 depression.

Ingenuity Canonical Pathways	-log(p-value)	z-score	Molecules
EIF2 Signaling	3.89E+00	−3.272	EIF2AK4,RPL22,DDIT3,ATF5,RPS18,RPLP2,EIF4A2,EIF2S1,RPL7A,EIF4G1,HSPA5,RPL7,RPS28,SHC1,RPL14,AKT1,MAP2K2,EIF4G2,RPL13,RPL18A,EIF5,RPL21,RPS17,RPS5,RPL18,-RPL4,RPS8,RPL17,RPS10,EIF3J,TRIB3,RPL12,XIAP,RPL9,RPL10A,EIF3G,PTBP1,SREBF1,EIF4A1,PIK3CB,PIK3CD,RPS15A,RPS25,RPL13A
Melatonin Signaling	3.74E+00	−3.3	PDIA3,PRKAR2A,GNAQ,PLCL2,RORC,PRKCZ,GNAI2,PLCD1,PLCB4,CAMK2D,PRKAR2B,MAP2K2,ARAF,PLCG2,PRKAG2,MAP2K3,CAMK2B,PRKAR1A,PRKCB
Neuregulin Signaling	2.84E+00	−3	ITGB1,RPS6KB1,STAT5A,BTC,ERBIN,NRG4,HBEGF,PRKCZ,CDK5R1,AREG,PTEN,SHC1,ITGA3,HSP90B1,AKT1,MAP2K2,HSP90AB1,PLCG2,ERRFI1,STAT5B,PRKCB
Superpathway of Cholesterol Biosynthesis	2.71E+00	−3	FDPS,MVD,DHCR7,IDI1,HADHB,MSMO1,LSS,SC5D,GGPS1
Sperm Motility	2.53E+00	−4.69	PAFAH1B2,PDIA3,PDE4A,PRKG2,PLA2G7,PRKCZ,STYK1,PLCD1,CLK1,LCAT,CLK3,DDR2,PDGFRA,PLA2G12A,GNAS,FES,ITPR2,CSK,EPHA1,PRKAR2A,TNK2,PLCL2,AXL,TEC,P-LA2G6,PLA2G4A,PLCB4,CLK4,PRKAR2B,PLCG2,CLK2,PRKAG2,MAP2K3,AATK,EPHA2,PRKCB,PAFAH1B3,PRKAR1A
Cdc42 Signaling	2.36E+00	−2.138	FGD3,MPRIP,ARPC1B,HLA-A,MYLK,EXOC6,PARD6A,PRKCZ,HLA-G,PAK1,ITGA3,ACTR3,PPP1R12A,HLA-DMA,EXOC2,EXOC5,EXOC3,RASA1,MYL12B,ITGB1,VAV2,TNK2,FOS,MAPK14,PARD3
AMPK Signaling	2.19E+00	−3.138	PBRM1,RAB1A,CHRNB1,PFKL,SMARCA4,EIF4EBP1,AKT1,TBC1D1,SMARCB1,RPTOR,ILKAP,SMARCC2,AKT1S1,RPS6KB1,PFKFB3,GNAS,SLC2A1,EEF2,STRADA,CREB3,SMAR-CE1,PRKAR2A,RAB7A,PFKP,CHRND,SMARCD3,MAPK14,PRKAR2B,PTPA,SMARCA2,PFKFB4,PRKAG2,AK4,PIK3CB,PIK3CD,MAP2K3,ACACA,PRKAR1A
Antioxidant Action of Vitamin C	2.18E+00	4	PAFAH1B2,STAT5A,SLC2A1,PDIA3,PLCL2,PLA2G7,GSTO1,TXNRD1,PLCD1,PLA2G4A,SLC23A2,PLA2G6,PLCB4,MAPK14,LCAT,PLCG2,CHUK,STAT5B,TXNRD2,PLA2G12A,PAFAH1-B3
Integrin Signaling	2.12E+00	−4.802	RAPGEF1,ITGA2B,MPRIP,ARHGAP26,ARPC1B,ITGA8,RHOT2,ILK,MYLK,NCK1,BCAR1,PTEN,SHC1,PAK1,ITGA3,ACTR3,AKT1,MAP2K2,PPP1R12A,ILKAP,ITGB4,CAPN10,MYL12B,I-TGB1,CAPN5,PARVA,PXN,CAPN6,TNK2,DOCK1,PLCG2,ZYX,PIK3CB,PIK3CD,ACTN4,CTTN,FNBP1
Ephrin Receptor Signaling	2.07E+00	−4.69	RAPGEF1,ITSN1,ARPC1B,AXIN1,PDGFA,NCK1,PDGFC,BCAR1,GNB4,SHC1,PAK1,ITGA3,AKT1,ACTR3,MAP2K2,RASA1,GNG12,ITGB1,PXN,NGEF,GNAS,ANGPT1,EPHA1,CREB3,GN-AQ,STAT3,FGF1,GNAI2,ABI1,MAP4K4,EPHA2
Regulation of eIF4 and p70S6K Signaling	2.03E+00	−2.53	RPS18,EIF4A2,PAIP2,EIF4G1,EIF2S1,PRKCZ,EIF4EBP1,RPS28,SHC1,ITGA3,AKT1,MAP2K2,EIF4G2,MKNK1,RPS17,RPS5,ITGB1,RPS6KB1,RPS8,RPS10,EIF3J,EIF3G,MAPK14,PTPA,E-IF4A1,RPS25,RPS15A,PIK3CD,PIK3CB
Role of NFAT in Cardiac Hypertrophy	``1.93E+00	−4.849	IL6ST,CAMK1,PDIA3,HDAC10,PRKCZ,PLCD1,GNB4,SHC1,AKT1,CAMK2D,MAP2K2,CACNG7,GNG12,PPP3CA,CAMK2B,CACNB1,GNAS,HDAC4,ITPR2,HDAC1,PRKAR2A,GNAQ,PLC-L2,HDAC5,GNAI2,RCAN1,PLCB4,MAPK14,PRKAR2B,PLCG2,PRKAG2,PIK3CB,PIK3CD,MAP2K3,MEF2C,PRKCB,PRKAR1A

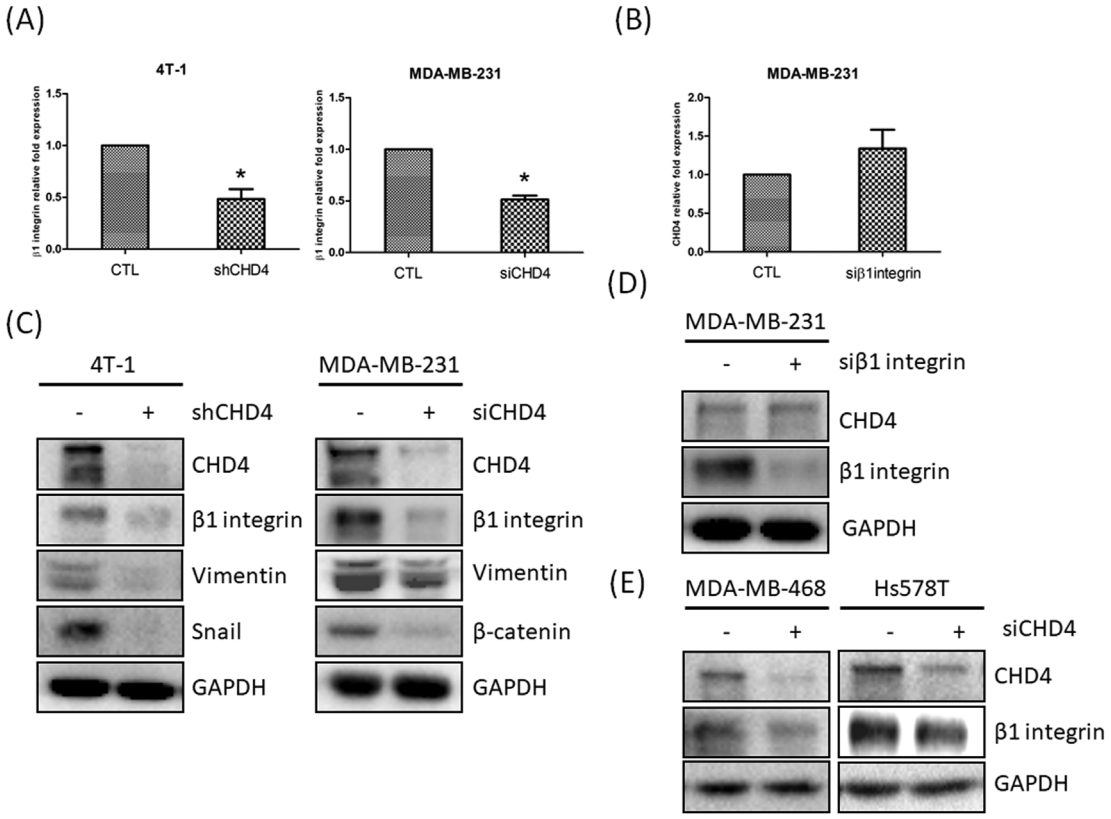
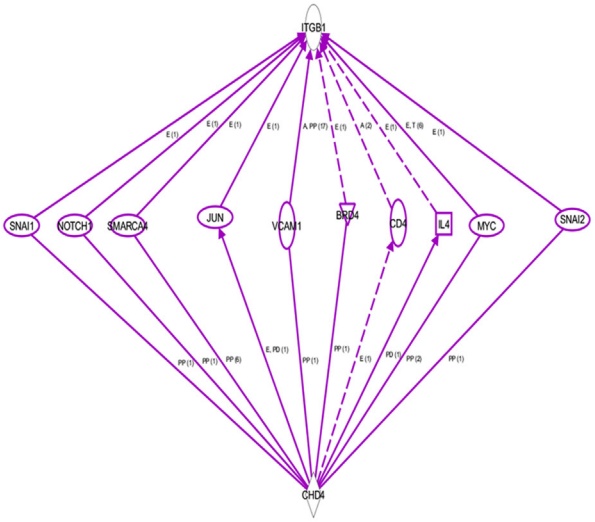


Fig 2. β1 integrin protein expression is modulated through gene regulation by CHD4 in TNBC cells. (A) CHD4 knockdown decreased expression of β1 integrin in 4T-1 and MDA-MB-231 cells. (B) β1 integrin knockdown did not significantly affect protein expression of CHD4. (C) Knockdown of CHD4 decreased β1 integrin and several EMT-related proteins (such as vimentin, β-catenin, and Snail) expression. (D) Knockdown of β1 integrin did not affect CHD4 protein expression. (E) Knockdown of CHD4 decreased β1 integrin protein expression in MDA-MB-468 and Hs578T cells.

that most of these mediators, such as Snail, BRD4, Notch, and Myc, could directly or indirectly bind to CHD4 to silence several tumor suppressor genes and regulate oncogenic functions. Several studies have indicated that β1 integrin could also be regulated by these molecules [25–31]. However, the dominant mediators and exact mechanism mediated by CHD4-β1 integrin axis in TNBC require further analysis. Surprisingly, CHD4 could also mediate β1 integrin through several immune response factors (Fig. 3). Therefore, this signaling transduction may be applied to immunotherapy in cancer treatment [32,33].



Gene symbol	Gene name	Subcellular location	Types	Canonical Pathway
SNAIL1	snail family transcriptional repressor 1	Nucleus	transcription regulator	Epithelial Adherens Junction Signaling; ILK Signaling; Regulation of the Epithelial-Mesenchymal Transition Pathway
NOTCH1	notch1	Plasma Membrane	transcription regulator	Epithelial Adherens Junction Signaling; Regulation of the Epithelial-Mesenchymal Transition Pathway; Immune response
SMARCA4	SWI/SNF related membrane 4	Nucleus	transcription regulator	AMPK Signaling; Aryl Hydrocarbon Receptor Signaling; Estrogen Receptor Signaling; Glucocorticoid Receptor Signaling; Hereditary Breast Cancer Signaling; RAR Activation; Role of BRCA1 in DNA Damage Response
JUN	Jun proto-oncogene	Nucleus	transcription regulator	TGF-β Signaling
VCAM1	vascular cell adhesion molecule 1	Plasma Membrane	transmembrane receptor	Atherosclerosis Signaling; Glucocorticoid Receptor Signaling; IL-8 Signaling; Leukocyte Extravasation Signaling; Neuroinflammation Signaling Pathway
BRD4	bromodomain containing 4	Nucleus	kinase	chromatin organization; positive regulation of cell cycle; positive regulation of transcription from RNA polymerase II promoterresponse to DNA damage stimulus
CD4	CD4 ANTIGEN	Plasma Membrane	transmembrane receptor	Immune response
IL4	Interleukin 4	Extracellular Space	cytokine	Inflammation Signaling Pathway; Cytokine Signaling; Immune response
MYC	MYC proto-oncogene	Nucleus	transmembrane receptor	Aryl Hydrocarbon Receptor Signaling; ERK/MAPK Signaling; ERK5 Signaling; Neuregulin Signaling; Opioid Signaling Pathway; P2Y Purigenic Receptor Signaling Pathway; STAT3 Pathway; Telomerase Signaling; Wnt/β-catenin Signaling
SNAIL2	snail family transcriptional repressor 2	Nucleus	transmembrane receptor	Endocannabinoid Cancer Inhibition Pathway; Epithelial Adherens Junction Signaling; ILK Signaling; p53 Signaling; Regulation of the Epithelial-Mesenchymal Transition Pathway

Fig. 3. Candidate proteins involved in CHD4-mediated β1 integrin signaling pathways analyzed by IPA Results from IPA suggest that several candidate proteins, including Snail1, Notch1, SMARCA4, JUN, VCAM1, BRD4, CD4, IL-4, MYC, and Snail2, participate in the regulation of CHD4-β1 integrin signaling pathways.

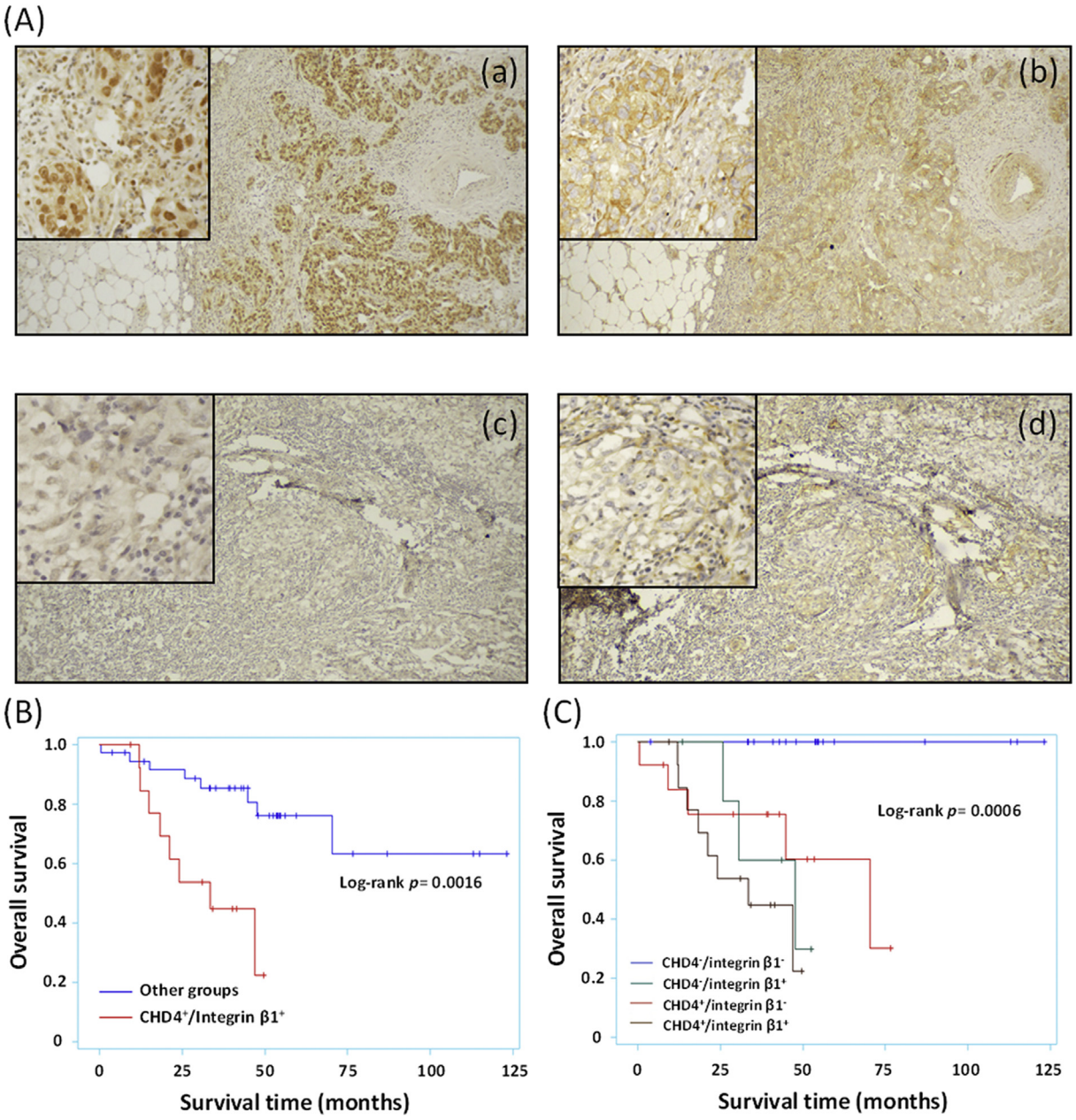


Fig. 4. Evaluation of CHD4-β1 integrin axis as a prognostic marker in TNBC patients. (A) Representative immunostaining images of CHD4 and β1 integrin expression in TNBC tissues (original magnification and high power insert are at 200x and 400x magnification, respectively). Expression of CHD4 and β1 integrin was classified as high ((a) and (b), respectively) or low ((c) and (d), respectively) according to staining observed in the nucleus and cytoplasm. (B) Kaplan-Meier survival curves for TNBC patients. Survival was significantly associated with co-expression of CHD4 and β1 integrin. (C) Kaplan-Meier survival curves for TNBC patients in four groups (co-expression of CHD4 and β1 integrin, expression of CHD4, expression of β1 integrin, and low expression of CHD4 and β1 integrin). Survival was significantly associated with co-expression of CHD4 and β1 integrin.

Table 3
Association of CHD4 and β1 integrin expressions in TNBC tissues.

		CHD4, n (%)		P-value
		high	low	
β1 integrin	High	14(27.46)	6(11.76)	
	Low	13(25.49)	18(35.29)	$P = 0.049$

Previous studies indicated that β1 integrin and its related signaling transduction affect cell proliferation, migration, invasion, survival, and drug resistance to contribute to the malignant characteristics. Additionally, high expression of β1 integrin is associated with poor outcomes in several types of cancer (Blandin et al., 2015, Sun et al., 2018, Yao et al., 2007). Disruption of β1 integrin may suppress tumor cell migration, intravasation, and anchorage-independent growth. Several studies have indicated that β1 integrin is a potential prognostic biomarker in TNBC [16,34–37], and some preclinical trials support the idea that β1 integrin antagonists are promising chemotherapeutics for inhibiting tumor growth and metastasis [38–40]. However, whether β1 integrin antagonists can be further applied in TNBC patients with high CHD4 expression remains unclear. Therefore, the correlations between co-expression of CHD4 and β1 integrin and several clinicopathological factors were evaluated. Our clinical data indicated that CHD4 was positively correlated with β1 integrin (Table 3), and this result was similar with several datasets from Ualcan and Oncomine databases (Fig. 5).

Table 4
Relationship between CHD4 expression and clinicopathological characteristics of TNBC patients (n = 51).

Parameters	n	CHD4-, n (%)	CHD4 + , n (%)	P-value	Integrin β1-, n (%)	Integrin β1 + , n (%)	P-value	[a]Other groups, n (%)	CHD4 + /Integrin β1 + , n (%)	P-value
Total	51	24(47.06)	27(52.94)		31(60.78)	20(39.22)		37(72.55)	14(27.45)	
Age										
≤ 40 yrs	45	22(91.67)	23(85.19)	0.4733	29(93.55)	16(80.00)	0.1426	35(94.59)	10(71.43)	0.0219[a]
> 40 yrs	6	2(8.33)	4(14.81)		2(6.45)	4(20.00)		2(5.41)	4(28.57)	
Size										
≤ 2.0 cm	20	8(33.33)	12(44.44)	0.4172	13(41.94)	7(35.00)	0.6204	15(40.54)	5(35.71)	0.7527
> 2.0 cm	31	16(66.67)	15(55.56)		18(58.06)	13(65.00)		22(59.46)	9(64.29)	
Grade										
I/II	19	11(45.83)	8(29.63)	0.2322	11(35.48)	8(40.00)	0.7447	15(40.54)	4(28.57)	0.4301
III	32	13(54.17)	19(70.37)		20(64.52)	12(60.00)		22(59.46)	10(71.43)	
Tumor stage										
T1	23	11(45.83)	12(44.44)	0.9207	16(51.61)	7(35.00)	0.2444	18(48.65)	5(35.71)	0.4074
T2/T3	28	13(54.17)	15(55.56)		15(48.39)	13(65.00)		19(51.35)	9(64.29)	
Nodal stage										
N0	30	14(58.33)	16(59.26)	0.9465	20(64.52)	10(50.00)	0.3038	23(62.16)	7(50.00)	0.4309
N1/N2/N3	21	10(41.67)	11(40.74)		11(35.48)	10(50.00)		14(37.84)	7(50.00)	
Metastatic stage										
M0	37	21(87.50)	16(59.26)	0.0241[a]	26(83.87)	11(55.00)	0.0241[a]	31(83.78)	6(42.86)	0.0035[a]
M1	14	3(12.50)	11(40.74)		5(16.13)	9(45.00)		6(16.22)	8(57.14)	
Recurrence										
Absent	46	24(100.00)	22(81.48)	0.0264[a]	31(100.00)	15(75.00)	0.0034[a]	37(100.00)	9(64.29)	0.0001[a]
Present	5	0(0.00)	5(18.52)		0(0.00)	5(25.00)		0(0.00)	5(35.71)	
Survival status										
Survival	35	21(87.50)	14(51.85)	0.0062[a]	26(83.87)	9(45.00)	0.0035[a]	29(78.38)	6(42.86)	0.0147[a]
Death	16	3(12.50)	13(48.15)		5(16.13)	11(55.00)		8(21.62)	8(57.14)	

[a] Other groups: means the groups excepting CHD4 + /Integrin 1 + (include CHD4-/Integrin 1-, CHD4-/Integrin 1 + , and CHD4 + /Integrin 1- groups).

Table 5
Univariate and multivariate logistic analysis of clinicopathological independent prognostic factors for survival of breast cancer patients (n = 51).

Factors	Univariate		Multivariate	
	HR(95%CI)	p-value	HR(95%CI)	p-value
CHD4 expression				
Low	1.0	0.0059*	1.0	0.0007*
High	5.928(1.672–21.020)		11.818(2.848–49.039)	
Age				
≤ 40 yrs	1.0	0.5151	1.0	0.4935
> 40 yrs	0.510(0.067–3.869)		0.475(0.056–4.000)	
Tumor stage				
T1	1.0	0.0114*	1.0	0.0407*
T2/T3	5.123(1.446–18.150)		5.123(1.072–24.484)	
Nodal stage				
N0	1.0	0.0007*	1.0	0.0022*
N1/N2/N3	13.088(2.965–57.768)		5.123(1.072–24.484)	
Tumor recurrent				
Absent	1.0	0.0025*	1.0	0.4694
Present	6.162(1.892–20.066)		0.607(0.157–2.348)	
β1 integrin expression				
Low	1.0	0.0024*	1.0	0.0111*
High	6.052(1.891–19.367)		4.547(1.413–14.634)	
Age				
≤ 40 yrs	1.0	0.5151	1.0	0.7660
> 40 yrs	0.510(0.067–3.869)		0.730(0.092–5.791)	
Nodal stage				
N0	1.0	0.0007*	1.0	0.0025*
N1/N2/N3	13.088(2.965–57.768)		10.379(2.278–47.287)	
CHD4/Integrin coexpression				
Other groups	1.0	0.0038*	1.0	0.0060*
CHD4 + /Integrin +	4.697(1.647–13.396)		4.525(1.541–13.290)	
Age				
≤ 40 yrs	1.0	0.5151	1.0	0.5775
> 40 yrs	0.510(0.067–3.869)		0.554(0.069–4.421)	
Nodal stage				
N0	1.0	0.0007*	1.0	0.0015*
N1/N2/N3	13.088(2.965–57.768)		11.509(2.544–52.072)	

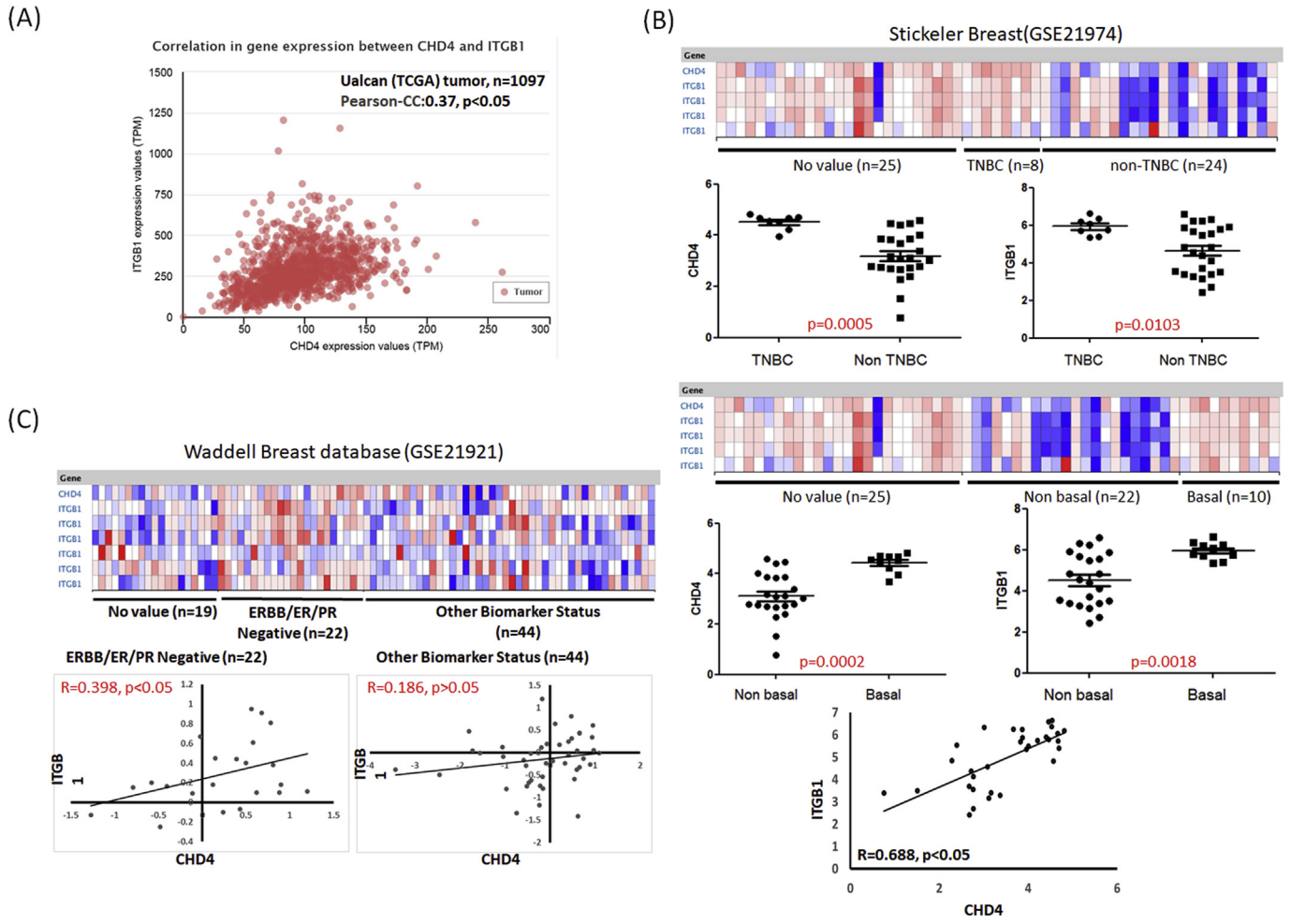


Fig. 5. β1 integrin expression accompanies with CHD4 expression in TNBC patients. (A) Positive correlation between CHD4 and β1 integrin (ITGB1) was evaluated by the UALCAN database in TCGA breast cancer samples. (B) Positive correlation between CHD4 and β1 integrin in TNBC patients and in basal-like breast cancer patients was evaluated by one dataset from Oncomine database. (C) Positive correlation between CHD4 and β1 integrin is significant in TNBC patients compared to non-TNBC patients from Oncomin database.

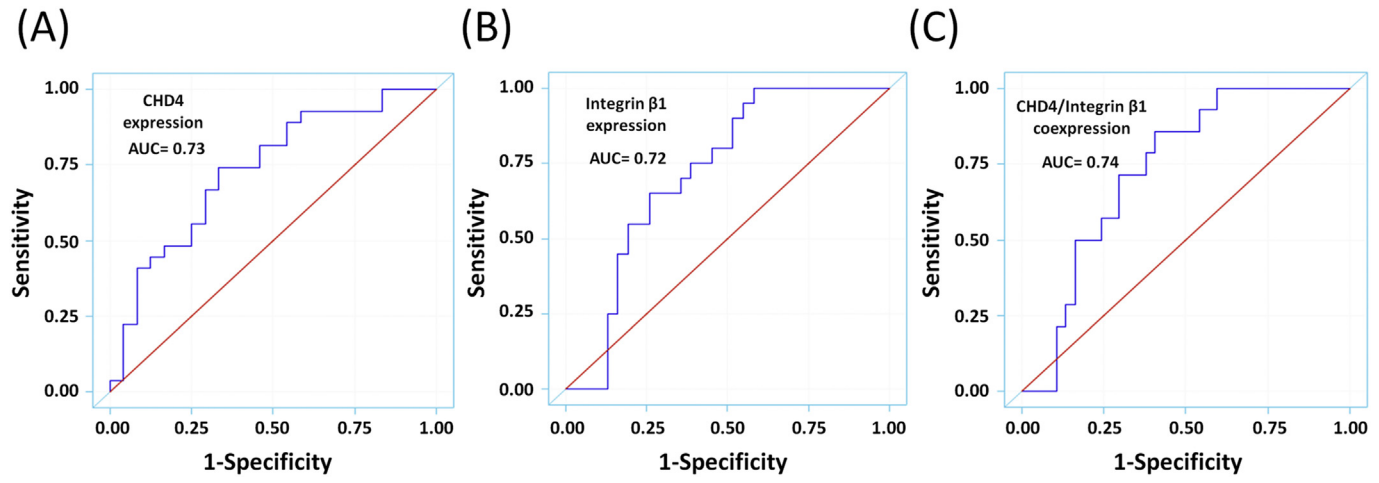


Fig. 6. Receiver operating characteristic (ROC) curve analysis of CHD4, β1 integrin, and co-expression of CHD4 and β1 integrin. (A) The ROC curve and the area under the curve (AUC) calculated for CHD4 expression. (B) The ROC curve and the AUC calculated for β1 integrin expression. (C) The ROC curve and the AUC calculated CHD4-β1 integrin co-expression.

This positive correlation was significant in TNBC and basal like type than other groups. Prior studies reported that about 71% of TNBC were found to be basal-like while 77% of basal-like cancers were TNBC [41,42]. Previous studies also suggested that CHD4 and β1 integrin could be prognostic markers in TNBC patients [15,16,19,43]. Thus, the role of CHD4 and β1 integrin should be more important in TNBC than in other subtypes of breast cancer. Co-expression of CHD4 and β1 integrin was significantly associated with metastasis, recurrence, and death, and

may be a significant predictor of overall survival (Table 4 & Table 5). Furthermore, co-expression of CHD4 and β1 integrin showed significantly higher positive correlation (r = 0.74) with survival than CHD4 (r = 0.73) and β1 integrin (r = 0.72) by receiver operating characteristic curve analysis (Fig. 6). Thus, this CHD4-β1 integrin axis has clinicopathological and prognostic significance in TNBC patients, implying that β1 integrin antagonists could be further applied in high CHD4 expression TNBC patients.

5. Conclusions

In summary, this is the first study to show that CHD4 transcriptionally regulates expression of β1 integrin in TNBC cells. In addition, CHD4-β1 integrin axis could potentially be a predictive marker in patients with TNBC and the use of β1 integrin inhibitors may be a therapeutic option for TNBC patients with high CHD4 expression.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116963.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 68 (2018) 394–424.
[2] O. Yersal, S. Barutca, Biological subtypes of breast cancer: prognostic and therapeutic implications, World J. Clin. Oncol. 5 (2014) 412–424.
[3] P. Eroles, A. Bosch, J.A. Perez-Fidalgo, A. Lluch, Molecular biology in breast cancer: intrinsic subtypes and signaling pathways, Cancer Treat Rev. 38 (2012) 698–707.
[4] S.A. O'Toole, J.M. Beith, E.K. Millar, R. West, A. McLean, A. Cazet, et al., Therapeutic targets in triple negative breast cancer, J. Clin. Pathol. 66 (2013) 530–542.
[5] C.M. Perou, Molecular stratification of triple-negative breast cancers, The Oncologist 15 (Suppl 5) (2010) 39–48.
[6] E.A. O'Reilly, L. Gubbins, S. Sharma, R. Tully, M.H. Guang, K. Weiner-Gorzel, et al., The fate of chemoresistance in triple negative breast cancer (TNBC), BBA Clin. 3 (2015) 257–275.
[7] H.A. Wahba, H.A. El-Hadaad, Current approaches in treatment of triple-negative breast cancer, Canc. Biol. Med. 12 (2015) 106–116.
[8] H. Easwaran, H.C. Tsai, S.B. Baylin, Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance, Mol. Cell 54 (2014) 716–727.
[9] S. Wee, D. Dhanak, H. Li, S.A. Armstrong, R.A. Copeland, R. Sims, et al., Targeting epigenetic regulators for cancer therapy, Ann. N. Y. Acad. Sci. 1309 (2014) 30–36.
[10] J. Wang, D.C. Dean, F.J. Hornicek, H. Shi, Z. Duan, RNA sequencing (RNA-Seq) and its application in ovarian cancer, Gynecol. Oncol. 152 (2019) 194–201.
[11] S.M. Kurian, T.C. Whisenant, J.M. Mathew, J. Miller, J.R. Leventhal, Transcriptomic studies in tolerance: lessons learned and the path forward, Hum. Immunol. 79 (2018) 395–401.
[12] M.F. Hou, C.W. Luo, T.M. Chang, W.C. Hung, T.Y. Chen, Y.L. Tsai, et al., The NuRD complex-mediated p21 suppression facilitates chemoresistance in BRCA-proficient breast cancer, Exp. Cell Res. 359 (2017) 458–465.
[13] C.W. Luo, C.C. Wu, H.J. Ch'ang, Radiation sensitization of tumor cells induced by shear stress: the roles of integrins and FAK, Biochim. Biophys. Acta 1843 (2014) 2129–2137.
[14] M.R. Pan, M.F. Hou, F. Ou-Yang, C.C. Wu, S.J. Chang, W.C. Hung, et al., FAK is required for tumor metastasis-related fluid microenvironment in triple-negative breast cancer, J. Clin. Med. 8 (2019).
[15] C.W. Luo, C.C. Wu, S.J. Chang, T.M. Chang, T.Y. Chen, C.Y. Chai, et al., CHD4-mediated loss of E-cadherin determines metastatic ability in triple-negative breast cancer cells, Exp. Cell Res. 363 (2018) 65–72.
[16] H.L. Yin, C.C. Wu, C.H. Lin, C.Y. Chai, M.F. Hou, S.J. Chang, et al., beta1 integrin as a prognostic and predictive marker in triple-negative breast cancer, Int. J. Mol. Sci. 17 (2016).
[17] L. Xia, W. Huang, M. Bellani, M.M. Seidman, K. Wu, D. Fan, et al., CHD4 has oncogenic functions in initiating and maintaining epigenetic suppression of multiple tumor suppressor genes, Cancer Cell 31 (2017) 653–668 e7.
[18] H. Hamidi, M. Pietila, J. Ivaska, The complexity of integrins in cancer and new scopes for therapeutic targeting, Br. J. Canc. 115 (2016) 1017–1023.
[19] C. D'Alesio, S. Punzi, A. Cicalese, L. Fornasari, L. Furia, L. Riva, et al., RNAi screens identify CHD4 as an essential gene in breast cancer growth, Oncotarget 7 (2016) 80901–80915.
[20] S. Guillemette, R.W. Serra, M. Peng, J.A. Hayes, P.A. Konstantinopoulos, M.R. Green, et al., Resistance to therapy in BRCA2 mutant cells due to loss of the nucleosome remodeling factor CHD4, Genes Dev. 29 (2015) 489–494.
[21] A. O'Shaughnessy, B. Hendrich, CHD4 in the DNA-damage response and cell cycle progression: not so NuRDy now, Biochem. Soc. Trans. 41 (2013) 777–782.
[22] M.R. Pan, H.J. Hsieh, H. Dai, W.C. Hung, K. Li, G. Peng, et al., Chromodomain helicase DNA-binding protein 4 (CHD4) regulates homologous recombination DNA repair, and its deficiency sensitizes cells to poly(ADP-ribose) polymerase (PARP) inhibitor treatment, J. Biol. Chem. 287 (2012) 6764–6772.
[23] J. Sperlazza, M. Rahmani, J. Beckta, M. Aust, E. Hawkins, S.Z. Wang, et al., Depletion of the chromatin remodeler CHD4 sensitizes AML blasts to genotoxic agents and reduces tumor formation, Blood 126 (2015) 1462–1472.
[24] Y. Cai, E.J. Geutjes, K. de Lint, P. Roepman, L. Bruurs, L.R. Yu, et al., The NuRD complex cooperates with DNMTs to maintain silencing of key colorectal tumor suppressor genes, Oncogene 33 (2014) 2157–2168.
[25] H.B. Koch, R. Zhang, B. Verdoodt, A. Bailey, C.D. Zhang, J.R. Yates 3rdet al., Large-scale identification of c-MYC-associated proteins using a combined TAP/MudPIT approach, Cell Cycle 6 (2007) 205–217.
[26] S. Rahman, M.E. Sowa, M. Ottinger, J.A. Smith, Y. Shi, J.W. Harper, et al., The Brd4 extraterminal domain confers transcription activation independent of pTEFb by recruiting multiple proteins, including NSD3, Mol. Cell. Biol. 31 (2011) 2641–2652.
[27] M.A. Shields, S.B. Krantz, D.J. Bentrem, S. Dangi-Garimella, H.G. Munshi, Interplay between beta1-integrin and Rho signaling regulates differential scattering and motility of pancreatic cancer cells by snail and Slug proteins, J. Biol. Chem. 287 (2012) 6218–6229.
[28] T. Takahara, A. Kasamatsu, M. Yamatoji, M. Iyoda, H. Kasama, T. Saito, et al., SIPA1 promotes invasion and migration in human oral squamous cell carcinoma by ITGB1 and MMP7, Exp. Cell Res. 352 (2017) 357–363.
[29] D. Tang, T. Yan, J. Zhang, X. Jiang, D. Zhang, Y. Huang, Notch1 signaling contributes to hypoxia-induced high expression of integrin beta1 in keratinocyte migration, Sci. Rep. 7 (2017) 43926.
[30] B. Xu, J. Lefringhouse, Z. Liu, D. West, L.A. Baldwin, C. Ou, et al., Inhibition of the integrin/FAK signaling axis and c-Myc synergistically disrupts ovarian cancer malignancy, Oncogenesis 6 (2017) e295.
[31] A. Yatim, C. Benne, B. Sobhian, S. Laurent-Chabalier, O. Deas, J.G. Judde, et al., NOTCH1 nuclear interactome reveals key regulators of its transcriptional activity and oncogenic function, Mol. Cell 48 (2012) 445–458.
[32] C.C. DeNucci, Y. Shimizu, beta1 integrin is critical for the maintenance of antigen-specific CD4 T cells in the bone marrow but not long-term immunological memory, J. Immunol. 186 (2011) 4019–4026.
[33] H. Hosokawa, T. Tanaka, Y. Suzuki, C. Iwamura, S. Ohkubo, K. Endoh, et al., Functionally distinct Gata3/Chd4 complexes coordinately establish T helper 2 (Th2) cell identity, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 4691–4696.
[34] D. Barkan, A.F. Chambers, beta1-integrin: a potential therapeutic target in the battle against cancer recurrence, Clin. Cancer Res. 17 (2011) 7219–7223.
[35] A.F. Blandin, G. Renner, M. Lehmann, I. Lelong-Rebel, S. Martin, M. Dontenwill, beta1 integrins as therapeutic targets to disrupt hallmarks of cancer, Front. Pharmacol. 6 (2015) 279.
[36] Q. Sun, C. Zhou, R. Ma, Q. Guo, H. Huang, J. Hao, et al., Prognostic value of increased integrin-beta 1 expression in solid cancers: a meta-analysis, OncoTargets Ther. 11 (2018) 1787–1799.
[37] E.S. Yao, H. Zhang, Y.Y. Chen, B. Lee, K. Chew, D. Moore, et al., Increased beta1 integrin is associated with decreased survival in invasive breast cancer, Cancer Res. 67 (2007) 659–664.
[38] E. Hedrick, S.O. Lee, R. Doddapaneni, M. Singh, S. Safe, NR4A1 antagonists inhibit beta1-integrin-dependent breast cancer cell migration, Mol. Cell. Biol. 36 (2016) 1383–1394.
[39] R. Huang, E.K. Rofstad, Integrins as therapeutic targets in the organ-specific metastasis of human malignant melanoma, J. Exp. Clin. Cancer Res. 37 (2018) 92.
[40] S. Raab-Westphal, J.F. Marshall, S.L. Goodman, Integrins as therapeutic targets: successes and cancers, Cancers 9 (2017).
[41] P. Alluri, L.A. Newman, Basal-like and triple-negative breast cancers: searching for positives among many negatives, Surg. Oncol. Clin. N. Am. 23 (2014) 567–577.
[42] E.A. Rakha, S.E. Elsheikh, M.A. Aleskandarany, H.O. Habashi, A.R. Green, D.G. Powe, et al., Triple-negative breast cancer: distinguishing between basal and nonbasal subtypes, Clin. Cancer Res. 15 (2009) 2302–2310.
[43] S. Klahan, W.C. Huang, C.M. Chang, H.S. Wong, C.C. Huang, M.S. Wu, et al., Gene expression profiling combined with functional analysis identify integrin beta1 (ITGB1) as a potential prognosis biomarker in triple negative breast cancer, Pharmacol. Res. 104 (2016) 31–37.