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YBX1 promotes the stemness and metastasis of NSCLC cells by promoting CDCA8 expression

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Cancer stemness is a major therapeutic challenge in oncology. This study investigated the functional role and molecular mechanism of cell division cycle-associated 8 (CDCA8) in non-small cell lung cancer (NSCLC) stem cells. In this study, NSCLC and paracancerous tissues were collected. The lung adenocarcinoma cell line A549 and the lung squamous cell carcinoma cell line NCI-H520 were used. The stem-like cell population in A549 and NCI-H520 was isolated by CD44+ fluorescence-activated cell sorting. Gene expression was detected by quantitative real-time PCR, western blotting, and immunohistochemical staining. Cell stemness was assessed by biomarker (SOX and NANOG) expression detection, colony formation assay, and sphere-formation assay. Cell migration and invasion ability were determined by the Transwell experiment. Our results showed that CDCA8 expression was higher in NSCLC tissues than in paracancerous tissues. CDCA8 overexpression enhanced stemness properties, as evidenced by increased biomarker expression and colony formation, larger sphere size, and enhanced migratory/invasive capacity. Conversely, CDCA8 knockdown had the opposite effect. Mechanistically, we identified Y-box binding protein 1 (YBX1) as a direct binding protein of CDCA8 mRNA that positively regulated CDCA8 expression. YBX1 overexpression had a similar effect to CDCA8. Furthermore, recovery experiments revealed that the stemness-promoting effect of YBX1 was reversed by CDCA8 knockdown. These findings were further validated in xenograft models, confirming that the YBX1/CDCA8 axis promoted tumorigenesis *in vivo*. Collectively, our study reveals that YBX1 enhances cell stemness and metastasis of NSCLC by promoting CDCA8 expression. Our findings established a new mechanism that maintains NSCLC stemness and may provide novel biomarkers.

Key words: non-small cell lung cancer; stemness; metastasis; YBX1; CDCA8

Lung cancer is a critical global health challenge with high morbidity and mortality. Global statistics indicate over 2 million newly diagnosed cases annually, with more than 50% occurring in Asia [1, 2]. Moreover, the new cases of lung cancer in China have still risen in recent years [3]. Histologically, lung cancer is categorized into small cell lung cancer (accounting for 15%) and non-small cell lung cancer (NSCLC, accounting for 85%) [4]. Moreover, NSCLC is further classified into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma subtypes; however, adenocarcinoma and squamous cell carcinoma are dominant [5]. Despite therapeutic advances, including targeting therapy and immunotherapy, the prognosis for advanced NSCLC remains poor, with a survival of 9–12 months due to acquired therapeutic resistance, metastasis, and recurrence [6]. Consequently, elucidating the molecular mechanisms underlying NSCLC progression is essential for identifying novel diagnostic biomarkers and therapeutic targets.

Cancer stem cells (CSCs) represent a minor subpopulation within tumors; however, they play a pivotal role in cancer recurrence, therapeutic resistance, and metastasis [7]. CSCs are considered a deadly subtype of cancer cells that significantly contribute to cancer-related mortality [8]. Characterized by the expression of specific stem cell markers (CD133, CD44, and CD90), CSCs exhibit high self-renewal capacity and differentiation potential, enabling them to drive tumor initiation, progression, and maintenance [9]. Furthermore, CSCs exhibit chemoresistance and metastatic potential through upregulated multidrug transporter expression, enhanced DNA damage repair mechanisms, and oxidative stress mitigation capability [10–12]. Besides, CSCs secrete extracellular vesicles and cytokines to remodel the tumor

microenvironment, promoting a more favorable microenvironment for their maintenance and expansion [9]. The remarkable properties of CSCs, facilitated by their heterogeneity, permit phenotypic switching that promotes environmental adaptability and contributes to therapeutic persistence [13]. In the last decade, CSCs have been described and isolated from human lung cancer [13]. Moreover, CSC-associated biomarkers have been increasingly associated with cancer diagnosis, treatment response, and prognosis [7]. Growing evidence implicates Wnt, Hedgehog, and Notch pathways as three important pathways maintaining their stemness [9]. The unique cell surface markers expressed by CSCs offer dual clinical value as targets for novel therapies and as detectable biomarkers for early relapse prediction and treatment optimization [14]. Given these properties, CSCs are regarded as critical therapeutic targets, and strategies targeting stemness inhibition may provide a promising approach for cancer therapy.

Cell division cycle associated 8 (CDCA8) is a critical component of the chromosomal passenger complex, a key regulatory factor of cell mitosis. This complex is essential for the sister chromatids segregation and cytokinesis production [15]. CDCA8 has been identified as an oncogene in various cancers, including hepatocellular carcinoma, ovarian cancer, and bladder cancer [16]. This gene affects diverse malignant tumor behaviors, including cell proliferation, metastasis, therapy resistance, and tumor microenvironment formation [16]. Current evidence indicates that CDCA8 promotes tumor progression through modulating multiple signaling pathways, including PI3K/AKT, P53, and mTORC pathways [16]. Notably, CDCA8 is implicated in cancer cell stemness. Silencing CDCA8 inhibits tumor growth and stemness, maintaining [17]. In lung cancer, CDCA8 promotes cell proliferation, migration, and invasion *in vitro* [18, 19]. Besides, CDCA8 has been recognized as a pivotal oncogene in lung cancer by several bioinformatics studies and may be associated with immune cell infiltration and cell stemness formation [20–22]. However, the detailed function of CDCA8 in NSCLC cell stemness remains unclear. This study aimed to explore the role and mechanism of CDCA8 in NSCLC stem cells, expected to deepen the understanding of NSCLC stem cells biology and provide a potential therapy target.

Materials and methods

Clinical samples collection. A total of 10 patients with NSCLC and without prior chemotherapy or radiotherapy were enrolled at our hospital. The tumor and paired paracancerous tissues were stored in liquid nitrogen for subsequent detection. This study is approved by the Medical Ethics Committee of NanFang Hospital Baiyun Branch of Southern Medical University (Approval No.2024-07-16), and all patients provided informed consent.

Immunohistochemical (IHC) staining. Tissue specimens were fixed in 4% paraformaldehyde, paraffin-embedded, and cut into 4-μm sections. After deparaffinization and antigen retrieval, the sections were blocked with 5% goat serum, and subsequently incubated with specific CDCA8 antibody (1:50; #LS-C168603, LifeSpan BioSciences, WA, USA) or Y-box binding protein 1 (YBX1) antibody (1:250; #ab76149, Abcam, Cambridge, MA, USA) at 4 °C overnight. The next day, the sections were incubated with HRP-conjugated goat anti-rabbit secondary antibody (1:5,000; #ab205718, Abcam) at 37 °C for 40 min. Immunoreactivity was visualized using DAB chromogen, followed by counterstaining with hematoxylin.

Cell culture and stem cell filtering. The lung adenocarcinoma cell line A549 and lung squamous cell carcinoma cell line NCI-H520 were obtained from Pricella Biotechnology Co., Ltd. (CL-0016 and CL-0402, Wuhan, China). A549 cells were cultured in Ham's F-12K medium (#PM150910, Pricella Biotechnology), and NCI-H520 cells were cultured in RPMI-1640 (#PM150110, Pricella Biotechnology), both of which were supplied with 10% fetal bovine serum (#164210, Pricella Biotechnology) and 1% penicillin-streptomycin (#PB180120, Pricella Biotechnology). Both cell lines were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. To obtain NSCLC stem cells, A549 and NCI-H520 cells were enzymatically dissociated into a single-cell suspension. Cells were labeled with medium with 1 μl anti-CD44 antibody (#ab243894, Abcam, Cambridge, MA, USA) for 30 min at 4 °C in the dark. Subsequently, cells were harvested at 300×g for 5 min, and the positive cells were subsequently isolated using fluorescence-activated cell sorting (FACS) with BD FACSAria™ III (BD Biosciences).

Cell transfection. The gene overexpressing pcDNA3.1 plasmid (CDCA8, YBX1) and short-hairpin RNA pLKO.1 plasmid (sh-CDCA8, sh-YBX1), and their respective negative control (empty vector for overexpression, non-targeting shRNA for knockdown) were synthesized by GenePharma (Suzhou, China). The plasmids were then transfected into A549 and NCI-H520 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) at 80–90% confluence, according to the manufacturer's instructions, followed by medium replacement after 6 h. Cells were typically harvested or assayed 48 h post-transfection for further analysis.

Quantitative real-time PCR (qPCR). To detect the gene mRNA expression, RNA was extracted from tissues or cells using TRIzol reagent (#15596026, Invitrogen, Carlsbad, CA, USA), and 1 μg total RNA was reverse transcribed into cDNA using the PrimeScript™ RT Kit (#RR036B, Takara, Japan). Sangon Biotech (Shanghai, China) provided the primers targeting YBX1 and CDCA8, which are presented in Table 1. The qPCR process was performed using the ABI ViiA7 system (Carlsbad, CA, USA). The relative mRNA level was quantified by the $2^{-\Delta\Delta Ct}$ method, with GAPDH serving as a reference gene.

Western blotting (WB). The protein expression from tissue and cells was evaluated by WB. Briefly, proteins were extracted using radioimmunoprecipitation assay lysis

buffer (Sigma, St. Louis, MO, USA). Protein concentration was determined using a BCA assay kit (Beyotime Biotechnology, Shanghai, China). Approximately 20 μg protein was resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then electrophoretically transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were then blocked with 5% bovine serum albumin diluted in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h at room temperature. Membranes were incubated with primary antibody targeting to YBX1 (1:1,000; #ab76149, Abcam), CDCA8 (1:1,000; #LS-C168603, LifeSpan BioSciences, WA, USA), SOX2 (1:1,000; #ab92494, Abcam), NANOG (1:2,000; #ab109250, Abcam), E-cad (1:5,000; #ab40772, Abcam) and vimentin (1:1,000; #ab92547, Abcam) overnight at 4 °C. After three washes in TBST (10 min each), the membranes were incubated with goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5,000; #ab205718, Abcam) for 1 h at room temperature. After three additional TBST washes, protein bands were visualized using an enhanced chemiluminescence (ECL) detection kit (Yeasen, Shanghai, China).

Colony formation experiment and sphere-formation assay. To assess cell stemness, colony formation and sphere-formation assays were conducted. For the colony formation experiment, cells were dissociated into single-cell suspensions and plated sparsely in 6-well plates with 2.5 ml of complete medium. The cells were cultured for seven days to allow colony development. The colonies were then fixed with 4% paraformaldehyde, stained with crystal violet dye solution, and counted under a microscope. For the sphere-formation assay, cells were plated in ultra-low attachment plates and cultured with serum-free medium supplemented. After one week, the size of the spheres was counted.

Transwell assay. Cell migration and invasion abilities were evaluated using a Transwell assay. The two experiments followed identical protocols, except that the invasion assay required pre-coating the chamber with Matrigel (BD Biosciences). The top chamber was seeded with 1×10^5 cells/well and supplied with 200 μl serum-free medium, while the lower chamber contained 0.7 ml complete medium as a chemoattractant. After 48 h of incubation, the cells on the lower surface were fixed with 4% paraformaldehyde, stained with crystal violet dye solution, and quantified under a microscope in five random fields.

RNA immunoprecipitation (RIP) and RNA pulldown assay. The binding protein of CDCA8 mRNA was predicted by RIPSeq (http://pridb.gdcb.iastate.edu/RPISeq/), PBPDB (https://rbpdb.ccbr.utoronto.ca/), and the starBase (https://rnasysu.com/encori/) database. Then, the interaction between the YBX1 protein and the CDCA8 mRNA was validated using RIP and RNA pulldown assays. For RIP assay, cells were crosslinked with 1% formaldehyde for 10 min at room temperature, quenched with 125 mM glycine, then lysed in RIP buffer for 30 min at 4 °C. Cleared lysates were incubated overnight at 4 °C with 5 μg anti-YBX1 antibody (1/30; #ab76149, Abcam) or IgG control, and the antibody complexes were captured using protein A/G magnetic beads for 2 h at 4 °C. The co-precipitated RNAs were isolated, purified, and quantified via qPCR to detect CDCA8 mRNA enrichment. For the RNA pulldown assay, a biotinylated CDCA8 mRNA probe (AAGAAGAACCCUGUGUUCUCAGGAAGACUGCCUCCACCACCGCUACCCAGAGAACCUCUGCAUCUGGC) was synthesized and incubated with cell lysates for 2 h at 4 °C. The protein-mRNA complexes were pulled down using streptavidin beads, and the bound proteins were eluted in buffer at 95 °C for 10 min. Finally, the YBX1 protein level was detected by WB.

Table 1. Primer pairs used for qPCR analysis.

Gene ID	Sequence (5'–3')
GAPDH-F	TGTTGCCATCAATGACCCCTT
GAPDH-R	CTCCACGACGTACTCAGCG
YBX1-F	TCCCAAAGTGCTGGGATTAC
YBX1-R	ACTCCCGACCCCTACTGTCT
CDCA8-F	CTCCTCGTCCCTACCCAGTT
CDCA8-R	GCCTCCGTAAGGAGTTGGTC

Abbreviations: F-forward primer; R-reverse primer

Xenograft model. To evaluate the gene function *in vivo*, a xenograft model was established. Thirty male BALB/c nude mice (6 weeks old) were obtained from the Animal Center of the Chinese Academy of Sciences (Shanghai, China) and housed in a SPF-level room under a 12 h light/dark cycle with *ad libitum* access to food and water. Following a one-week adaptive feeding period, 1×10^7 A549 and NCI-H520 cells were subcutaneously injected into the forelimb axilla of mice. In the next 35 days, the tumor volume was monitored weekly using the formula of length × $(\text{width})^2/2$. The mice were then anesthetized with pentobarbital sodium and euthanized by cervical dislocation, and the tumors were collected and weighed. All animal procedures were approved by our institutional ethical committee and performed in accordance with the national guidelines for the care and use of laboratory animals.

Statistical analysis. All statistical analyses were performed and graphs generated using the GraphPad Prism software. The data are presented as mean ± standard deviation. Differences between groups were assessed using Student's t-test (for two-group comparisons) or one-way analysis of variance (for multi-group comparisons). A $p<0.05$ was considered statistically significant.

Results

CDCA8 was upregulated in NSCLC tissues compared with paracancerous tissues. CDCA8 expression and prognostic significance were analyzed using databases and clinical validation. GEPIA2 database revealed significantly elevated CDCA8 expression in LUAD and LUSC samples,

compared with normal lung tissue (Figure 1A). Besides, patients with lung cancer and high CDCA8 level had a poorer prognosis than those with low CDCA8 level, as exhibited by Kaplan–Meier Plotter database (Figure 1B). These findings were validated in our clinical cohort. qPCR experiment revealed that the mRNA level of CDCA8 was significantly elevated in NSCLC tissues, compared with paracancerous tissues (Figure 1C). Consistently, WB and IHC assays confirmed CDCA8 protein upregulation in NSCLC tissues relative to paracancerous tissues (Figures 1D, 1E). These experiments implied the potential pro-tumor role of CDCA8.

CDCA8 overexpression enhanced the cell stemness of NSCLC cell lines. To isolate stem-like NSCLC cells, A549 and NCI-H520 cell lines were sorted for CD44-positive populations using fluorescence-activated cell sorting. After sorting, the cell population exhibited a high percentage of CD44-positive cells (Figure 2A). These cells also exhibited elevated stemness markers (SOX2 and NANOG) expression at the protein level, as confirmed by WB (Figure 2B). To study the role of CDCA8, gain and loss-of-function models were generated via plasmid transfection. qPCR and WB experiments revealed that the mRNA and protein levels of CDCA8 were enhanced in the CDCA8 overexpressing group compared with the vector group; however, their levels were inhibited in the CDCA8 knockdown group compared with the sh-NC group (Figures 2C, 2D). These results confirmed the successful transfection. The expression of stemness biomarkers (SOX2 and NANOG) was increased by CDCA8 overexpression transfection and decreased by sh-CDCA8 transfection (Figure 2D). Moreover, CDCA8-overexpressing cells formed more colonies and larger spheres, whereas CDCA8-inhibited cells revealed reduced clonogenicity and spheroid size (Figures 2E, 2F). Besides, the Transwell assay indicated that the cell migration and invasion abilities were enhanced in the CDCA8 group; however, suppressed in the sh-CDCA8 group (Figure 2G). These experiments suggested that CDCA8 overexpression enhanced the cell stemness of NSCLC cell lines, while CDCA8 knockdown had an opposite effect.

YBX1 stabilized CDCA8 mRNA and was upregulated in NSCLC tissues. After exploring the CDCA8 role, its upstream regulator was further studied. YBX1, a well-characterized post-transcriptional regulator, was predicted as a binding protein of CDCA8 mRNA by RIPSeq, PBPDB, and the starBase database. Moreover, the GEPIA2 database exhibited that YBX1 and CDCA8 expression levels were significantly positively correlated (Figure 3A). Kaplan-Meier analysis revealed that patients with lung cancer and high YBX1 expression exhibited a poorer prognosis, suggesting a potential oncogenic role (Figure 3B). To validate the binding between CDCA8 mRNA and YXB1 protein, RIP and RNA pulldown assays were performed. RIP results disclosed that the YBX1 antibody enriched significantly more CDCA8 mRNA sequence compared with the IgG control (Figure 3C). Conversely, RNA pulldown assays confirmed that the CDCA8 mRNA probe also bound more YBX1 protein, with minimal interaction observed using the mutated probe (Figure 3D).

Furthermore, YBX1 expression was evaluated in our clinical NSCLC cohort. As revealed by qPCR, the mRNA level of YBX1 increased in NSCLC tissues compared to paracancerous tissues (Figure 3E). Consistently, WB and IHC assays revealed its upregulation in protein level in NSCLC tissue

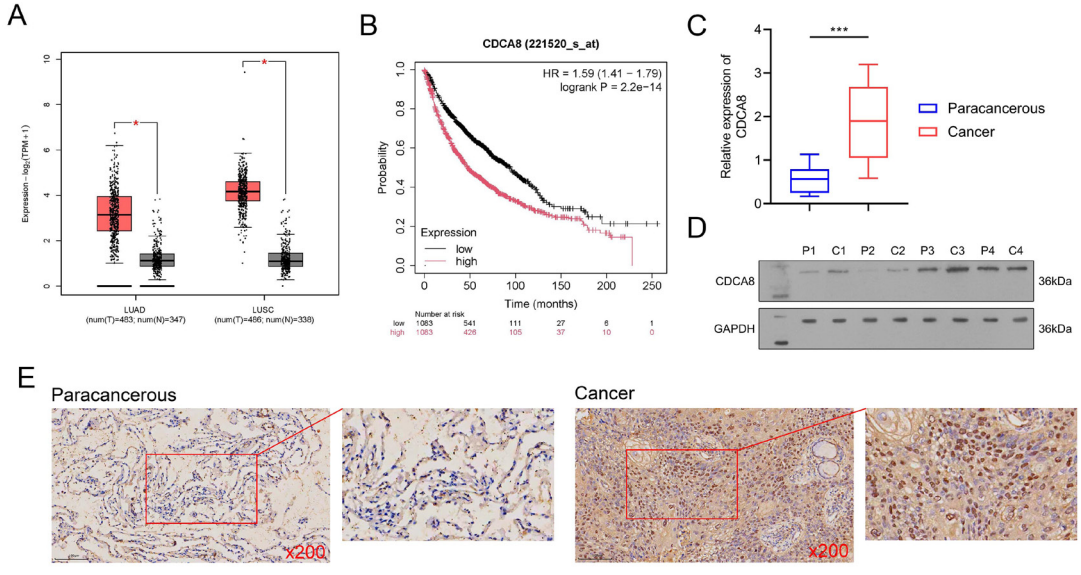

Figure 1. CDCA8 is upregulated in NSCLC tissues compared with paracancerous tissues. A) Analysis of CDCA8 mRNA expression levels in LUAD and LUSC tissues using the GEPIA2 database. B) Prognostic significance of CDCA8 expression in patients with lung cancer, as determined using the Kaplan-Meier Plotter database. Validation of CDCA8 expression in our collected sample detected by qPCR (C), WB (D), and IHC (E). ***p<0.001

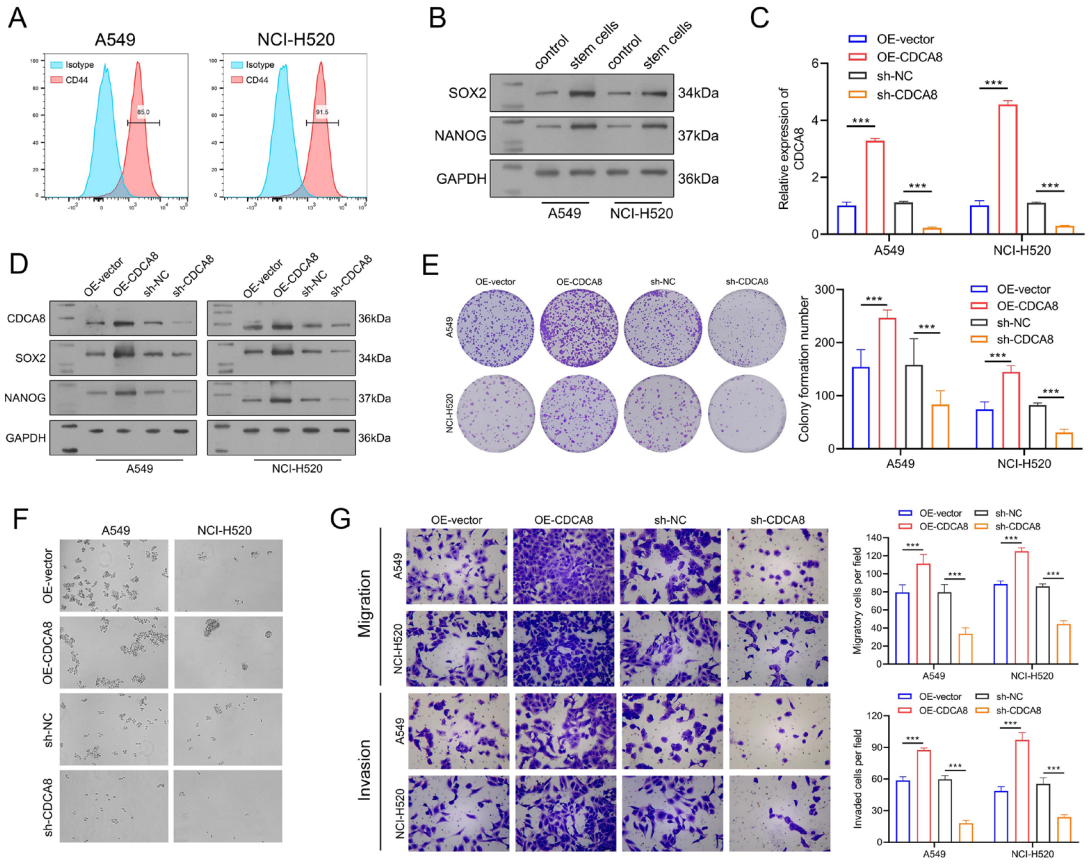

Figure 2. CDCA8 overexpression enhances cell stemness of NSCLC cell lines. A) CD44+ stem cells were isolated using fluorescence-activated cell sorting. B) Expression of stemness markers detected by WB. C) A qPCR assay was used to confirm the transfection efficiency. D) WB experiment was used to detect the protein expression of CDCA8 and stem cell biomarkers. E–F) Colony formation and sphere-formation assay were performed to evaluate stemness properties. G) Cell migration and invasion ability were analyzed by the Transwell assay. ***p<0.001

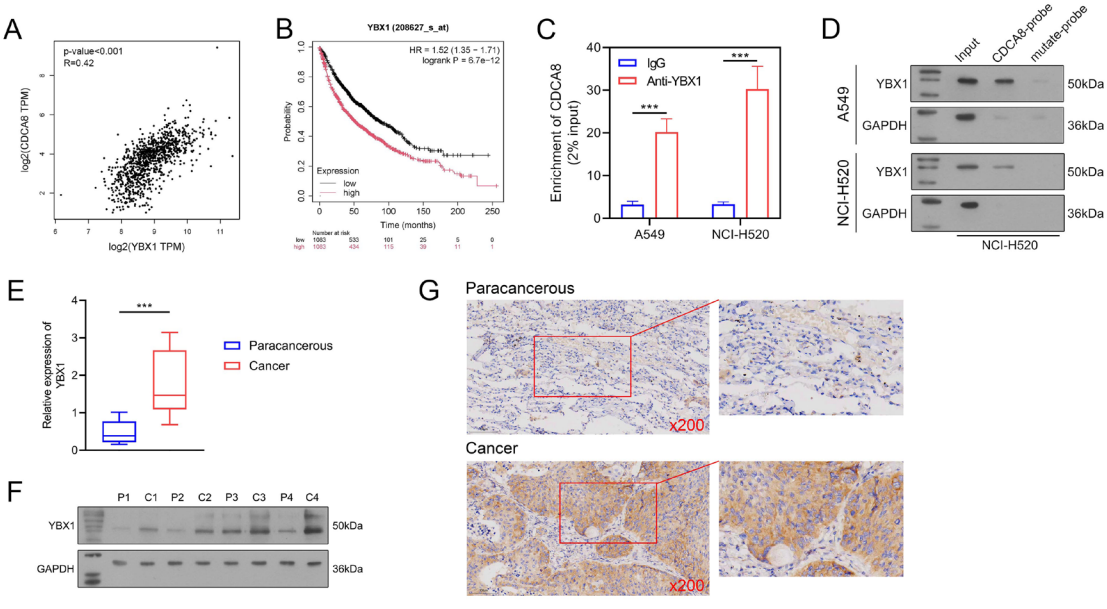

Figure 3. YBX1 stabilizes CDCA8 mRNA and is upregulated in NSCLC tissues. A) Correlation between YBX1 and CDCA8 expression in lung cancer exhibited by the GEPIA2 database. B) Prognostic significance of YBX1 expression in patients with lung cancer, as determined by the Kaplan-Meier Plotter database. RIP (C) and RNA pulldown assay (D) were used to validate the interaction between CDCA8 mRNA and YBX1 protein. Validation of YBX1 expression in our collected sample detected by qPCR (E), WB (F), and IHC (G). ***p<0.001

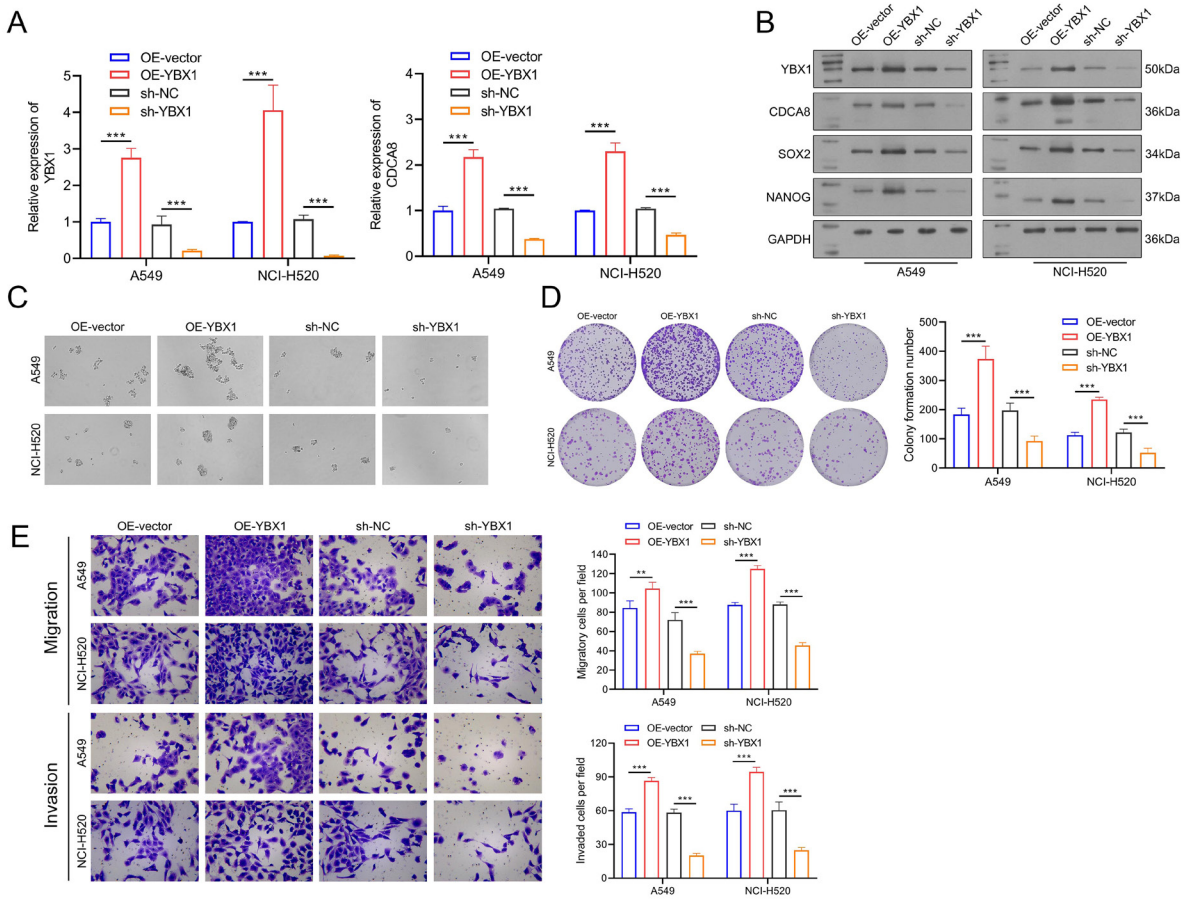

Figure 4. YBX1 increases CDCA8 expression and enhances NSCLC stemness. A) A qPCR assay was used to detect YBX1 and CDCA8 levels. B) WB was used to detect the protein expression of YBX1, CDCA8, and stem cell biomarkers. C–D) Colony formation and sphere-formation assay were performed to evaluate stemness properties. E) Cell migration and invasion ability were analyzed by the Transwell assay. ***p<0.001

relative to paracancerous tissues (Figures 3F, 3G). The above assays suggested that YBX1 interacted with CDAC8 mRNA and served as an oncogene.

YBX1 increased CDCA8 expression and enhanced NSCLC stemness. To further validate the YBX1 function in NSCLC stemness, YBX1-overexpressing and knockdown cell lines were then established. As exhibited by qPCR and WB experiments, the YBX1 level and the CDCA8 level were elevated by YBX1 overexpression plasmid transfection, while inhibited by YBX1 shRNA plasmid transfection (Figures 4A, 4B). Consistent with CDCA8's role in stemness, YBX1 overexpression enhanced the expression of stem cell markers (SOX2 and NANOG); however, YBX1 inhibition reduced their levels (Figure 4B). Functionally, the sphere formation size and cell colony number were increased in YBX1-overexpressing cells, while decreased in YBX1-suppressing cells (Figures 4C, 4D). Cell migration and invasion abilities were also promoted in the YBX1 overexpressing group, while inhibited in the sh-YBX1 group (Figure 4E). These experiments confirmed the YBX1 role in NSCLC stemness, which might function by regulating CDCA8.

YBX1 enhanced NSCLC cell stemness by promoting CDCA8 expression. To confirm the YBX1/CDCA8 axis in NSCLC cell stemness, the following recovery assay was conducted by co-transfecting a YBX1 overexpression plasmid and sh-CDCA8. qPCR and WB experiments identified YXB1 and CDCA8 upregulation in the YBX1 overexpression plasmid-transfected group; however, the CDCA8 level was downregulated by CDCA8 shRNA transfection (Figures 5A, 5B). Furthermore, YBX1 overexpression increased the expression of the stemness biomarkers SOX2 and NANOG, which were reversed by sh-CDCA8 co-transfection (Figure 5B). The colony numbers and sphere size were enhanced by YBX1 overexpression, which were attenuated by sh-CDCA8 co-transfection (Figures 5C, 5D). Additionally, the promoted cell migration and invasion ability in the YBX1 group was recovered in the YBX1+sh-CDCA8 group (Figure 5E). These results suggest that YBX1 enhances NSCLC cell stemness by regulating CDCA8 levels.

YBX1/CDCA8 axis promoted tumorigenesis in xenograft models. To assess the role of YBX1/CDCA8 *in vivo*, NSCLC cells co-transfected with YBX1 and sh-CDCA8

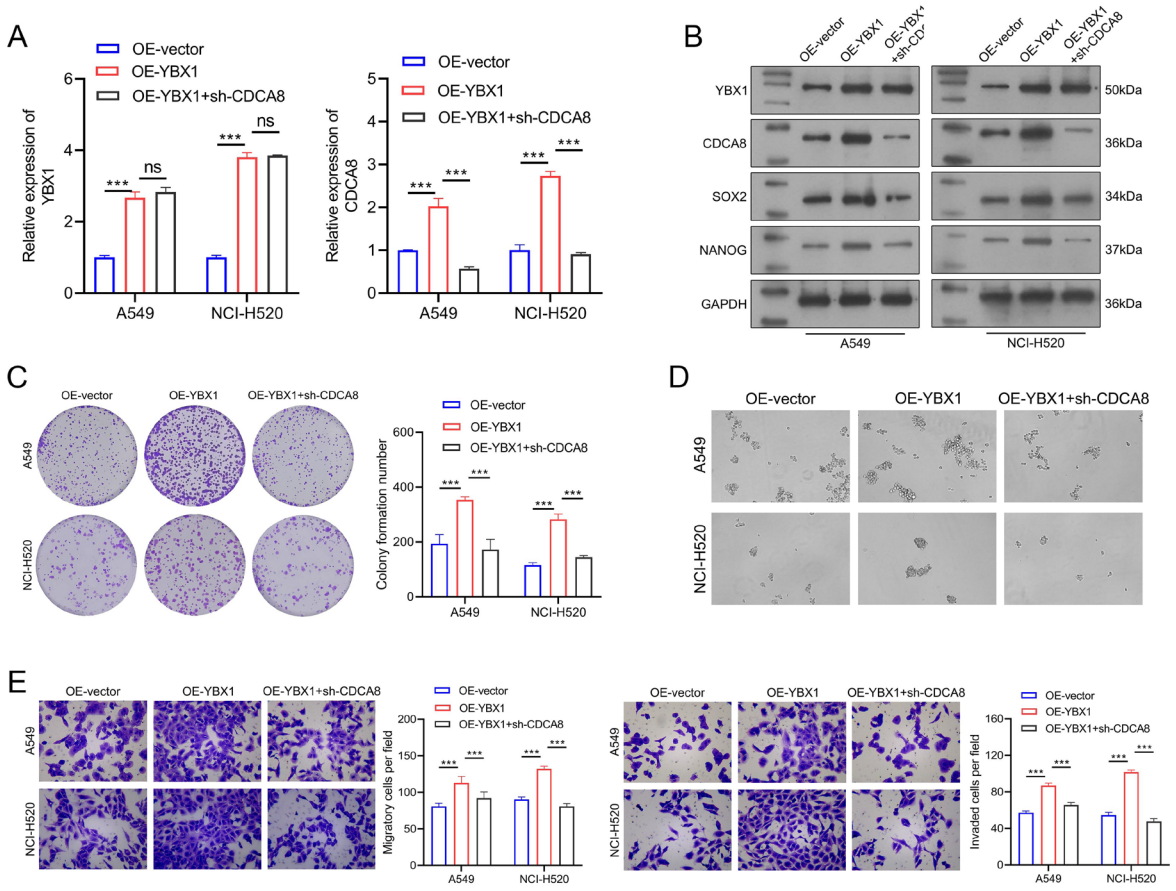

Figure 5. YBX1 enhances NSCLC cell stemness by promoting CDCA8 expression. A) A qPCR assay was used to detect YBX1 and CDCA8 levels. B) WB experiment was used to detect the protein expression of YBX1, CDCA8, and stem cell biomarkers. C–D) Colony formation and sphere-formation assay were performed to evaluate stemness properties. E) Cell migration and invasion ability were analyzed by the Transwell assay. ***p<0.001; Abbreviation: ns-no significant difference

plasmids were used to establish a xenograft model. As demonstrated in Figures 6A–6C, the tumor size, volume, and weight increased in the YBX1 group and decreased in the YBX1+sh-CDCA8 group. Moreover, the mRNA and protein levels of YBX1 and CDCA8 were enhanced by YBX1 overexpression, whereas the CDCA8 level was reversed by shCDCA8 co-inhibition, which was consistent with the *in vitro* assay (Figures 6D, 6E). The biomarkers of cell stemness (SOX2 and NANOG) and mesenchymal biomarker vimentin were promoted, while epithelial biomarker E-cad was inhibited by YBX1 overexpression, which was attenuated by sh-CDCA8 (Figures 6E, 6F). These experiments further validated the pro-tumor function of YBX1/CDCA8 *in vivo* studies.

Discussion

Cancer cell stemness poses a significant challenge in cancer treatment due to its contribution to cancer recurrence, therapeutic resistance, and metastasis. Therefore, targeting and inhibiting stemness is a promising therapeutic strategy. A deeper exploration of the mechanisms underlying the maintenance of CSCs may help us find a way to eradicate this cancer cell population. In this study, we investigated the function of CDCA8 and its upstream regulator, YBX1, in regulating the stemness of NSCLC cells.

CDCA8 has been implicated in promoting tumor progression in various cancers by enhancing cell proliferation, inducing metastasis, and augmenting cell stemness [16]. A bioinformatic study reveals CDCA8 as an important cancer stem cell biomarker in bladder cancer, with significantly elevated expression in stem-like subtypes compared to normal subtypes [23]. Consistent findings across multiple studies have further positioned CDCA8 as a stemness-related risk gene in lower-grade glioma [24], hepatocellular carcinoma [25], and lung adenocarcinoma [26]. However, most research remains limited to bioinformatic predictions, lacking experimental validation. In our study, we identified that CDCA8 overexpression enhanced cell stemness in NSCLC, representing elevated stemness biomarker expres-

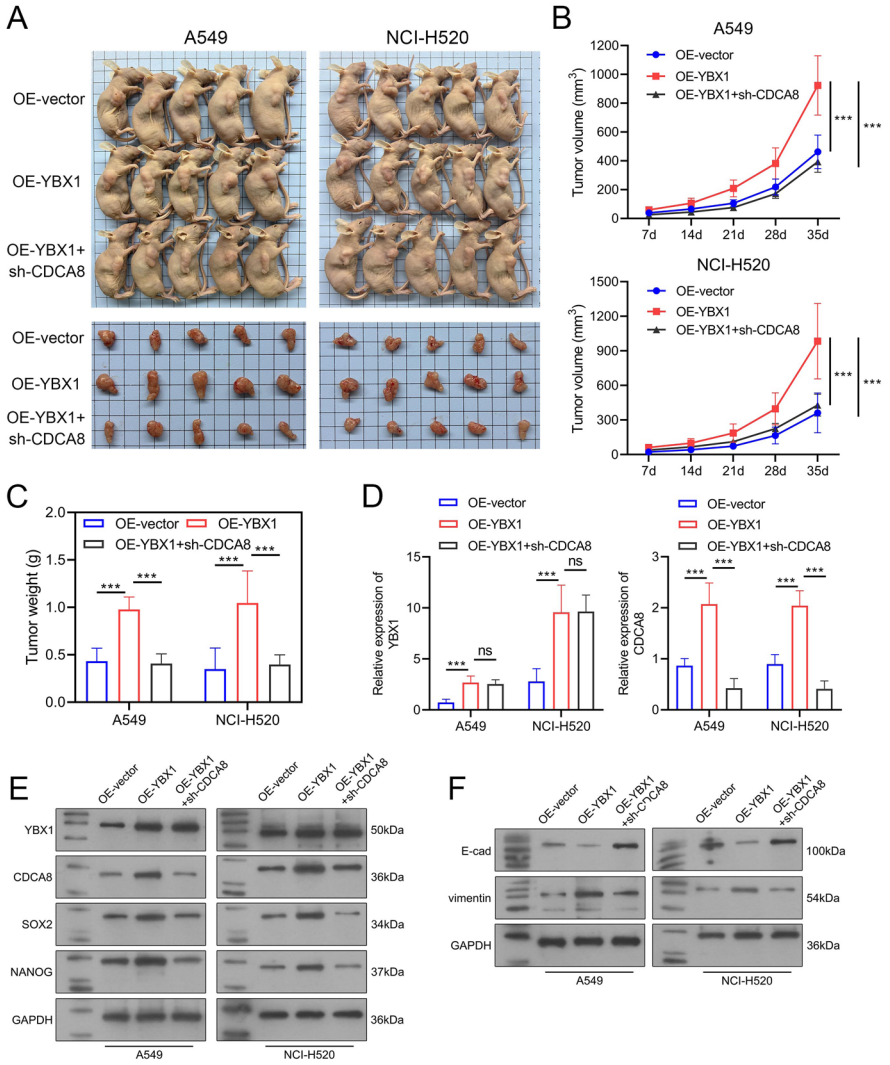

Figure 6. YBX1/CDCA8 axis promotes tumorigenesis in xenograft models. A–C) Tumor size, volume, and weight in xenograft models. D) A qPCR assay was used to detect YBX1 and CDCA8 levels. E) WB experiment was used to detect the protein expression of YBX1, CDCA8, and stem cell biomarkers. F) WB experiment was performed to detect the protein expression of E-cad and vimentin. ***p<0.001; Abbreviation: ns-no significant difference

sion, enhanced colony formation, and sphere formation, while CDCA8 knockdown had an opposite effect. Consistent with our study, Jeon et al. demonstrate that CDCA8 knockdown inhibits cancer progression and cell stemness by inactivating the AKT/β-catenin pathway in hepatocellular carcinoma [17]. As a characteristic of cancer stem cells, cell migration and invasion abilities were also identified to have a consistent trend with cancer cell stemness affected by CDCA8. Prior studies have documented CDCA8's role in promoting lung cancer cell proliferation, migration, and invasion [18, 19]. Our study provides the first experimental evidence for its stemness-enhancing function in NSCLC.

Furthermore, we explored the upstream regulatory mechanism of CDCA8 and identified YBX1 as a key regulator that enhanced CDCA8 mRNA stability. YBX1, a member of the cold-shock domain protein family, has been implicated in driving tumorigenesis across multiple cancer types, including pancreatic [27], lung [28], prostate [29], and ovarian cancers [30]. Its pro-tumorigenic role is attributed to its interactions with DNA and RNA, thereby modulating critical processes such as cell proliferation, apoptosis resistance, genome instability, and metastatic potential [31]. Regulation of the stability of binding mRNA represents a key functional mechanism of YBX1. For instance, in breast cancer, YBX1 interacts with HGH1 mRNA and enhances its stability, contributing to cancer progression [32]. Similarly, YBX1 stabilizes PFKFB4 mRNA to facilitate LUSC development [28]. Accumulating evidence also highlights YBX1's role in promoting cancer stem cell properties. The study of Shi et al. reveals that YBX1 drives stemness and cisplatin resistance in cholangiocarcinoma [33]. Xie et al. identified its involvement in LUAD metastasis and stemness forma-

tion [34]. Consistent with these findings, our research revealed that YBX1 overexpression enhanced stemness in NSCLC cells. Importantly, we identified CDCA8 mRNA as a novel binding target of YBX1, providing a direct mechanistic link between YBX1-mediated mRNA stabilization and CDCA8-driven stemness enhancement.

In conclusion, our study demonstrates that YBX1 binds to and stabilizes CDCA8 mRNA, thereby enhancing stemness in NSCLC cells. These findings reveal a novel mechanism underlying NSCLC cell stemness and identify CDCA8/YBX1 as a promising therapeutic target for NSCLC treatment.

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