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Jab1/Cops5 contributes to chemoresistance in breast cancer by regulating Rad51

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ABSTRACT

Jab1 overexpression correlates with poor prognosis in breast cancer patients, suggestting that targeting the aberrant Jab1 signaling in breast cancer could be a promising strategy. In the current study, we investigate the hypothesis that Jab1 positively regulates the DNA repair protein Rad51 and, in turn, the cellular response of breast cancer to chemotherapy with adriamycin and cisplatin. High-throughput mRNA sequencing (RNA-Seq) data from 113 normal and 1109 tumor tissues (obtained from TCGA) were integrated to our analysis to give further support to our findings. We found that Jab1 was overexpressed in adriamycin-resistant breast cancer cell MCF-7R compared with parental MCF-7 cells, and that knockdown of Jab1 expression conferred cellular sensitivity to adriamycin and cisplatin both *in vivo* and *in vitro*. By contrast, exogenous Jab1 expression enhanced the resistance of breast cancer cells to adriamycin and cisplatin. Moreover, we discovered that Jab1 positively regulated Rad51 in p53-dependent manner and that overexpression of Rad51 conferred cellular resistance to adriamycin and cisplatin in Jab1-deficient cells. Data from TCGA further validated an correlation between Jab1 and Rad51 in breast cancer, and elevated Jab1 and Rad51 associated with poor survival in breast cancer patients. Our findings indicate that Jab1 association with Rad51 plays an important role in cellular response to chemotherapy in breast cancer.

1. Introduction

Breast cancer is one of the most commonly occurring female malignancies in women worldwide. Despite chemotherapy have improved breast cancer survival rates [1], chemoresistance is a major obstacle for the effective treatment in breast cancer. Multiple mechanisms have been proposed in chemoresistance, including increased drug efflux [2], altered enzymatic activity of glutathione transferase, and increased expression of anti-apoptotic proteins [3]. Most chemotherapeutic agents cause cell death *via* induction of DNA damage. Anthracycline antibiotics such as adriamycin induce DNA damage through embedding between the DNA double-stranded bases. In addition, platinum drugs such as cisplatin forming DNA adducts to produce inter-strand and intrastrand DNA crosslinks to induce DNA damage [4]. Therefore, increased DNA damage repair (DDR) is an important route to enhance tumor cells resistance to chemotherapy [5,6].

Rad51 involves in DNA repair by forming nucleoprotein filaments and mediating strand exchange between DNA duplexes [7,8]. Aberrantly expressed Rad51 have been reported in numerous transformed cells which may promote malignant transformation [9]. In addition, elevated Rad51 increases spontaneous recombination frequency and enhances resistance to cancer therapies [10,11]. Suppressing Rad51 induces sensitivity of cancer cells to radiotherapy and chemotherapy [10,11]. It was reported that RECK inhibited the Her2 signaling and attenuated the expression of Jab1 and Rad51 to impede DNA repair in SKBR3 breast cancer cells. Ectopic expression of Jab1 counteracted RECK-induced Rad51 reduction [12]. However, the direct linkage between Jab1 and Rad51, and their role in chemotherapy response need to be elicited.

Jab1, also known as COPS5 or CSN5, regulates several signaling

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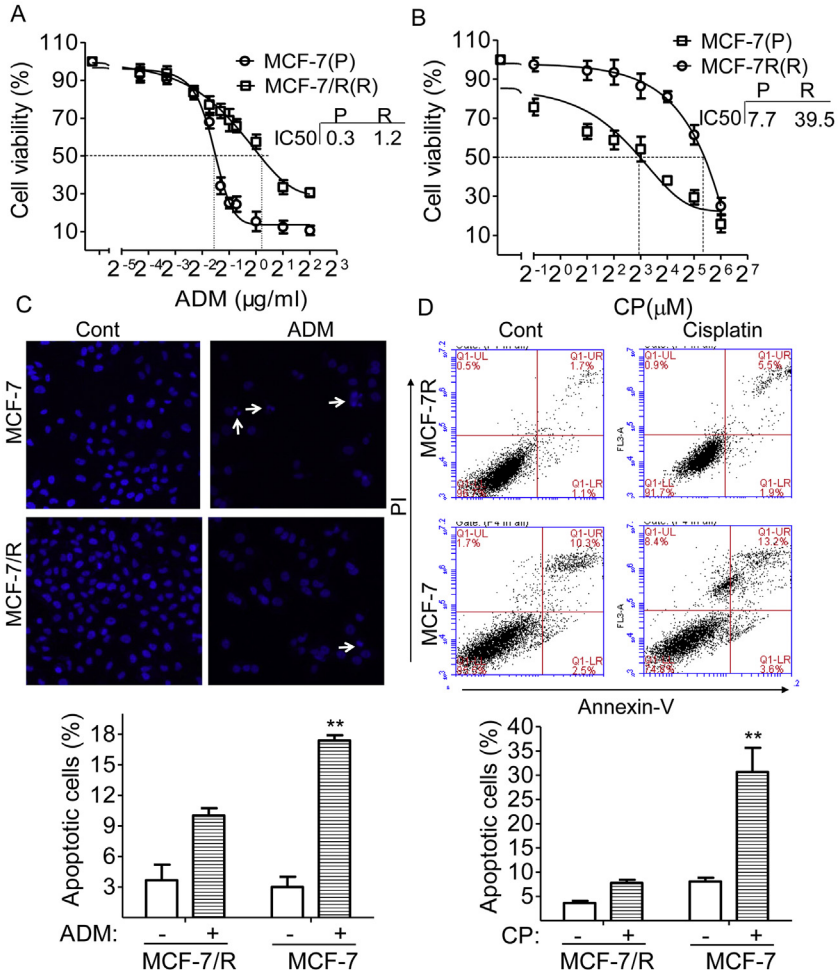

Fig. 1. Characterization of the adriamycin-resistant MCF-7 and derivative subline MCF-7R cells. (A, B) Cell viability assay. MCF-7R and its parental MCF-7 cells were treated with various doses of adriamycin (ADM) (A) or cisplatin (CP) (B) for 48 h. Cell viability was measured by MTT assay. (C) Apoptosis was measured by Hoechst 33342 staining. (Top) MCF-7R and its parental MCF-7 cells were treated with 1 μg/ml ADM for 48 h, nuclei were stained with Hoechst 33342, and imaging analysis was performed as described in the Methods and Materials section. The white arrows indicate apoptotic cells. Original magnification, ×200. (Bottom) Quantification of the stained cells. (D) Measurement of apoptosis by Annexin V/propidium iodide (PI) staining. (Top) MCF-7R and its parental MCF-7 cells were treated with 10 μM CP for 48 h, followed by Annexin V/PI staining as described in the Methods and Materials section. (Bottom) Quantification of PI staining. All data represent three independent experiments, mean ± SD**P < .01.

factors by influencing their subcellular localization, degradation, and deneddylation [13]. Jab1 inactivates several key tumor-related genes, such as p53, p27, and thioredoxin [14,15]. Increased Jab1 has been observed in various cancers and associates with poor survival [16–18]. Overexpression of Jab1 also leads to tamoxifen-resistance by degrading of NCoR in breast cancer [19]. Jab1 also suppresses the ubiquitination and degradation of programmed cell death-ligand 1 (PD-L1). Inhibition of Jab1 diminish cancer cell PD-L1 expression and sensitize cancer cells to anti-CTLA4 therapy [20]. It was recently reported that CSN5i-3, a Jab1/CSN5 inhibitor, differentially decreased the viability of tumor cells and delayed growth of human xenograft in mice [21]. Given the potential role of Jab1 in cancer, exploring the mechanism by which Jab1 regulates chemoresistance is very important.

In this study, we explored the role of Jab1 in drug resistance. Jab1 expression was found to be substantially upregulated in the adriamycin-resistant breast cancer cell line MCF-7R. Inhibiting Jab1 caused a marked reduction in Rad51 expression, leading to a reversal of chemotherapy resistance in MCF-7R cells. In animal models inoculated with MCF-7R, we suppressed Jab1 expression, which reversed chemotherapy resistance in the solid tumors that formed. *In vivo* data from TCGA further validated that Jab1 correlated with Rad51, and both are novel prognostic markers in breast cancer. These findings suggest a novel mechanism of Jab1 in chemoresistance and provide novel links with Rad51 in breast cancer.

2. Methods and materials

2.1. Reagents

Cell culture medium and fetal bovine serum (FBS) were obtained from Life Technologies (Carlsbad, CA). The antibodies used were Jab1 and Rad51 (Abcam, Hongkong, China); poly ADP ribose polymerase (PARP) (BD Pharmingen, San Jose, CA); p53, and Myc-tag and β-actin (Cell Signaling Technology, Beverly, MA). Lipofectamine 2000 reagents were purchased from Life Technologies. The Annexin V/propidium iodide (PI) kit and MTT assay kit were purchased from KeyGEN Company (Nanjing, China).

2.2. Cell cultures

Human breast cancer cell lines MCF-7 and adriamycin resistant MCF-7 (MCF-7/R) cells were purchased from KeyGEN Company (Nanjing, China). ZR-75-1 cells (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco's modified Eagle medium. Media were supplemented with 10% FBS and penicillin-streptomycin sulfate. All cell lines were incubated at 37 °C in an atmosphere of 5% carbon dioxide.

2.3. DNA and siRNA transfection

The MYC-Jab1 plasmid has been previously described [22] and transfected with the Lipofectamine 2000 reagent. The negative control gene and siRNA targeting the human Jab1 (Jab1 siRNA#1: forward: 5′-CUACAAACCUCCUGAUGAADTDT-3′, reverse:

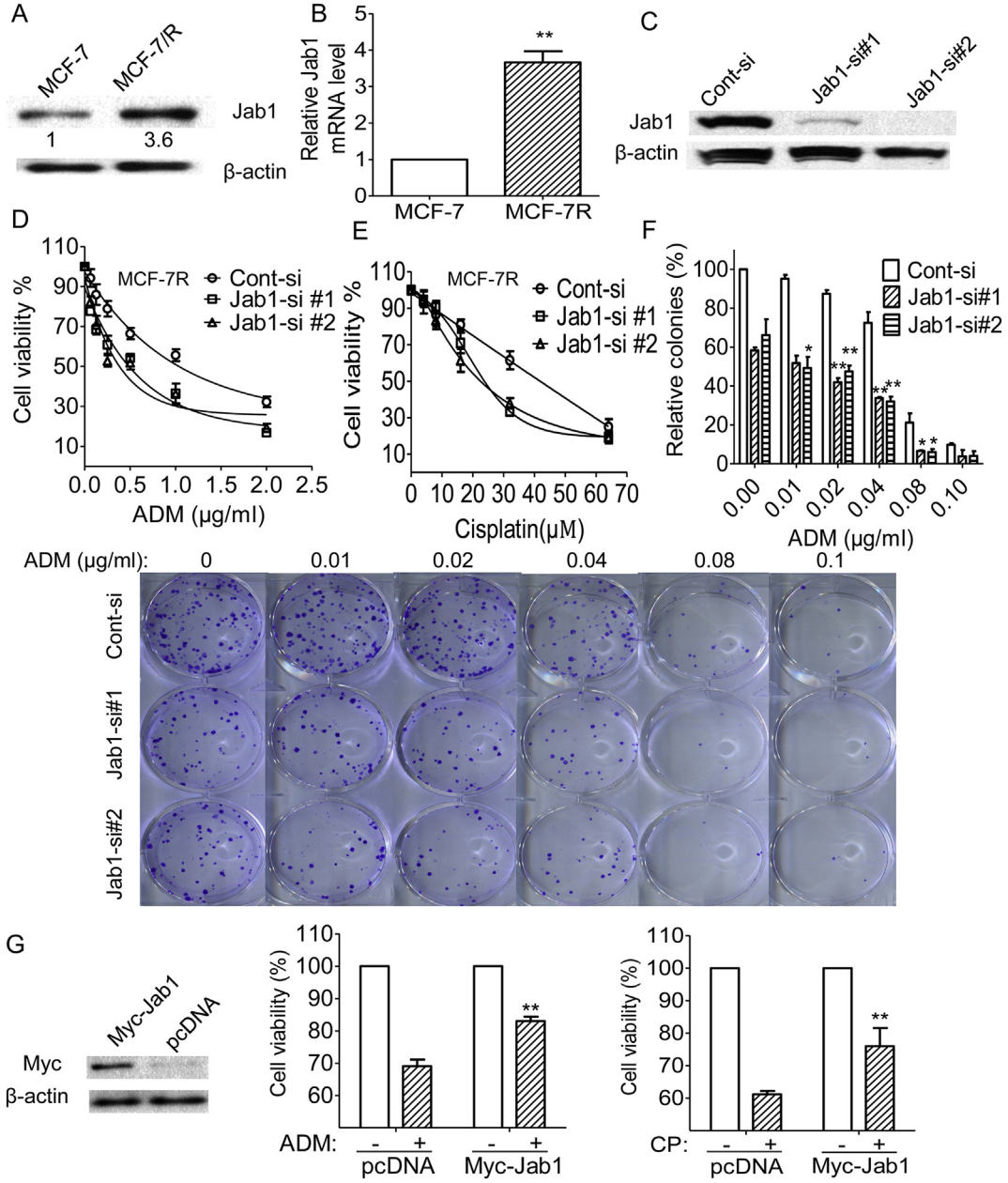

Fig. 2. Effect of Jab1 depletion on the sensitivity of breast cancer cells to adriamycin and cisplatin. (A, B) Jab1 expression in MCF-7R and MCF-7 cells. MCF-7 and MCF-7R cells in the logarithmic growth phase were collected and lysed, followed by Western blotting (A) or real-time polymerase chain reaction (PCR) (B) for Jab1. β-actin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. (C–F) Effect of knockdown of Jab1 on the sensitivity of MCF-7R cells to adriamycin and cisplatin. MCF-7R cells (C) were transiently transfected with Jab1 siRNA (Jab1-si) or scrambled control siRNA (Cont-si). Cells were then analyzed for their Jab1 expression level (C) and their cell viability response to adriamycin (ADM) (D) or cisplatin (CP), or clone formation (F) as described in the Methods and Materials section. (G) Effect of overexpression of Jab1 on the resistance of MCF-7 cells to adriamycin and cisplatin. (left) MCF-7 cells were transfected with ectopic Jab1 (Myc-Jab1) or a control vector (pcDNA). Cells were then analyzed for their Jab1 expression level (left) and response to adriamycin (0.3 μg/ml ADM) (middle) or cisplatin (2 μM CP) (right) as described in the Methods and Materials section. Data represent three independent experiments, mean ± SD $^{*}P < .05$, $^{**}P < .01$.

3′-DTDTGAUGUUUGGAGGA CUACUU-5′; Jab1 siRNA#2: forward: 5′-GGACU AAGGAUCACCAUUADTDT-3′, reverse: 3′-DTDTCCUGAUUCC-UAGUGGUAAU-5′) and p53 (p53 siRNA: forward: 5′-GCACAGAGGA-AGAGAAUCUDTDT-3′, reverse: 3′-DTDTCGUGU CUCCUUCUCUU AGA-5′) genes were purchased from RIOBOBIO (Guangzhou, China). Transient transfections of breast cancer cells were performed using the Lipofectamine (Life Technologies) protocol, as described previously [22].

2.4. Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to examine cell viability [17]. Briefly, cells were seeded in 96-well plates (5000 cells/well). MTT (0.5 mg/ml) was added after chemotherapy, and the absorbance was obtained using a microplate reader at 570 nm.

2.5. Colony formation assay

Colony formation assay was done as previously described [22].

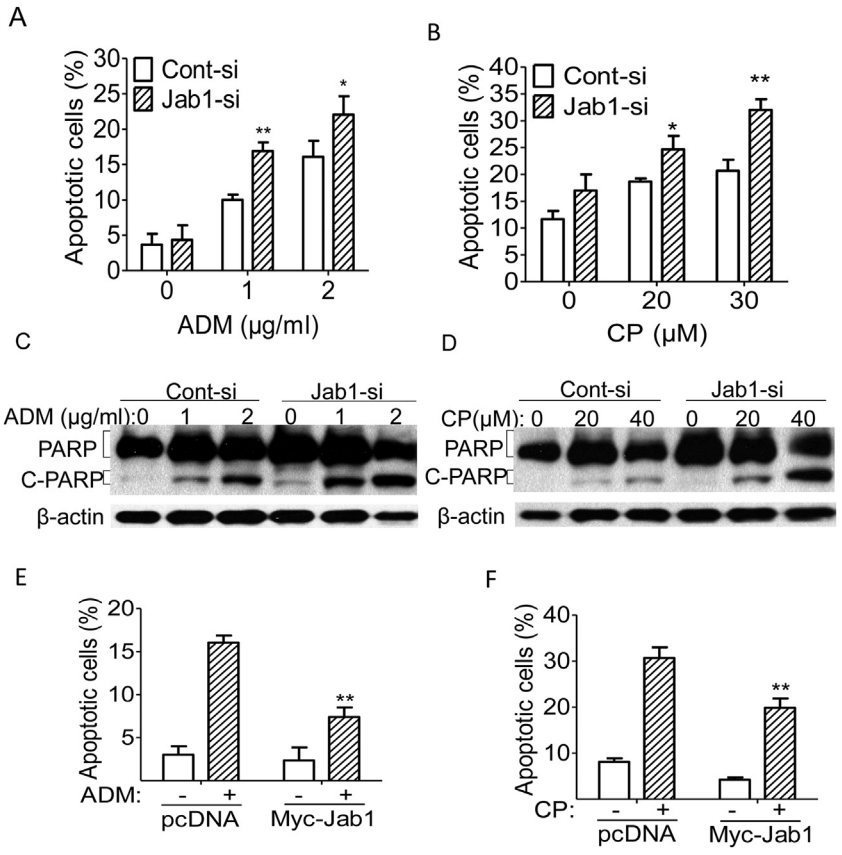

Fig. 3. Effect of Jab1 on adriamycin- and cisplatin-induced apoptosis. MCF-7R cells were transiently transfected with either Jab1 siRNA (Jab1-si) or scrambled control siRNA (Cont-si) and treated with adriamycin (ADM) or cisplatin (CP) at the indicated doses. Apoptosis was analyzed by Hoechst 33342 staining (A), Annexin V/propidium iodide (PI) staining (B) or detection of cleaved poly ADP ribose polymerase (PARP) by Western blotting (right) (C, D). MCF-7 cells were transfected with ectopic Myc-Jab1 plasmid and treated with adriamycin or cisplatin for 48 h. Apoptosis was analyzed by Hoechst 33342 staining (E) and Annexin V/PI staining (F). Data represent three independent experiments, mean ± SD $^{*}P < .05$, $^{**}P < .01$.

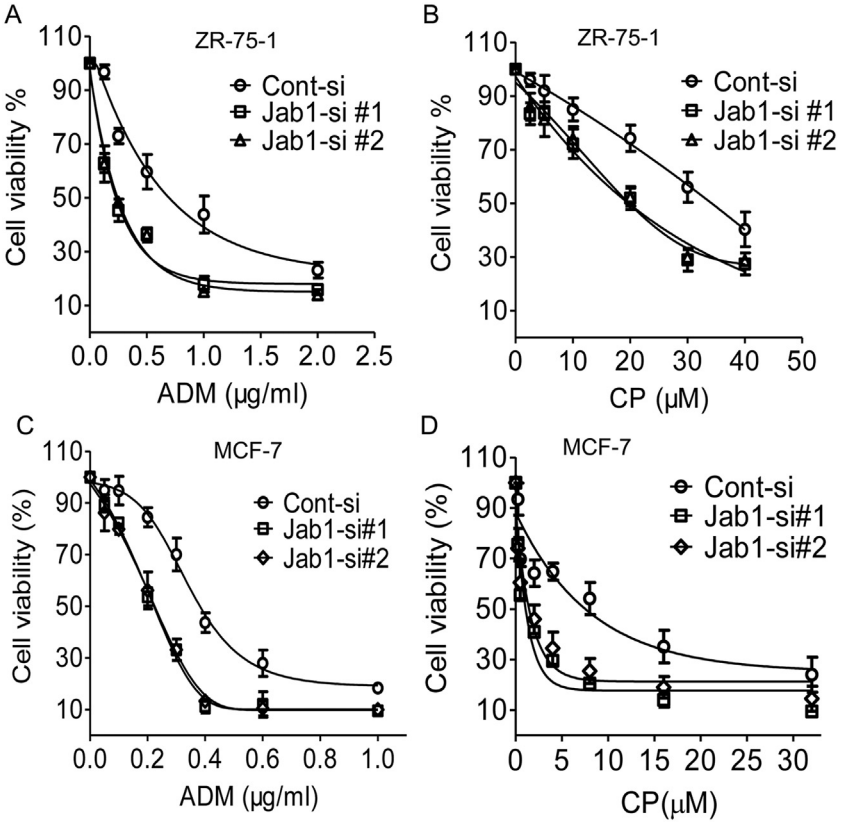

Fig. 4. Role of Jab1 in the response of breast cancer cells to adriamycin and cisplatin. ZR-75-1 and MCF-7 cells were transiently transfected with Jab1 siRNA (Jab1-si) or scrambled control siRNA (Cont-si), followed by MTT assay of cellular response to adriamycin (ADM) (A, B) and cisplatin (CP) (C, D).

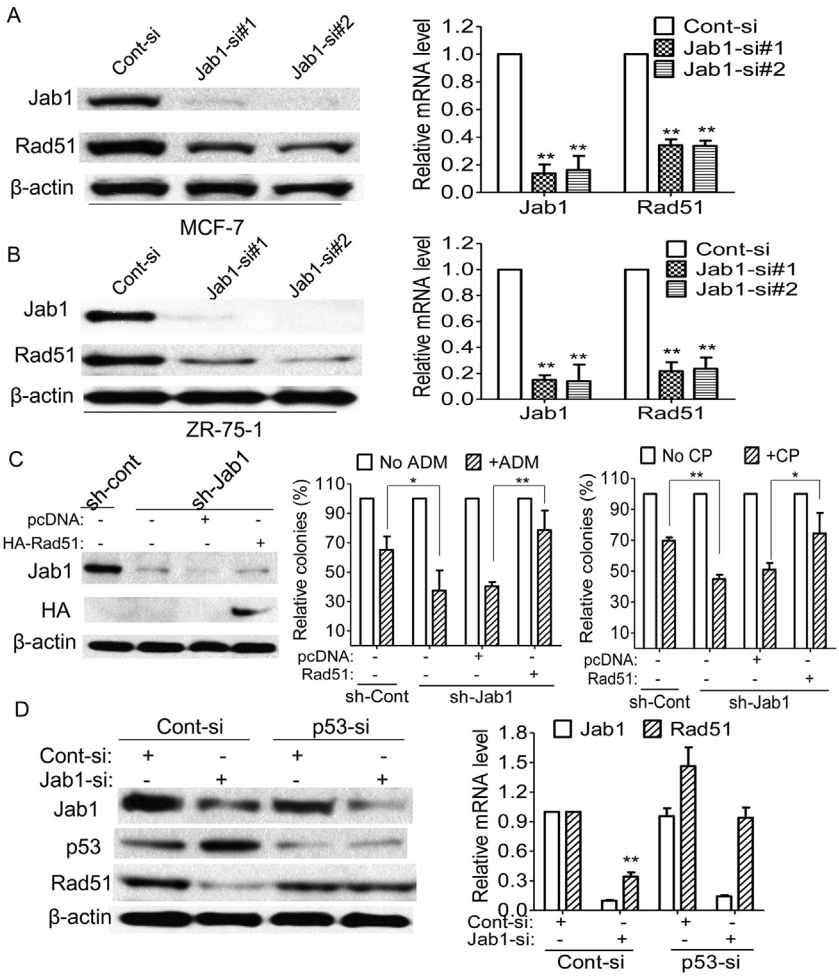

Fig. 5. Jab1 regulates Rad51 through p53 pathway. (A, B) MCF-7 (A) and ZR-75-1 (B) cells were transfected with Jab1 siRNA for 48 h. Jab1 and Rad51 protein (left) and RNA (right) levels were evaluated by Western blot or real-time polymerase chain reaction (PCR). (C) MCF-7R cells stably expressing sh-Jab1 were transfected with pcDNA or HA-Rad51 plasmid DNA and then exposed to adriamycin (ADM) or cisplatin (CP). Western blot analysis results demonstrated the effective knockdown of Jab1 and ectopic expression of Rad51 (left). Colonies were stained with crystal violet after ADM (middle) and CP exposure (right). (D) MCF-7R cells were transfected with siRNAs for 48 h, cells lysates were prepared, and the protein levels of Jab1, p53, and Rad51 were determined by Western blotting (left), or RNA levels were quantified by real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) (right). All data represent three independent experiments, mean ± SD $^{*}P < .05$, $^{**}P < .01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Briefly, cells (500 cells/well) were plated in 6-well plates and were treated with either adriamycin or cisplatin for 48 h. After 12 days, cells were stained with crystal violet, colonies consisted of 50 or more cells were counted.

2.6. Measurement of apoptosis

To detect apoptosis, we performed nuclear staining using 10 μg/ml Hoechst 33342 [22]. Apoptotic cells were identified by morphologic features and by the presence of condensed and fragmented nuclei.

Dual Annexin V and PI staining was also performed as described previously [16]. Briefly, cells were collected and suspended in binding buffer containing Annexin V and PI, and apoptotic cells were quantified by a flow cytometer.

2.7. Establishment of small hairpin RNA (shRNA) stable cells

We generated shRNA stable cell as previously described [17]. Briefly, Jab1 shRNA–vector DNA and the helper vectors pCGP and pVSVG293T were transfected into 293 T cells. The supernatant was collected 48 h after transfection, added with polybrene (1.2 μg/ml), and used to infect target cells. Stable clones were selected following treatment with puromycin (0.8 μg/ml) for 2 weeks. Positive clones were further confirmed by immunoblot and cultured with puromycin (0.2 μg/ml).

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

Total RNA was extracted using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. Reverse transcription was performed using transcriptase (Life Technologies), and real-time PCR was performed in a LightCycler 480 System (Roche) using a SYBR Premix Ex Taq kit (KAPA). Fold changes were calculated according to the supplier's protocol. The sequences of the quantitative real-time PCR primers are as follow: Jab1 forward: 5′-CCAGGAACCATTTGTAGCAG TGG-3′, Jab1 reverse: 5′-GTCTGGTACTCAGAAGGTCCTTC-3; Rad51 forward: 5′-TCTCTGGCAGTGAT GTCCTGGA-3′, 5′-TAAAGGGCGGTGG CACTGTCTA-3′.

2.9. Western blotting

Cells were collected and lysed as described previously [22]. Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and probed with anti-Jab1, anti-Rad51, anti-p53, anti-MYC, and anti-PARP. β-actin served as the internal control. Immunoreactive bands were examined using horseradish peroxidase–conjugated secondary antibodies. The protein levels were quantified using Image J software.

2.10. Tumorigenicity assay in nude mice

Four-week-old athymic nude (nu/nu) mice were bred and maintained under defined conditions at the Animal Experiment Center of Wuhan University, and all procedures were approved by the Animal

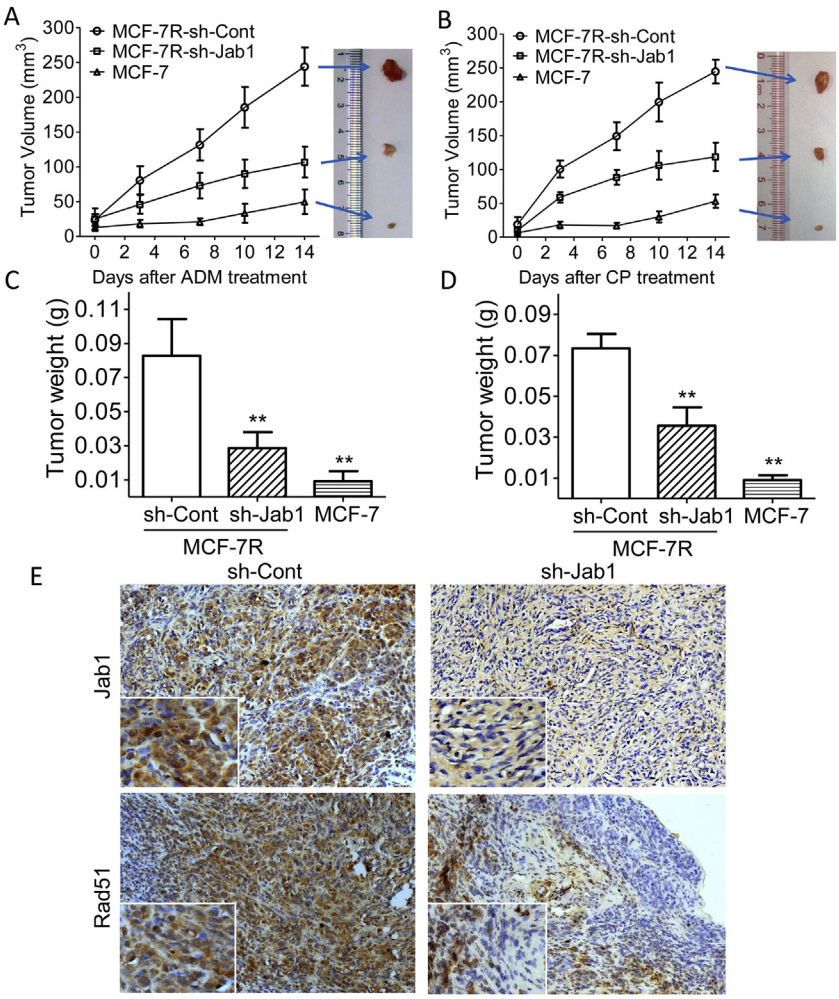

Fig. 6. Inhibiton of Jab1 reverses adriamycin and cisplatin resistance of human breast tumor xenografts in athymic nude mice. (A, B) Female nude mice bearing xenograft tumors derived from MCF-7R stably knockdown of Jab1 and control MCF-7R or parental MCF-7 were given intraperitoneal injections of adriamycin (3 mg/kg) or cisplatin (6.15 μmol/kg) every 3 d. And corresponding tumor growth curves (left) measured at indicated time points. (right) representative photographs of harvested tumors 14 d after adriamycin (ADM) and cisplatin (CP) treatment. (C, D) Tumor weight was measured at the end of the experiment. (E) Overall Jab1 and Rad51 immunoreactivity was low in tumor xenograft tissue derived from MCF-7R cells with stably knockdown Jab1 (sh-Jab1) compared to that derived from MCF-7R control cells (sh-Cont).*P < .05, **P < .01.

Care and Use Committee of Wuhan University University. MCF-7 and MCF-7/R (5×10^6) cells that stably knockdown of Jab1 (sh-Jab1) or control vector (sh-Cont) were inoculated into the mammary fat pads of the mice ($n = 4$/group). Mice were checked every 3 d for xenograft development. Treatment started when tumors became palpable (about 0.1 mm^3): adriamycin (3 mg/kg) or cisplatin (0.00615 mmol/kg) was administrated by intraperitoneal injection once every 3 d. Tumor growth was evaluated by monitoring tumor volume twice weekly. Tumor volume was calculated using the formula: tumor volume (mm^3) = $(A \times B^2)/2$, where A and B represent the tumor length and width (in mm), respectively. After 3 weeks, the mice were killed; the tumors were excised and weighed, and necropsy was done.

2.11. Immunohistochemical analyses

The Jab1/COPS5 levels in the FFPE tissue sections were detected by immunohistochemical analysis as described in our previous work [18]. Briefly, the specimens were sectioned and mounted on slides, and then deparaffinized in two xylene. The slides were boiled in sodium citrate for antigen retrieval. The slides were incubated with the primary antibody Jab1 or Rad51 overnight. The sections were incubated with secondary antibody and were then counterstained with hematoxylin.

2.12. TCGA dataset and the human protein Altas dataset

To validate the potential role of Jab1/COPS5 and Rad51 in breast cancer, we analyzed The Caner Genome Atlas database (TCGA dataset, http://cancergenome.nih.gov/) which provides > 1000 breast cancer cases including gene expression data and follow-up information. A total of 1109 cases of breast cancer patients were included in our study. Besides, 113 cases of non-tumor breast tissues were also extracted from TCGA dataset. The data was analyzed using R software. In addition, we extracted data from The Human Protein Atlas database (https://www.proteinatlas.org/) [23] to validate the Jab1/COPS5 and Rad51 protein levels in breast cancer tissue and normal breast tissue.

2.13. Statistical analysis

Differences between groups were compared using student's *t*-test or one-way analysis of variance by SPSS 22.0 statistics software (SPSS, Chicago, IL). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Adriamycin and cisplatin sensitivity/resistance patterns of MCF-7R cells

As adriamycin and cisplatin are the main treatments for breast cancer, we exposed MCF-7 and adriamycin-resistant MCF-7 (MCF-7R) cell lines to various doses of adriamycin. MCF-7R is approximately four times more resistant to adriamycin than the parental MCF-7 cells, with an IC_{50} of 0.3 μg/ml for MCF-7 cells *versus* 1.2 μg/ml for MCF-7R cells (Fig. 1A). As was the case with adriamycin, MCF-7R cells were approximately 5 times more resistant to cisplatin than were MCF-7 cells (Fig. 1B), indicating a multidrug resistance in MCF-7R cells.

To determine whether the adriamycin and cisplatin resistance of

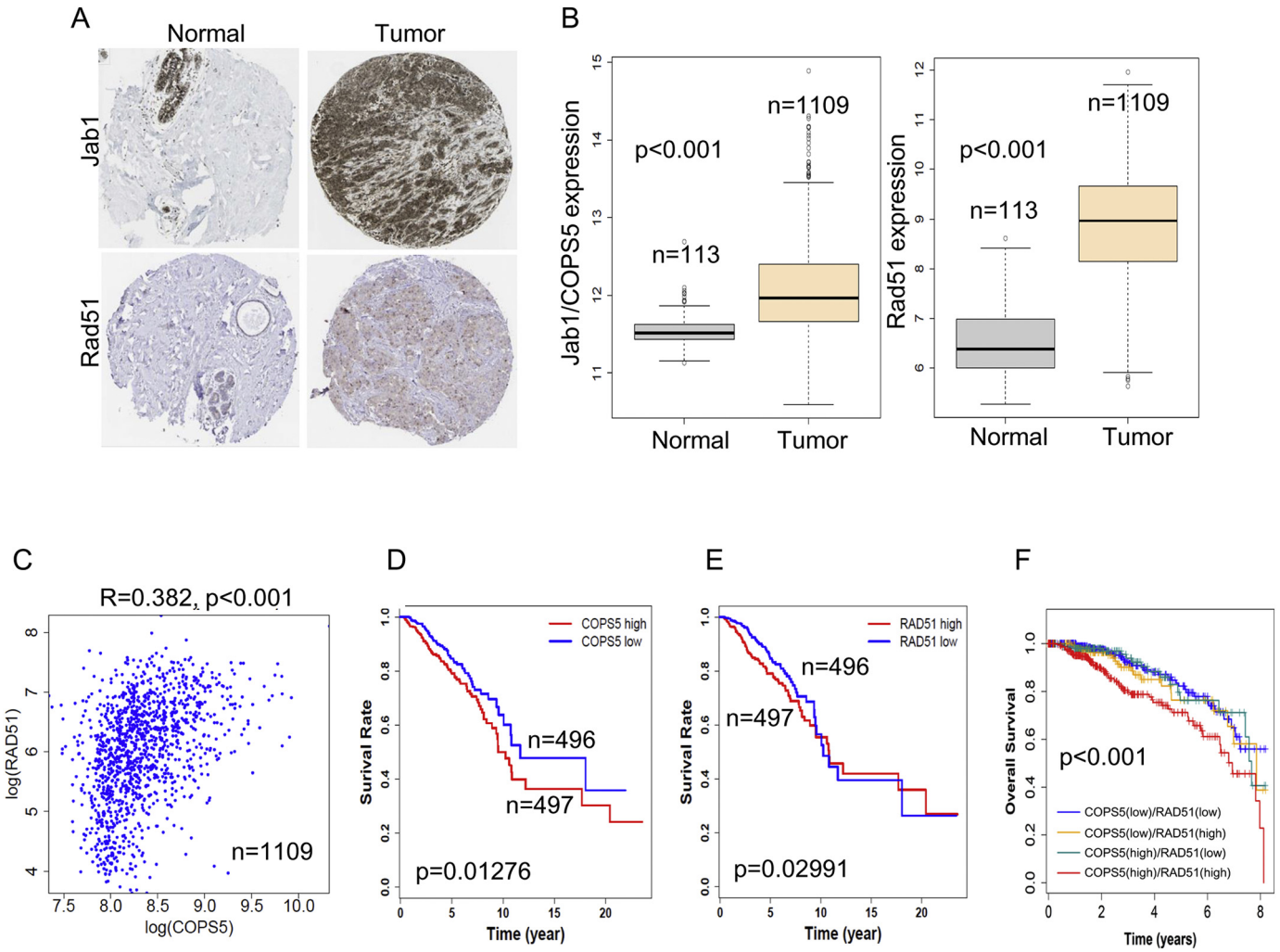

Fig. 7. Correlation of the expression of Jab1 and Rad51 with the outcome of patients with breast cancers. (A) Jab1 and Rad51 protein was strongly up-regulated in breast cancer tissues compared with normal breast based on The Human Protein Atlas database. The normal breast tissue of Jab1 staing was from a female, aged 25 (patient ID: 2419; staining: medium; intensity: moderate; quantity: 75%- 25%; location: nuclear; https://www.proteinatlas.org/ENSG00000121022-COPS5/tissue/breast#img) and the breast cancer tissue of Jab1 staing was from a female, aged 40 (patient ID: 2091; staining: medium; intensity: moderate; quantity: > 75%; location: cytoplasm and nuclear; https://www.proteinatlas.org/ENSG00000121022-COPS5/pathology/tissue/breast+cancer#img). The normal breast tissue of Rad51 staing was from a female, aged 23 (patient ID: 2773; staining: not detected; https://www.proteinatlas.org/ENSG00000051180-RAD51/tissue/breast#img) and the breast cancer tissue of Rad51 staing was from a female, aged 62 (patient ID: 1916; staining: medium; intensity: moderate; quantity: > 75%; location: nuclear; https://www.proteinatlas.org/ENSG00000051180-RAD51/pathology/tissue/breast+cancer#img). (B) *Jab1* and *Rad51* gene expression in normal breast and breast cancer using the TCGA data. (C) Jab1/COPS5 was associated with Rad51 expression in breast cancer from the TCGA data. (D, E) Kaplan-Meier analyses of the association between Jab1/COPS5 or Rad51 expression and survival as well as association of combined Jab1/COPS5 and Rad51 (F) and survival.

MCF-7R cells is followed by decreased apoptosis, we treated MCF-7 and MCF-7R cells with Adriamycin (1 μg/ml) for 48 h and analyzed the cells with Hoechst 33342 staining, which detects disintegrated nuclei (an indicator of apoptosis). In addition, the cells were exposed to cisplatin (10 μM) followed by Annexin V and PI staining. Treatment of breast cancer cells with adriamycin or cisplatin resulted in a marked increase in apoptotic cells (Fig. 1C and D). However, after being treated with adriamycin or cisplatin, significantly more MCF-7 cells (17% and 30%, respectively) underwent apoptosis than did MCF-7R cells (10% and 8%, respectively) (Fig. 1C and D).

3.2. Contribution of Jab1 depletion to adriamycin and cisplatin sensitivity of breast cancer cells

We first detected Jab1 protein and RNA levels in MCF-7 and MCF-7R cells by western blotting and quantitative real-time PCR. MCF-7R cells expressed approximately 3.6 times higher levels of Jab1 than did MCF-7 cells (Fig. 2A and B). We next investigated if inhibition of Jab1 sensitized breast cancer cells to adriamycin and cisplatin. Western blotting indicated successful knockdown of Jab1 expression in the Jab1 siRNA–treated MCF-7R cells (Fig. 2C). MCF-7R cells with reduced Jab1 expression showed an increase in the efficacy of adriamycin and cisplatin compared with control cells transfected with a scrambled siRNA (Fig. 2D and E).

We also tested the effects of Jab1 depletion on the cells' response to adriamycin using colony formation assay, which eliminated the possible contribution of enhanced cell proliferation to drug resistance. We observed similar results to those described above; MCF-7R cells transfected with Jab1 siRNA had worse survival rates when exposed to adriamycin (Fig. 2F). Thus, downregulation of Jab1 likely contributed to the breast cancer cells increased sensitivity to adriamycin and cisplatin.

To confirm this conclusion, we investigated whether elevated Jab1 in MCF-7 cells would enhance the resistance of MCF-7 cells to adriamycin and cisplatin treatments. MCF-7 cells were transfected with Myc-Jab1 cDNA (Fig. 2G, left), and cell viability was detected. The MCF-7

cells overexpressing Jab1 displayed decreased adriamycin-induced cell inhibition and 20% higher cell viability than did control cells (Fig. 2G, middle). Similar results were observed in cells treated with cisplatin, with 25% higher cell viability than that of control cells (Fig. 2G, right). Thus, we conclude that Jab1 inhibition sensitize breast cancer cells to adriamycin and cisplatin.

3.3. Depletion of Jab1 leads to increased apoptosis induced by adriamycin and cisplatin in breast cancer

As discussed above, MCF-7 cells displayed increased adriamycin and cisplatin-induced apoptosis compared with MCF-7R cells. To examine if Jab1 downregulation mediates this process, we investigated if knockdown of Jab1 in MCF-7R cells enhanced adriamycin- and cisplatin-induced apoptosis. MCF-7R cells transfected with Jab1 siRNA showed significantly more apoptosis in response to adriamycin (40% to 70% increase of apoptosis, as measured by Hoechst 33342 staining) than did control cells (Fig. 3A). Similar results were observed in cells treated with cisplatin (35% to 52% increase of apoptosis; Fig. 3B). Because cleavage of PARP is a hallmark of the apoptosis [18], we investigated the effect of Jab1 on adriamycin and cisplatin-induced apoptosis in breast cancer cells. After exposure to adriamycin and cisplatin, MCF-7R cells transfected with Jab1 siRNA displayed increased adriamycin and cisplatin-induced PARP cleavage compared with control cells (Fig. 3C and D). In contrast, overexpression of Jab1 in MCF-7 cells inhibited adriamycin- and cisplatin-induced apoptosis. Myc-Jab1–treated MCF-7 cells had decreased 56% and 35% apoptotic cells than did the control pcDNA-treated MCF-7 cells after 48 h of adriamycin or cisplatin treatment (Fig. 3E and F).

3.4. The contribution of Jab1 depletion to chemosensitivity is not cell line-specific

To rule out the possibility that the increase in adriamycin and cisplatin sensitivity upon Jab1 depletion was specific to the MCF-7, we examined another breast cancer cell line, ZR-75-1, by knocking down Jab1 expression and by performing an MTT assay. For this experiment, we also included the parental MCF-7 cells to detect whether knockdown of Jab1 would further increase MCF-7 chemosensitivity. As shown in Fig. 5A and B, Jab1 expression was successfully inhibited by siRNA in both cell lines according to western blot analysis results. Both cell lines with decreased Jab1 levels were also more sensitive to adriamycin and cisplatin (Fig. 4A–D), compared with the respective control cells transfected with scrambled siRNA when exposed to adriamycin- and cisplatin. Thus, the role of Jab1 in adriamycin and cisplatin response is likely not cell line–specific, and reducing Jab1 levels in parental MCF-7 cells could further enhance the chemosensitivity of MCF-7 cells.

3.5. Jab1 regulates Rad51 in p53-dependent manner

Adriamycin and cisplatin cause cytotoxicity by damaging DNA. Increased DNA repair confers cancer cells resistance to adriamycin or cisplatin [24]. Rad51 is recruited to sites of DNA damage to mediate repair [25]. Thus, we hypothesized that Jab1 depletion may impair Rad51 activity, which in turn increases chemosensitivity and chemotherapy-induced apoptosis. To test our hypothesis, we transfected breast cancer cells with Jab1 siRNA. Jab1 protein and RNA were decreased in Jab1-deficient cells compared with that in the controls (Fig. 5A–B). The decreased Jab1 protein or RNA was correlated with a decrease in Rad51 protein and RNA levels. Further, Jab1 inhibition not only reduced Rad51 expression but also influenced its repair function. Jab1-deficient cells formed less colonies after chemotherapy, and this effect was attributable to the decrease of Rad51 (Fig. 5C). Additionally, transfection of ectopic Rad51 into Jab1-deficient cells reversed their proliferation: the colonies in these cells were similar to thoes of the control cells, suggesting that replenishing Rad51 rescue the DNA repair function (Fig. 5C). Ectopic expression of Rad51 in Jab1-deficient cells was confirmed by western blotting (Fig. 5C, left). These data indicated that Jab1 regulated Rad51, thus sensitizing breast cancer cells to adriamycin and cisplatin.

Because p53 was demonstrated to regulate Rad51 [26,27], we examined whether the effect of Jab1 on Rad51 expression was mediated by p53. Rad51 expression was decreased in Jab1-deficient MCF-7 cells than in control cells (Fig. 5D). However, after knocking down p53 expression, Rad51 levels were not reduced in the Jab1 siRNA-treated breast cancer cells (Fig. 5D, left). Similarly, we found that Rad51 RNA and Jab1 RNA levels were lower in siRNA- treated MCF-7 cells than in controls; but the Rad51 RNA was less reduced in response to p53 siRNA transfection, regardless of Jab1 siRNA treatment (Fig. 5D, right). These data suggested that Jab1 regulated Rad51 in p53-dependent manner.

3.6. Jab1 depletion sensitizes breast cancer xenografts to chemotherapy

Our *in vitro* results showed the critical role of Jab1 in breast cancer cells' chemoresistance. To further confirm the function of Jab1 in breast cancer, we established breast cancer cells with and without stably knocked down Jab1 by infecting MCF-7R with a retrovirus shRNA-Jab1 or shRNA-control, and has verified the Jab1 expression by western blot (Fig. 5C, left). We then transplanted the MCF-7 and MCF-7R cells with stably knocked down Jab1 into nude mice and treated the mice with adriamycin and cisplatin when tumors were palpable. As shown in Fig. 6, tumor growth was observed in all the mice. Remarkably, adriamycin and cisplatin effectively suppressed the tumor growth in the MCF-7 group (Fig. 6A and B); however, the tumors grew stably in the MCF-7R group, indicating adriamycin and cisplatin resistance in the MCF-7R cells. Furthermore, MCF-7R sh-Jab1 mice had significantly less tumor growth than did MCF-7R sh-Cont mice. Consistently, the xenograft mouse model also showed the suppressive effects of chemotherapy on tumor weight in the MCF-7R sh-Jab1 group. These mice had 65% (adriamycin) and 51% (cisplatin) lower tumor weights than did the MCF-7R sh-control group (Fig. 6C and D). Our *in vivo* findings suggest that Jab1 depletion sensitizes breast cancer to chemotherapy.

We further examined the Jab1 and Rad51 expression in tumor xenograft tissue. Immunostaining revealed that Jab1 and Rad51 were abundantly expressed in the tumor xenograft tissue derived from sh-control MCF-7R cells, whereas both proteins were lower in the tissue derived from MCF-7R cells with stably knockdown Jab1 (Fig. 6E), confirming that Jab1 associated with Rad51 in breast cancer.

3.7. Correlation of the expression of Jab1 and Rad51 with the outcome of breast cancer patients

Data from the Human Protein Atlas database showed that immunohistochemistry staining of Jab1/COPS5 and Rad51 protein were higher in breast cancer tissue compared with normal breast tissue (Fig. 7A). We also analyzed the expression of Jab1/COPS5 and Rad51 in TCGA breast cancer datasets. The results revealed that both Jab1/COPS5 and Rad51 were upregulated in breast cancer tissues than in normal breast tissues ($P < .001$) (Fig. 7B). In addition, *Jab1/COPS5* gene is positively associated with *Rad51* gene analyzed from 1109 breast cancer patients ($R = 0.382$, $p < .001$).

Survival analysis demonstrated that high expression of either *Jab1/COPS5* or *Rad51* associated with poor prognosis in breast cancer (Fig. 7D and E). In the present study, we also analyzed the combined phenotypes of *Jab1/COPS5* and *RAD51*, and patients with *COPS5*(high)/*RAD51*(high) expression had the worst survival among all phenotypes ($p < .001$) (Fig. 7F).

4. Discussion

Chemotherapy is a powerful tool to treating cancer. Nevertheless, in many cases, initially sensitive cancer cells rapidly develop acquired

resistance when apoptosis pathways fail to activate [28,29]. Identification of effective strategies to sensitize breast cancer cells to chemotherapy will allow the use of lower drug doses by reducing therapy-associated side effects while increasing cancer cell death. Chemotherapy drugs, adriamycin and cisplatin, can directly act on DNA in cells, resulting in DNA base mismatch, chain crosslinking, and doublestrand breaks (DSBs) [30,31]. The major repair mechanisms of DSBs are non-homologous end-joining (NHEJ) repair and homologous recombination (HR) repair [31]. HR repair occurs during G2 or late S phases of the cell cycle [32]. NHEJ, in contrast, occurs rapidly throughout the cell cycle [32]. Cancer tissues have an elevated ratio of cells in G2 and S phase, leading them to favor HR repair over NHEJ repair [33]. Rad51 is the key protein controlling the HR repair process [34], as it as it mediates pairing of homologous DNA sequences and strand invasion [35]. Rad51 functions by forming nucleoprotein filaments in single-stranded DNA, mediating homologous pairing and strand exchange reactions between single and double stranded DNA during repair [35].

Aberrant overexpression of Jab1 has been observed in various human cancers and it is closely related to tumorgenesis [13]. Jab1 involves in cell cycle regulation, transcriptional activation and signal transduction of tumor development. For example, Jab1 promotes the degradation of p27, an inhibtor of cyclin *E*-Cdk2, through accelerating its translocation from the nucleus to the cytoplasm [16]. Jab1 is also involved in ubiquitination and degradation of tumor-inhibiting factors including P53 and Smad4/7 [36,37]. Limited studies available indicated that Jab1 could participate in cell proliferation and poor prognosis of breast cancer [18,19,38]. In this study, overexpression of Jab1 has been confirmed in both breast cancer tissues. Since the higher expression of Jab1 was found in chemo-resistant breast cancer cell lines compared with parental cell lines, Jab1 might be a chemo-resistant enhancer. Our study demonstrated that suppressing Jab1 results in elevated adrimaycin and cisplatin sensitivity by suppressing the DNA repair gene *Rad51*, which, in turn, contributes to increased adriamycin- and cisplatin-induced apoptosis. These findings illustrate Jab1 as a biomarker for predicting chemotherapy response in breast cancer.

Our results show that Jab1 depletion is associated with decreased Rad51, suggesting that DNA repair defect accounted for the increased spontaneous DNA damage in Jab1 knockdown cells. These data explain the delayed cell proliferation and elevated apoptosis in Jab1 knockdown cells treated with adriamycin and cisplatin. Further, Jab1 positively regulates Rad51 in breast cancer, which explains why Jab1 depletion sensitizes breast cancer cells to adriamycin and cisplatin. By contrast, ectopic expression of Rad51 conferred resistance to adriamycin and cisplatin. The decreased Jab1 resulted in deregulation of the Rad51 and defects in DNA repair and sensitized cancer cells to adriamycin and cisplatin. Jab1 expression facilitates MDM2-mediated p53 ubiquitination and promotes p53 nuclear export [39,40]. Thus, suppression of Jab1 expression could lead to the reduced translocation of p53 from the nucleus to the cytoplasm. We observed that reduced Jab1 levels was associated with a significant elevated p53 levels, implying that p53, at least in part, participates in the Jab1-mediated regulation of Rad51.

According to the data from TCGA database, mRNA expression of both Jab1 and Rad51 are significantly elevated in breast cancer patient specimens compared with normal breast tissues. This phenomenon was further validated by the protein levels from the Human Protein Atlas database. Importantly, either Jab1 or Rad51 expression is highly associated with survival in breast cancer. The data obtained from the online database also agrees with previous reports that Jab1 is overexpressed in breast cancer patients [19,41]. An association between Jab1 and Rad51 has been consistently implicated in breast cancer patients, supporting our *in vitro* findings.

In conclusion, our findings demonstrate that depletion of Jab1 sensitize breast cancer cells to adriamycin and cisplatin. Jab1 positively regulates Rad51 through p53 pathway. Our findings indicates that evaluating Jab1 and Rad51 levels could help to predict response to adriamycin and cisplatin based therapy, allowing to design personalized medicine for breast cancer patients.

Competing interests

None.

Acknowledgements

The results (Fig.7) are in part based upon data generated by the TCGA Research Network (http://cancergenome.nih.gov/) and The Human Protein Atlas (https://www.proteinatlas.org/).

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Authors' contributions

Y. Pan conceived and designed the study; G. Liu, M. Yu, B. Wu, S. Guo, X. Huang and Y. Pan performed the experiments; G. Liu and Y. Pan analyzed and interpreted the data; G. Liu and Y. Pan drafted the manuscript; F. Zhou and FX. Claret provided technical and material support.

References

[1] M. Abotaleb, P. Kubatka, M. Caprnda, E. Varghese, B. Zolakova, P. Zubor, et al., Chemotherapeutic agents for the treatment of metastatic breast cancer: an update, Biomed. Pharmacother. 101 (Mar 1, 2011) 458–477 PubMed PMID: 29501768.

[2] C.F. Higgins, Multiple molecular mechanisms for multidrug resistance transporters, Nature 446 (7137) (Apr 12, 2007) 749–757 PubMed PMID: 17429392.

[3] N.A. Johnson, G.W. Slack, K.J. Savage, J.M. Connors, S. Ben-Neriah, S. Rogic, et al., Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone, J. Clin. Oncol. 30 (28) (Oct 1, 2012) 3452–3459 PubMed PMID: 22851565. Pubmed Central PMCID: 3454768.

[4] L.A. Gilbert, M.T. Hemann, DNA damage-mediated induction of a chemoresistant niche, Cell 143 (3) (Oct 29, 2010) 355–366 PubMed PMID: 21029859. Pubmed Central PMCID: 2972353.

[5] J. Stingele, R. Bellelli, S.J. Boulton, Mechanisms of DNA-protein crosslink repair, Nat. Rev. Mol. Cell Biol. 18 (9) (Sep 2017) 563–573 PubMed PMID: 28655905.

[6] S. Wang, Z. Zou, X. Luo, Y. Mi, H. Chang, D. Xing, LRH1 enhances cell resistance to chemotherapy by transcriptionally activating MDC1 expression and attenuating DNA damage in human breast cancer, Oncogene 16 (Mar 2018) . PubMed PMID: 29545602.

[7] T. Haaf, E.I. Golub, G. Reddy, C.M. Radding, D.C. Ward, Nuclear foci of mammalian Rad51 recombination protein in somatic cells after DNA damage and its localization in synaptonemal complexes, Proc. Natl. Acad. Sci. U. S. A. 92 (6) (Mar 14, 1995) 2298–2302 PubMed PMID: 7892263. Pubmed Central PMCID: 42471.

[8] W. Zhao, J.B. Steinfeld, F. Liang, X. Chen, D.G. Maranon, C. Jian Ma, et al., BRCA1-BARD1 promotes RAD51-mediated homologous DNA pairing, Nature 550 (7676) (Oct 19, 2017) 360–365 PubMed PMID: 28976962. Pubmed Central PMCID: 5800781.

[9] M. Gachechiladze, J. Skarda, A. Soltermann, M. Joerger, RAD51 as a potential surrogate marker for DNA repair capacity in solid malignancies, Int. J. Cancer 141 (7) (Oct 1, 2017) 1286–1294 PubMed PMID: 28477336.

[10] C. Maranto, V. Udhane, D.T. Hoang, L. Gu, V. Alexeev, K. Malas, et al., STAT5A/B Blockade Sensitizes Prostate Cancer to Radiation through Inhibition of RAD51 and DNA Repair, Clin. Cancer Res. 26 (Feb 2018) PubMed PMID: 29483142.

[11] H. Dungrawala, K.P. Bhat, R. Le Meur, W.J. Chazin, X. Ding, S.K. Sharan, et al., RADX promotes genome stability and modulates chemosensitivity by regulating RAD51 at Replication forks, Mol. Cell 67 (3) (Aug 3, 2017) 374–386 e5. PubMed PMID: 28735897. Pubmed Central PMCID: 5548441.

[12] K.J. Hong, M.C. Hsu, W.C. Hung, RECK impedes DNA repair by inhibiting the erbB/JAB1/Rad51 signaling axis and enhances chemosensitivity of breast cancer cells, Am. J. Cancer Res. 5 (8) (2015) 2422–2430 PubMed PMID: 26396917. Pubmed

Central PMCID: 4568777.
[13] G. Liu, F.X. Claret, F. Zhou, Y. Pan, Jab1/COPS5 as a novel biomarker for diagnosis, prognosis, therapy prediction and therapeutic tools for human cancer, Front. Pharmacol. 9 (2018) 135 PubMed PMID: 29535627. Pubmed Central PMCID: 5835092.
[14] Y. Pan, F.X. Claret, Targeting Jab1/CSN5 in nasopharyngeal carcinoma, Cancer Lett. 326 (2) (Dec 30, 2012) 155–160 PubMed PMID: 22867945. Pubmed Central PMCID: 3474602.
[15] F. Zhou, Y. Pan, Y. Wei, R. Zhang, G. Bai, Q. Shen, et al., Jab1/Csn5-thioredoxin signaling in relapsed acute monocytic leukemia under oxidative stress, Clin. Cancer Res. 23 (15) (Aug 01, 2017) 4450–4461 PubMed PMID: 28270496.
[16] Y. Pan, Q. Zhang, L. Tian, X. Wang, X. Fan, H. Zhang, et al., Jab1/CSN5 negatively regulates p27 and plays a role in the pathogenesis of nasopharyngeal carcinoma, Cancer Res. 72 (7) (Apr 1, 2012) 1890–1900 PubMed PMID: 22350412. Pubmed Central PMCID: 3460549.
[17] Y. Pan, S. Wang, B. Su, F. Zhou, R. Zhang, T. Xu, et al., Stat3 contributes to cancer progression by regulating Jab1/Csn5 expression, Oncogene 36 (8) (Feb 23, 2017) 1069–1079 PubMed PMID: 27524414. Pubmed Central PMCID: 5311075.
[18] Y. Pan, G. Liu, Y. Yuan, J. Zhao, Y. Yang, Y. Li, Analysis of differential gene expression profile identifies novel biomarkers for breast cancer, Oncotarget 8 (70) (Dec 29, 2017) 114613–114625 PubMed PMID: 29383106. Pubmed Central PMCID: 5777718.
[19] R. Lu, X. Hu, J. Zhou, J. Sun, A.Z. Zhu, X. Xu, et al., COPS5 amplification and overexpression confers tamoxifen-resistance in ERalpha-positive breast cancer by degradation of NCoR, Nat. Commun. 7 (Jul 04, 2016) 12044 PubMed PMID: 27375289. Pubmed Central PMCID: 4932188.
[20] S.O. Lim, C.W. Li, W. Xia, J.H. Cha, L.C. Chan, Y. Wu, et al., Deubiquitination and Stabilization of PD-L1 by CSN5, Cancer Cell 30 (6) (Dec 12, 2016) 925–939 PubMed PMID: 27866850. Pubmed Central PMCID: 5171205.
[21] A. Schlierf, E. Altmann, J. Quancard, A.B. Jefferson, R. Assenberg, M. Renatus, et al., Targeted inhibition of the COP9 signalosome for treatment of cancer, Nat. Commun. 7 (Oct 24, 2016) 13166 PubMed PMID: 27774986. Pubmed Central PMCID: 5078989 Novartis Pharma AG.
[22] Y. Pan, Q. Zhang, V. Atsaves, H. Yang, F.X. Claret, Suppression of Jab1/CSN5 induces radio- and chemo-sensitivity in nasopharyngeal carcinoma through changes to the DNA damage and repair pathways, Oncogene 32 (22) (May 30, 2013) 2756–2766 PubMed PMID: 22797071. Pubmed Central PMCID: 3566273.
[23] P.J. Thul, L. Akesson, M. Wiking, D. Mahdessian, A. Geladaki, H. Ait Blal, et al., A subcellular map of the human proteome, Science 356 (6340) (May 26, 2017) PubMed PMID: 28495876.
[24] M.T. Esposito, C.W. So, DNA damage accumulation and repair defects in acute myeloid leukemia: implications for pathogenesis, disease progression, and chemotherapy resistance, Chromosoma 123 (6) (Dec 2014) 545–561 PubMed PMID: 25112726.
[25] N. Suwaki, K. Klare, M. Tarsounas, RAD51 paralogs: roles in DNA damage signalling, recombinational repair and tumorigenesis, Semin. Cell Dev. Biol. 22 (8) (Oct 2011) 898–905 PubMed PMID: 21821141.
[26] C. Arias-Lopez, I. Lazaro-Trueba, P. Kerr, C.J. Lord, T. Dexter, M. Iravani, et al., p53 modulates homologous recombination by transcriptional regulation of the RAD51 gene, EMBO Rep. 7 (2) (Feb 2006) 219–224 PubMed PMID: 16322760. Pubmed Central PMCID: 1369244.
[27] J.A. Hannay, J. Liu, Q.S. Zhu, S.V. Bolshakov, L. Li, P.W. Pisters, et al., Rad51 overexpression contributes to chemoresistance in human soft tissue sarcoma cells: a role for p53/activator protein 2 transcriptional regulation, Mol. Cancer Ther. 6 (5) (May 2007) 1650–1660 PubMed PMID: 17513613.
[28] X. Chen, Y. Wu, H. Dong, C.Y. Zhang, Y. Zhang, Platinum-based agents for individualized cancer treatment, Curr. Mol. Med. 13 (10) (Dec 2013) 1603–1612 PubMed PMID: 24206132.
[29] K. Gogineni, A. Demichele, Current approaches to the management of Her2-negative metastatic breast cancer, Breast Cancer Res. 14 (2) (2012) 205 PubMed PMID: 22429313. Pubmed Central PMCID: 3446361.
[30] N. Lipinska, A. Romaniuk, A. Paszel-Jaworska, E. Toton, P. Kopczynski, B. Rubis, Telomerase and drug resistance in cancer, Cell. Mol. Life Sci. 74 (22) (Nov 2017) 4121–4132 PubMed PMID: 28623509. Pubmed Central PMCID: 5641272.
[31] T. Helleday, E. Petermann, C. Lundin, B. Hodgson, R.A. Sharma, DNA repair pathways as targets for cancer therapy, Nat. Rev. Cancer 8 (3) (Mar 2008) 193–204 PubMed PMID: 18256616.
[32] Z. Mao, M. Bozzella, A. Seluanov, V. Gorbunova, Comparison of nonhomologous end joining and homologous recombination in human cells, DNA Repair 7 (10) (Oct 1, 2008) 1765–1771 PubMed PMID: 18675941. Pubmed Central PMCID: 2695993.
[33] C.M. Hine, A. Seluanov, V. Gorbunova, Use of the Rad51 promoter for targeted anticancer therapy, Proc. Natl. Acad. Sci. U. S. A. 105 (52) (Dec 30, 2008) 20810–20815 PubMed PMID: 19106292. Pubmed Central PMCID: 2634908.
[34] A. Quinet, D. Lemacon, A. Vindigni, Replication fork reversal: players and guardians, Mol. Cell 68 (5) (Dec 7, 2017) 830–833 PubMed PMID: 29220651.
[35] L. Krejci, V. Altmannova, M. Spirek, X. Zhao, Homologous recombination and its regulation, Nucleic Acids Res. 40 (13) (Jul 2012) 5795–5818 PubMed PMID: 22467216. Pubmed Central PMCID: 3401455.
[36] M. Wan, X. Cao, Y. Wu, S. Bai, L. Wu, X. Shi, et al., Jab1 antagonizes TGF-beta signaling by inducing Smad4 degradation, EMBO Rep. 3 (2) (Feb 2002) 171–176 PubMed PMID: 11818334. Pubmed Central PMCID: 1083965.
[37] D. Bech-Otschir, R. Kraft, X. Huang, P. Henklein, B. Kapelari, C. Pollmann, et al., COP9 signalosome-specific phosphorylation targets p53 to degradation by the ubiquitin system, EMBO J. 20 (7) (Apr 2, 2001) 1630–1639 PubMed PMID: 11285227. Pubmed Central PMCID: 145508.
[38] Y. Wei, G. Liu, B. Wu, Y. Yuan, Y. Pan, Let-7d inhibits growth and metastasis in breast cancer by targeting Jab1/Cops5, Cell. Physiol. Biochem. 47 (5) (2018) 2126–2135 PubMed PMID: 29975923.
[39] X.C. Zhang, J. Chen, C.H. Su, H.Y. Yang, M.H. Lee, Roles for CSN5 in control of p53/MDM2 activities, J. Cell. Biochem. 103 (4) (Mar 1, 2008) 1219–1230 PubMed PMID: 17879958.
[40] W. Oh, E.W. Lee, Y.H. Sung, M.R. Yang, J. Ghim, H.W. Lee, et al., Jab1 induces the cytoplasmic localization and degradation of p53 in coordination with Hdm2, J. Biol. Chem. 281 (25) (Jun 23, 2006) 17457–17465 PubMed PMID: 16624822.
[41] M.A. Kouvaraki, G.Z. Rassidakis, L. Tian, R. Kumar, C. Kittas, F.X. Claret, Jun activation domain-binding protein 1 expression in breast cancer inversely correlates with the cell cycle inhibitor p27(Kip1), Cancer Res. 63 (11) (Jun 1, 2003) 2977–2981 PubMed PMID: 12782606.