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GBP1 promotes non-small cell lung carcinoma malignancy and chemoresistance *via* activating the Wnt/β-catenin signaling pathway

J. SONG[1], Q.-Y. WEI[2]

[1]Department of Geriatrics, DaLian Friendship Hospital, Dalian, China
[2]Department of Allergy, General Hospital of Northern Theater Command, Shenyang, China

Abstract. – OBJECTIVE: Non-small cell lung cancer (NSCLC) is one of the most ordinary cancers worldwide. Recent studies have discovered many oncogenes play vital roles in the tumorigenesis of malignant tumors. The purpose of our study was to uncover the role of GBP1 in NSCLC and the underlying mechanism.

PATIENTS AND METHODS: GBP1 expression in NSCLC samples was detected by Real Time quantitative-Polymerase Chain Reaction (RT-qPCR). Function assays were performed in NSCLC cells transfected with GBP1 shRNA. Furthermore, RT-qPCR and Western blot assay were conducted to explore the target signaling pathway of GBP1.

RESULTS: GBP1 expression was significantly upregulated in NSCLC tissue samples compared with adjacent normal tissues. Function assays showed that the proliferation of NSCLC cells was significantly inhibited *via* knockdown of GBP1, while cell apoptosis was promot sistance to paclitaxel was reversed afte knockdown in paclitaxel resistance A549 lls (A549/Taxol). In addition, Wnt/β-catenin si ing pathway was repressed *via* kdow GBP1 in NSCLC cells and A54 axo ells.

CONCLUSIONS: In our st GBP firs ly identified as a novel on ge ur thermore, it could prom NSC velopment and paclitaxel resista y induc /nt/β-catenin signaling path y.

Key Words:
GBP1, De pme Chemot rapy, Wnt/β-catenin signalin th y, Non-small cell lung cancer (NSCLC).

ntroduction

Lung c cer is one of the most common cancers globally both in terms of morbidity and mortality[1]. Non-small cell lung cancer (NSCLC) is the major subtype of lung cancer. Current studies have indicated that NSCLC contributes to 85% of all lung cancer cases. It has be rted that the incidence and mortality of CLC continue to increase for the next s al decad ue to the characteristics of cell mi on and vasion[2,3]. Most patients have eady in advanced stage at the ti of di gnos oreover, the median survi m these patients barely exceeds 18 nth herefo there is an urgent need to idate th lying mechanism of NSCL a prove t poor prognosis for unfo ate pati

Chemoresista remains one of the major bstacles to the success of cancer therapies. In past se ral decades, chemoresistance has fou losely associated with the expression o genes and complex biological mechanisms, including epithelial-mesenchymal tran n (ETM), enrichment of cancer stem cells (CSCs), autophagy, drug efflux mechanism, and so on[5,6]. Consistently, the MDR1 gene is one of the determinants involved in the chemoresistance of ovarian cancer[7]. CSCs contribute to chemoresistance in breast cancer. Furthermore, it may lead to a novel therapy to prevent the relapse and improve the prognosis of breast cancer patients[8]. By interacting with Notch1, DDR1 induces ineffective induction of cell death, eventually leading to a chemo-resistant phenotype[9]. Paclitaxel, as a clinically common chemotherapy drug, cannot avoid drug resistance during the treatment. However, the mechanism of paclitaxel resistance in malignancies remains unclear.

Recently, GBP1 has been reported as a novel oncogene that promotes the development of multiple cancers. However, the role of GBP1 in NSCLC development and chemotherapy resistance has not been fully elucidated. In our study, we

Corresponding Author: Qingyu Wei, Ph.D; e-mail: weiqingyu1964@163.com

discovered that GBP1 was involved in cell proliferation, metastasis, and chemotherapy resistance by inducing Wnt/β-catenin signaling pathway in NSCLC. Our findings might offer new insight into the therapy of NSCLC.

Patients and Methods

Clinical Samples

Totally, 56 paired NSCLC tissues and matched paracancerous tissues were collected from patients who underwent surgical resection at General Hospital of Northern Theater Command from June 2016 to December 2018. Collected tissues were quickly placed in liquid nitrogen and immediately stored in -80°C cryogenic refrigerator. This study was approved by the Ethics Committee of the General Hospital of Northern Theater Command. All the enrolled patients were diagnosed with NSCLC by two independent pathologists without any controversial. Written informed consent was obtained from each patient before surgery.

Cell Culture

Four NSCLC cell lines (A549, SPCA1, [illegible] and H1299) and one normal human bronc[illegible] epithelial cell line (16HBE) were purchased fro[illegible] the Institute of Biochemistry an[illegible]ll Biology, Shanghai Institutes for Bio[illegible]iences (Shanghai, China). All cells [illegible]e cu[illegible]d in Dulbecco's Modified Eagle's [illegible]dium (Hyclone, Waltham, MA, U[illegible]ng 10% fetal bovine serum (FBS, Invitr[illegible]rlsbad, CA, USA) in an incubat[illegible]ith 5% C[illegible]°C. A549/Taxol cells were [illegible]ured in DMEM [illegible]edium added with 10% [illegible]

Cell Transfection

For transfe[illegible]n, lentivirus [illegible]expressing short-hairpin [illegible]A (shRNA) targeting GBP1 was compounded [illegible]d clo[illegible]to pLenti-EF1a-EGFP-F2A-Puro [illegible] (B[illegible]ttia In[illegible]San Diego, CA, USA). GBP1 [illegible]r neg[illegible]e control shRNA (NC) was transfe[illegible]CLC cells according to [illegible]tructio[illegible]Lipofectamine 2000 (Inv[illegible]rlsbad, CA, USA).

[illegible]ime[illegible]ative Polymerase Ch[illegible]tion (RT-qPCR)

The [illegible]A from tissues and cells was extracted t[illegible] TRIzol reagent (Invitrogen, Carlsbad, CA, USA), followed by measurement of RNA concentration using [illegible] spectrophotometer (Hitachi, T[illegible], Japa[illegible]BR green (Roche, Basel, Swit[illegible]nd) was co[illegible]ed to measure the relative e[illegible]ion levels o[illegible]es. β-actin was used as an [illegible]eferen[illegible] The primers used in this study w[illegible]s follows: GBP1, forw[illegible]3'-ACTTC[illegible]ACAG-GAGCAAC-5' a[illegible]evers[illegible]3'-TATG[illegible]TACAT-GCCTTTCGTC[illegible]β-ac[illegible]forwa[illegible]: 5′-GATG-GAAATCGTC[illegible]3′ and[illegible]erse: 5′-TG-GCACTTAC[illegible]C-3′. The specific thermal cy[illegible]was as [illegible] sec at 95°C, 5 sec for 40[illegible]es at 95°C, a[illegible] sec at 60°C. The relative[illegible]n was calculated by the $2^{-\Delta\Delta CT}$ metho[illegible]

[illegible]hyl Thiazolyl Tetrazolium [illegible]TT] Assay

[illegible] × 10^3 tran[illegible]ted cells were seeded into [illegible]ell plates [illegible]aining 100 μL medium with-[illegible]s was to ensure that the number of [illegible]ell was about 2000. 10 μL MTT reagent (Roche, Basel, Switzerland) was added to [illegible]h well at 0 h, 24 h, 48 h, and 72 h, respectively, [illegible]y incubation in an incubator. Optical [illegible]sity (OD) value at 490 nm was measured by [illegible] ELISA reader system (Multiskan Ascent, Lab-Systems, Helsinki, Finland).

[illegible]olony Formation Assay

1.5 × 10^3 of cells were seeded into 6-well plates and cultured. 10 day later, the formed colonies were fixed with 10% formaldehyde for 30 min and stained with 0.5% crystal violet for 5 min. Nikon camera was used for taking a photograph of colonies. Finally, the number of formed colonies was counted and compared.

Flow Cytometry Assay

1-5 × 10^5 cells in logarithmic growth phase were digested with trypsin and made into cell suspension. After washing twice with Phosphate-Buffer Saline (PBS) and centrifugation at 1000 rpm/min for 5 min), 100 μL Binding Buffer was added to suspend the cells. Later, 5 μL of 7-aad and 5 μL of PE dye were added to each tube, followed by mixing with 400 μL of Binding Buffer. At the same time, blank control (no dye) and single dye (5 μL 7-aad and 5 μL PE respectively) were set for 15 min at room temperature. Flow cytometry was performed, and the results were expressed as the percentage of total apoptosis.

Western Blot Analysis

The cells were first washed with pre-cooled PBS and lysed with cell lysis solution (RIPA; Beyotime, Shanghai, China). The protein concentration was detected by the bicinchoninic acid (BCA; Thermo Fisher Scientific Inc., Waltham, MA, USA). The total proteins were separated and transferred on polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking in Tris-Buffered Saline and Tween 20 (TBST; 25 mM Tris, 140 mM NaCl, and 0.1% Tween 20, pH 7.5) containing 5% skimmed milk for 2 h, the membranes were incubated with primary antibodies of Wnt3a, β-catenin, C-myc, and Survivin (Abcam Inc., Cambridge, MA, USA) in Wnt/β-catenin signaling pathway and β-actin (Abcam Inc., Cambridge, MA, USA) at 4°C overnight. After washing (3 × 10 min) with TBST, the membranes were incubated with corresponding secondary antibody at room temperature for 1 h. The immunoreactive band were exposed and analyzed by Image J software (Media Cybernetics, Silver Springs, MD, USA).

Statistical Analysis

The Statistical Product and Service So[illegible]s (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) [illegible] used for all statistical analyses [illegible] sample *t*-test was selected when [illegible]ropria[illegible]05 was considered statistically [illegible]nificant.

Results

GBP1 Expressio[illegible]n NS[illegible]C Tissues and C[illegible]

GBP1 expre[illegible]t dete[illegible]in 56 pairs of NSCLC ti[illegible]ues a[illegible]CLC[illegible]ls. As shown in Figure 1A, GBP1 was [illegible]ly upregulated in NSCL[illegible]ssues when c[illegible]ared with adjacent nor[illegible]s through RT-qPCR detection. Weste[illegible]ot a[illegible]dicated that the protein level of GBP1 was sign[illegible]pregulated in NSCLC tis[illegible]as well (Figure[illegible]). Moreover, the expression level of GBP1 in four NSCLC cell lines was shown in Figure 1C.

GBP1 Knock[illegible]n Repressed Cell [illegible] and Promoted Cell Apo[illegible] NSCLC

To determine whether GBP1 exerted a vital [illegible]ation in NSCLC, A549 cells were chosen for [illegible]n of GBP1. GBP1 shRNA and negative [illegible]ol shRNA were synthesized and trans-

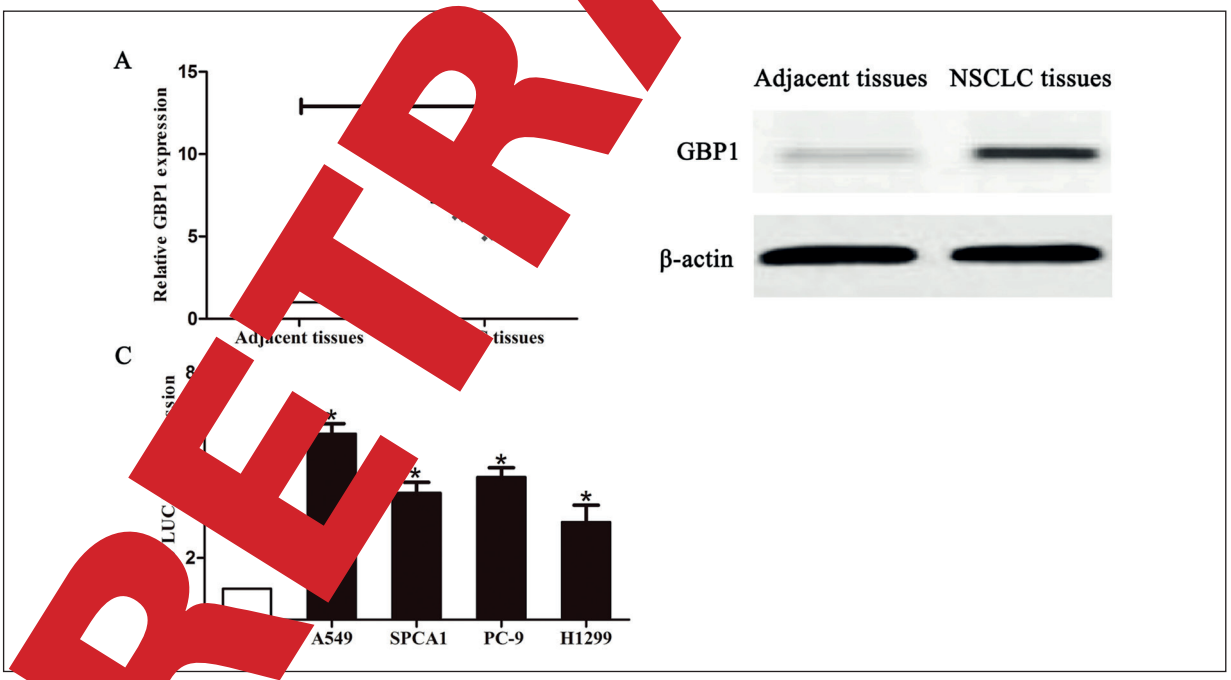


Figure [illegible]ion level of GBP1 in NSCLC tissues and cell lines. **A,** GBP1 expression was significantly upregulated in NSCLC tiss[illegible]d with adjacent tissues. **B,** The protein level of GBP1was significantly upregulated in NSCLC tissues through Western[illegible]C, Expression levels of GBP1 relative to β-actin in human NSCLC cell lines were determined by RT-qPCR. Data were presented as mean ± standard error of the mean. *$p<0.05$.

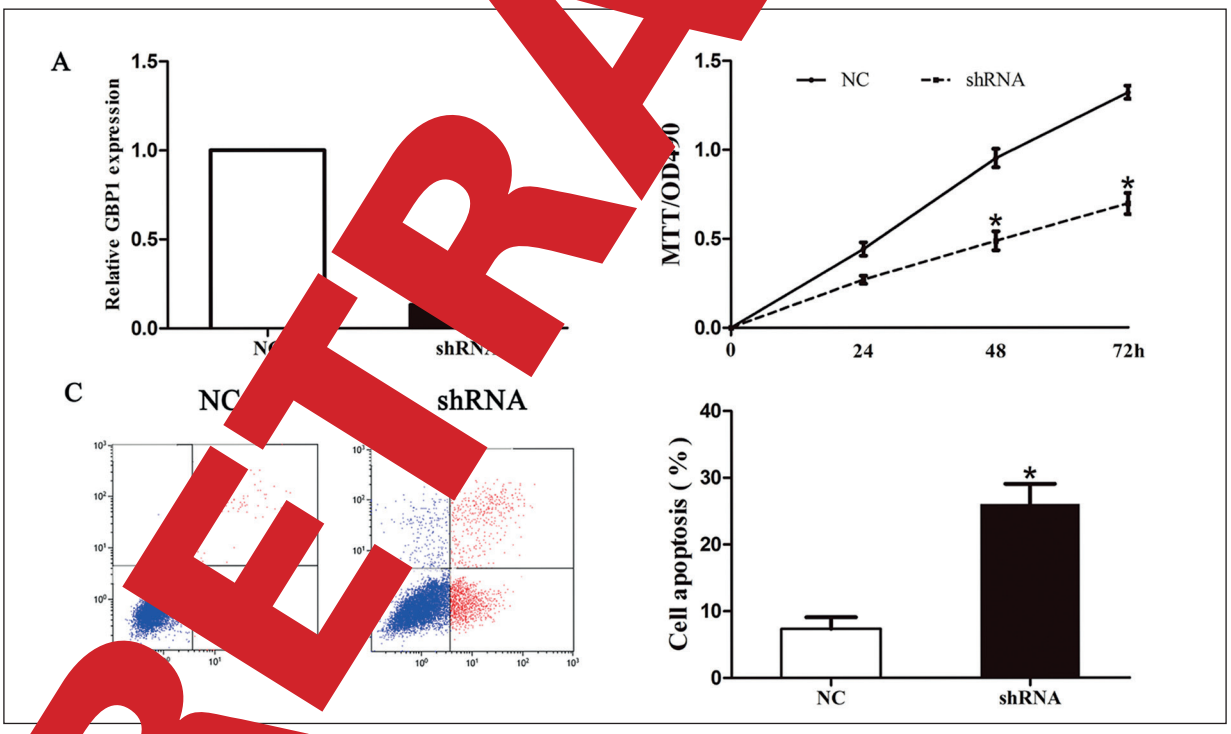
duced into SW780 cells. Transfection efficiency was verified by RT-qPCR (Figure 2A). As shown in Figure 2B, the MTT assay showed that the knockdown of GBP1 significantly inhibited the viability of NSCLC cells. To further confirm the effect of GBP1 on the apoptosis of NSCLC cells, the flow cytometry assay was performed. As shown in Figure 2C, the percentage of cell apoptosis rate remarkably increased after GBP1 was knocked down.

Knockdown of GBP1 Reversed Paclitaxel Resistance of A549/Taxol NSCLC Cells

To investigate the effect of GBP1 on the paclitaxel resistance of A549/Taxol NSCLC cells, MTT, and colony formation assay were applied in A549/Taxol NSCLC cells after transfection of GBP1 shRNA. Transfection efficiency was monitored by RT-qPCR (Figure 3A). MTT assay found that paclitaxel sensitivity increased remarkably through knockdown of GBP1 in A549/Taxol cells (Figure 3B). The colony formation assay demonstrated that the colony formation ability was significantly su[illegible] the knockdown of GBP1 in A[illegible] Taxol [illegible] Figure 3C).

Knockdown of GBP1 Inhi[illegible] Development a[illegible] Reversed [illegible] axel Resistance Thr[illegible] gh W[illegible] β-Cate[illegible] n Signaling Pat[illegible] ay in [illegible] SCLC

To explore t[illegible] g mec[illegible] sm of GBP1 function in [illegible] de[illegible] t of [illegible] SCLC, RT-qPCR and W[illegible] n blot a[illegible] conducted. The mRNA a[illegible] rotein expres[illegible] of Wnt3a, β-catenin, C[illegible] d Survivin were determined, which [illegible] re [illegible] proteins of the Wnt/β-catenin signaling pa[illegible] As shown in Figure 4A a[illegible] gure 4B, the [illegible] NA and protein expression of the above molecules were remarkably [illegible] wnregulated *via* knockdown of GBP1 in A549 [illegible] s. Conversel[illegible] s shown in Figure 4C and [illegible] e 4D, the [illegible] ressions of these proteins were [illegible] nregulated *via* knockdown of GB[illegible] 9/Taxol cells at both mRNA and protein levels. These results suggested that the



F[illegible] [illegible]BP1 inhibited NSCLC cell proliferation and promoted cell apoptosis. **A,** GBP1 expression in N[illegible] ansduced with negative control shRNA (NC) or GBP1 shRNA (shRNA) was detected by RT-qPCR. β-actin was us[illegible] ternal control. **B,** MTT assay showed that the viability of NSCLC cells was significantly inhibited by the knockdown[illegible] C, Flow cytometry assay results showed that the percentage of cell apoptosis rate increased remarkably after GBP1 was [illegible] ed down. The results represented the average of three independent experiments (mean ± standard error of the mean). *p<0.05.

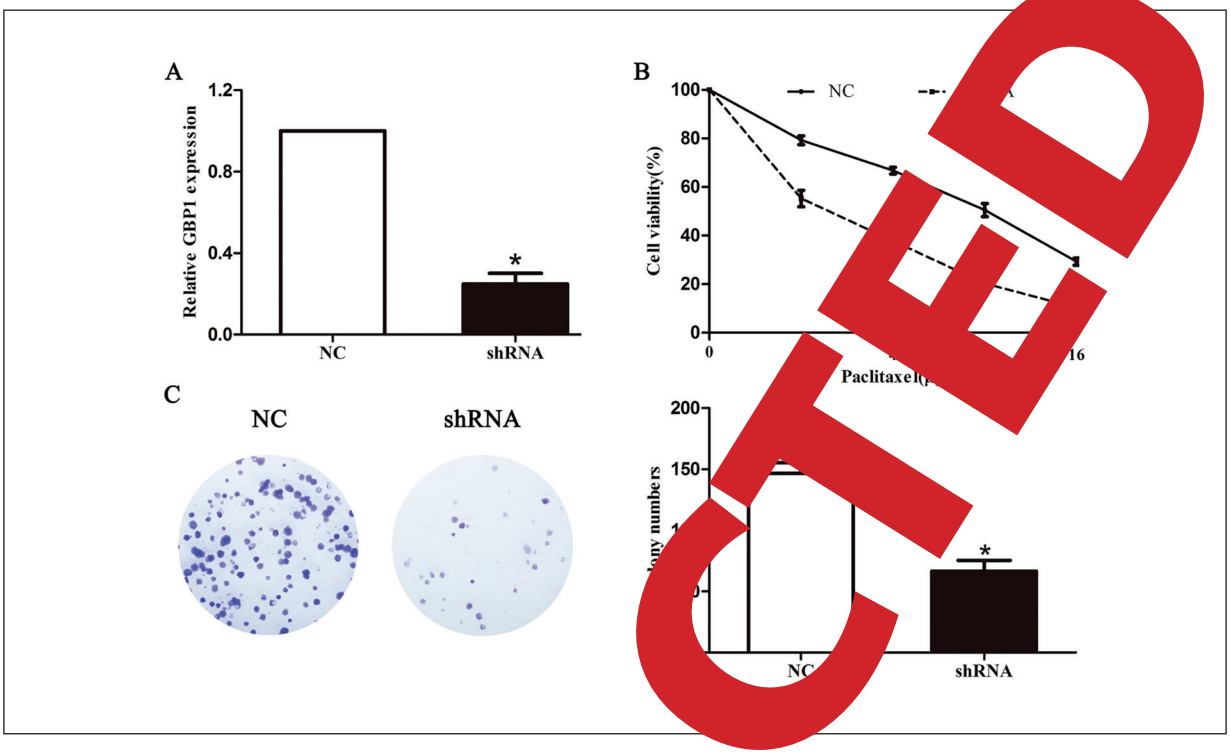


Figure 3. Knockdown of GBP1 reversed paclitaxel re of A549/Taxol NSCLC cells. **A,** 48 h after A549/Taxol NSCLC cells were transfected with GBP1 shRNA, transfecti ed by RT-qPCR. GAPDH was used as an internal control. **B,** Paclitaxel sensitivity of A549/Taxol N cell th control or GBP1 shRNA was analyzed by MTT assay. **C,** Colony formation assay showed that lony f bility of cells was significantly suppressed after the treatment of paclitaxel and GBP1 shRNA (magnifi he results represented the average of three independent experiments. *p<0.05.

knockdown of GBP1 participat n th la- tion of Wnt/β-catenin signalin thwa inhibiting NSCLC develop versing paclitaxel resistance.

Dis

Guanine nucleotide-binding p (GBP1) belongs to the e GTPase fam which is encoded by a e clu er located on chromosome[10]. As FN-g na-response mediator, GBP-1 inhi ll ferati by suppressing the Hippo sig nscri factor TEAD[11]. *Via* mediating EC P-1 enhances the progre glioblas *n vivo*[12]. The knockdow affects the growth of triple-negati reast serves as a novel po- t er [13]. The overexpression of GBP ficantly associated with resistance to paclita oor prognosis of ovarian cancer patients[14]. In dy, we explored the function of GBP1 in the proliferation and apoptosis of NSCLC cells. The results showed that GBP1 expression was significantly upregulated in NSCLC tissues. After GBP1 was knocked down, NSCLC cell proliferation was suppressed, while cell apoptosis was promoted. These data indicated that GBP1 functioned as an oncogene in NSCLC and promoted its tumorigenesis.

Although chemotherapy is effective for NSCLC patients at an early stage of treatment, many patients develop resistance to chemotherapeutic drugs during the period of treatment. This brings a huge burden to the patient's family and the society. The possible mechanisms underlying drug resistance include DNA damage repair, drug transport system error, anti-apoptosis, self-protection of thiol molecules inside the cells, etc. All of them are related to dysregulated genes and activation or inhibition of signaling pathways in NSCLC cells. Paclitaxel, as a clinically common chemotherapy drug, cannot avoid drug resistance during the treatment. However, the paclitaxel resistance remains unclear currently. In the present study, the target genes and related signaling

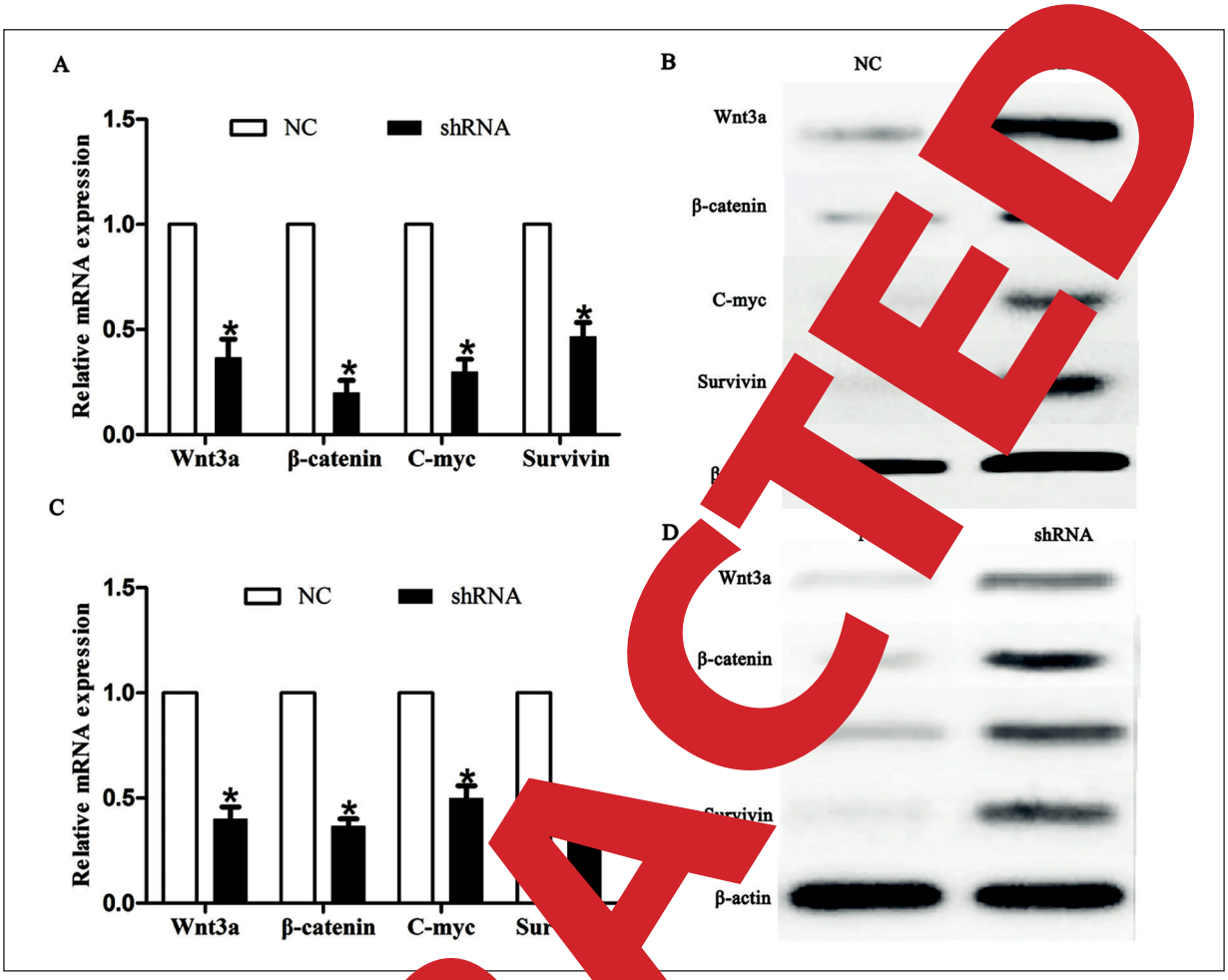


Figure 4. Knockdown of GBP1 inhib ment and re sed paclitaxel resistance through Wnt/β-catenin signaling pathway in NSCLC. **A,** RT-qPCR re revea mRNA expression of target proteins in Wnt/β-catenin signaling pathway was downregulated in A5 ls of mpared with NC group. **B,** Western blot assay revealed that the expression of the target protei in sig g pathway was downregulated in A549 cells of shRNA group compared with NC group. **C,** RT-qP revealed that the mRNA expression of target proteins in Wnt/β-catenin signaling pathway was downr ed in A cells of shRNA group compared with NC group. **D,** Western blot assay revealed that the expression target protei β-catenin signaling pathway was downregulated in A549/Taxol cells of shRNA group compared e NC group. Th sults represented the average of three independent experiments. Data were presented as mean ± r of the mean. *p<0.05.

pathways ass ted w th paclitaxel resistance were explor Our ings showed that cell proliferati si icantl ppressed after knockdown o A54 xol cells.

Canonical Wn ignaling functions as an nt path volved in phenotype and stance of ancer-initiating cells. W -cater pathway plays an ex- t in embryonic develop- me proliferation, cell differentiation, cancer, gene was originally named Int-1 gene in 198 resent, it has been identified as an oncogene by numerous researches[15,16]. There are 19 members in the Wnt gene family. Many previous studies have demonstrated that the Wnt signaling pathway plays an extremely important role in the development of various malignant cancers[17,18]. The most common mechanism of activation of the Wnt signaling pathway is through regulating β-catenin. Briefly, β-catenin excessively accumulates in the cytoplasm and activates TCF/LEF inside the nucleus, further regulating the related pathways. Dysregulated β-catenin is discovered in different types of human tumors. Whether the Wnt/β-catenin signaling pathway plays a key role in the drug resistance of NSCLC cells still

requires more comprehensive researches[19,20]. In our study, we explored the association between Wnt/β-catenin pathway and GBP1. GBP1 knockdown significantly downregulated the mRNA and protein expressions of target proteins in Wnt/β-catenin signaling pathway *in vitro*. These results above suggested that the knockdown of GBP1 might inhibit tumorigenesis and reverse paclitaxel resistance of NSCLC *via* regulating Wnt/β-catenin signaling pathway.

Conclusions

GBP1 served as a novel biomarker in the development of NSCLC. Furthermore, it could enhance NSCLC tumorigenesis and chemoresistance through activation of the Wnt/β-catenin signaling pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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