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Running title: CDH17 reduces cisplatin sensitivity in gastric cancer cells

CDH17 facilitates β-catenin nuclear translocation to reduce drug sensitivity in cisplatin-resistant gastric cancer cells

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Chemoresistance greatly impairs the effectiveness of chemotherapy in gastric cancer (GC) patients. According to our prior results, Cadherin-17 (CDH17) contributes to chemoresistance in GC through activating the Wnt/β-catenin pathway; however, its specific molecular mechanisms require further elucidation. We compared the Wnt/β-catenin pathway activation levels between cisplatin (DDP)-resistant GC cell lines and their parental cell lines. Subsequently, we carried out loss-of-function and gain-of-function tests to investigate CDH17 for its effect on regulating β-catenin expression, nuclear transport, as well as transcriptional activity within DDP-resistant GC cells. Additionally, CDH17 was examined for its role in the expression of four ABC transporters using molecular assays. Finally, rescue experiments were carried out using the Wnt signaling pathway agonist CP21R7 and inhibitor IWR-1 to elucidate the specific mechanism of CDH17 in promoting chemotherapy resistance of GC cells. The results showed that the activation level of the Wnt/β-catenin signaling pathway was significantly elevated in DDP-resistant GC cell lines compared to their parental cell lines. Silencing CDH17 resulted in reduced expression, impaired nuclear translocation, and decreased transcriptional activity of β-catenin, whereas overexpression of CDH17 had the opposite effects. Notably, CDH17 was shown to specifically regulate the expression of ABCB1 (protein name: P-glycoprotein, P-gp) in resistant cells, with no observable impact on the other three ABC transporters (ABCC1, ABCG2, and ABCC2) examined. Importantly, treatment with IWR-1 effectively reversed the enhancing effect of CDH17 overexpression on P-gp protein expression, as well as its suppressive effects on DDP accumulation and chemosensitivity. Conversely, administration of CP21R7 attenuated the inhibitory consequences of CDH17 silencing on P-gp expression, DDP efflux, and drug resistance. In conclusion, CDH17 promotes the expression and nuclear translocation of β-catenin in GC cells, leading to activation of the Wnt/β-catenin signaling pathway, which subsequently upregulates ABCB1/P-gp expression and enhances cellular capacity for DDP efflux. These findings imply that targeting CDH17 could be a potential strategy for overcoming chemotherapy resistance in GC.

Key words: gastric cancer; chemotherapy resistance; Cadherin-17; Wnt/β-catenin signaling pathway; ABCB1

Gastric cancer (GC) shows the highest prevalence among malignant tumors globally. Based on

GLOBOCAN 2022 data, GC takes the fifth place with regard to global morbidity and mortality of all cancer types [1]. Driven by socioeconomic factors, the disease continues to exhibit increasing morbidity and mortality rates in Asia [2, 3]. Overall, factors including improvements in dietary patterns, the eradication of Helicobacter pylori infection, and advances in healthcare consistently decline GC morbidity and mortality rates recently [4, 5]. Despite these advancements, advanced GC patients still have unfavorable prognostic outcome, with tumor metastasis, recurrence and chemoresistance representing major contributors to treatment failure [6].

Chemotherapy represents one of the standard therapeutic approaches for GC, with commonly administered agents including cisplatin (DDP), paclitaxel, and 5-fluorouracil [7]. However, the clinical efficacy of these chemotherapeutic agents is usually hampered by multidrug resistance (MDR), which constitutes an important barrier to favorable therapeutic outcomes in GC [8, 9]. P-glycoprotein (P-gp), belonging to the ATP binding cassette (ABC) transporter family, represents an ATP-dependent transmembrane efflux transporter predominantly encoded by the gene ABC subfamily B member 1 (ABCB1, called multidrug resistance 1 (MDR1) as well), in humans [10]. It mainly functions as a "drug efflux pump", harnessing ATP-derived energy to actively transport various chemotherapeutics outside cells. This process decreases drug concentration within cells, thus decreasing the antitumor therapeutic effects and inducing the development of MDR [11]. Inhibitors targeting *ABCB1* can restore the sensitivity of cancer cells to chemotherapeutic agents by suppressing P-gp- mediated drug efflux, representing a promising strategy to overcome clinical chemotherapy resistance in tumor patients [12]. However, due to challenges such as inherent toxicity and the complexity of multidrug resistance mechanisms, no *ABCB1* inhibitor has yet been approved for clinical use in MDR.

As a highly-conserved pathway across multicellular organisms, Wnt/β-catenin pathway exerts an essential effect on embryogenesis, cell proliferation and tissue homeostasis [13]. Nonetheless, when this tightly regulated pathway is persistently abnormally activated, it can strongly promote tumorigenesis and tumor progression [14, 15]. Consequently, it is frequently characterized as an "oncogenic pathway". In normal cells, β-catenin stability can be tightly regulated via the destruction complex comprising APC, Axin, GSK-3β, and CK1, which promotes its phosphorylation and subsequent degradation [16]. In contrast, in tumor cells, this regulatory pathway is frequently impaired, leading to cytoplasmic β-catenin accumulation, nuclear transport, and interaction with transcription factors TCF/LEF. The aberrant activation causes downstream target gene transcription, thereby promoting malignant characteristics of tumor cells [17, 18]. The Wnt/β-catenin pathway is activated inside GC cells, thereby enhancing tumor cell growth, invasion, migration, as well as tumorigenesis [19, 20]. Furthermore, this pathway is related to chemoresistance occurrence among

tumor cells [21-23]. The *ABCB1* promoter harbors a binding site for β-catenin, with evidence confirming a direct interaction between the two [24]. Consequently, activation of the Wnt/β-catenin signaling pathway can enhance both the expression and efflux activity of *ABCB1*, thereby contributing to increased chemoresistance of tumor cells to chemotherapeutic agents [25, 26]. However, it remains unclear whether inhibition of *ABCB1* transcription through modulation of the Wnt/β-catenin signaling pathway can effectively reverse chemoresistance in GC cells.

Cadherin-17 (CDH17) is a member of the calcium-dependent adhesion protein superfamily and is specifically expressed on the plasma membrane of gastrointestinal epithelial cells, where it mediates intercellular adhesion. Its expression is significantly upregulated in GC [27, 28]. CDH17, serving as a specific marker for the gastrointestinal tract, exhibits a positive correlation with the stage, metastasis, and tumor size of GC patients. It also stands as an independent adverse prognostic factor [29, 30]. Moreover, researches have verified that CDH17 can facilitate the biological behaviors of GC cells, including proliferation, migration, invasion, and adhesion, through modulation of the Wnt/β-catenin signaling pathway [31, 32]. Furthermore, our previous studies have demonstrated that silencing CDH17 in GC cells suppresses activation of the Wnt/β-catenin signaling pathway, thereby inhibiting the Warburg effect and ultimately restoring DDP sensitivity [33]. In line with the above results, CDH17 is hypothesized to promote chemoresistance of GC cells by promoting the transcription of *ABCB1* and drug efflux via regulating Wnt/β-catenin pathway. Building upon prior research findings, we further investigated the molecular mechanism through which CDH17 modulates Wnt/β-catenin pathway to contribute to drug resistance of GC cells, thus providing a foundation for targeting CDH17 in treating chemotherapy-resistant GC.

Materials and Methods

Cell culture and transfection. Human GC cells (HGC-27 and AGS) were provided by Cell Resource Center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. DDP-resistant sublines HGC-27/DDP and AGS/DDP were established in our laboratory [33], and their resistance indices were 5.74 and 6.42, respectively. Cells were cultivated within RPMI-1640 (Gibco, Carlsbad, USA) that contained 10% fetal bovine serum (Gibco) at 37 °C with 5% CO_2. Based on the CDH17 coding sequence (NM_001144663.2), the full-length cDNA was cloned and inserted into pcDNA3.1 plasmids to generate the CDH17 overexpression construct (oe-CDH17). Additionally, short interfering RNA sequences targeting the CDH17 coding region (sense strand 5'-GGAAUGUUACAGUUAGCUAAA-3' and antisense strand 5'-UAGCUAACUG UAACAUUCCAG-3'), identified by our previous study, were incorporated into pLKO.1 plasmids to construct the CDH17 knockdown vector (si-CDH17). Subsequently, oe-CDH17 and the control

empty vector (Vector) were transfected into AGS/DDP cells using Lipofectamine^{TM} 3000 reagent (Invitrogen, Carlsbad, USA), while si-CDH17 and its negative control (si-NC) were transfected into HGC-27/DDP cells. After 48 h of transfection, HGC-27/DDP cells were transferred to complete medium supplemented with 5 μM of the Wnt signaling pathway agonist CP21R7 [34] (Selleck, Houston, USA) and cultured for an additional 24 h. Similarly, AGS/DDP cells were transferred to complete medium containing 5 μM of the Wnt signaling pathway inhibitor IWR-1 [35] (MedChem Express, Monmouth Junction, USA) and incubated for 24 h.

Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. The DDP-resistant cell lines (3×10^3/well), following transfection, were inoculated in 96-well plates for overnight incubation. Subsequently, CP21R7 or IWR-1 was administered for a 24 h treatment period. Thereafter, cells were exposed to varying concentrations (0, 0.5, 1, 2, 4, 8, and 16 μg/ml) of DDP (Aladdin, Shanghai, China) for an additional 24 h. Following this, 5 mg/ml MTT solution (Beyotime, Shanghai, China) was added to incubate cells for a 4 h duration away from light. Formazan crystals formed were subjected to dissolution using dimethyl sulfoxide, with absorbance being read with the microplate reader at 490 nm. Data analysis was performed with SPSS 23.0 software to determine the median inhibition concentration (IC_{50}) values for DDP.

Immunofluorescence staining. GC cell lines and DDP-resistant GC cell lines were seeded into 12-well plates at a density of 1×10^5 cells/well and cultured overnight. Following fixation with 4% paraformaldehyde, antigen retrieval and permeabilization were carried out. Cells were blocked for a 30 min duration under ambient temperature before overnight incubation using an anti-active β-catenin primary antibody (#8814, 1:500; Cell Signaling Technology, Danvers, USA) under 4 °C. A CoraLite488-labeled secondary antibody (1:100) was applied for additional 5 min of incubation under ambient temperature away from light. DAPI was employed for nuclear counterstaining. Finally, the expression and subcellular localization of active β-catenin were examined using a laser scanning confocal microscope, and the nuclear expression level of active β-catenin were quantitatively analyzed with Image Pro Plus software.

Quantitative Real-time polymerase chain reaction (qRT-PCR) analysis. Total RNA was isolated from GC cell lines and DDP-resistant GC cell lines using TRIzol reagent (Beyotime). Following the manufacturer's instructions for the BeyoFast^{TM} SYBR Green One-Step qRT-PCR kit (Beyotime), 1 μg of total RNA was used as a template for cDNA synthesis and subsequent qRT-PCR. The thermal cycling conditions were as follows: reverse transcription at 50 °C for 20 min, initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 30 s. Primer sequences are listed in Table 1. β-actin was employed as an internal reference gene, and gene expression levels were normalized and analyzed using the $2^{-\Delta\Delta Ct}$ method.

Co-immunoprecipitation. HGC-27/DDP and AGS/DDP cells were harvested and lysed using Triton X-100 (Beyotime, Shanghai, China). Subsequently, the protein lysates were collected, and the protein concentration within the lysates was ascertained via the BCA method. Protein A/G magnetic beads (MedChem Express, Monmouth Junction, USA) were incubated with the CDH17 antibody (24339-1-AP; Proteintech, Wuhan, China) or an equivalent type of IgG at room temperature for 1.5 h. Thereafter, equal quantities of protein lysates were added to these pre-treated magnetic beads and incubated at 4 °C overnight. Next, the complexes were eluted from the magnetic beads and underwent high-temperature denaturation. A separate portion of the protein lysate was employed as Input. Ultimately, the expression of CDH17 and β-catenin proteins in the eluted products and Input was detected through Western blot analysis.

Western blot analysis. Proteins from cytoplasm and nucleus were sequentially extracted from GC cells together with DDP-resistant GC cells with a Nuclear and Cytoplasmic Protein Extraction kit (Beyotime). RIPA lysis buffer (Beyotime) was utilized to isolate total cell proteins. After determining protein concentrations, samples underwent sodium dodecyl sulfate polyacrylamide gel electrophoresis to separate target proteins before transfer on polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, USA). Membranes later underwent 1 h of blockage using a blocking solution under ambient temperature prior to overnight primary antibody incubation (dilution 1:1000) under 4 °C and 1 h of secondary antibody (dilution 1:10000) probing under ambient temperature. Chemiluminescent detection reagents (Beyotime) were employed to visualize the protein bands on the membrane. Finally, the gray values of the protein bands were quantitatively analyzed using Image J software to evaluate protein expression levels. The primary antibodies used in this study were purchased from Cell Signaling Technology, Inc., including total β-catenin (#9582), phospho-β-catenin (Ser552) (#9566), cellular myelocytomatosis oncogene (c-Myc, #5605), Cyclin D1 (#2978), CDH17 (#85724), P-gp (#13978), multidrug resistance-associated protein 1 (MRP1, #72202), breast cancer resistance protein (BCRP, #42078), multidrug resistance protein 2 (MRP2, #4446), protein kinase B (AKT) (#9272), phospho-AKT (Ser473) (#9271), Lamin B (#13435), and β-actin (#8457).

Luciferase reporter analysis. In this study, the TOPFlash reporter gene plasmid was employed to assess β-catenin-mediated transcriptional activity of TCF/LEF. Briefly, DDP-resistant GC cell lines were seeded into 96-well plates at a density of 3×10^3 cells/well following transfection and cultured overnight. Subsequently, cells were transfected with FOPFlash or TOPFlash plasmids (150 ng, Beyotime) and Renilla plasmid (50 ng) through utilizing Lipofectamine™ 3000. At 24 h later, Reporter Lysis Buffer was added for complete cell lysis, while Dual Luciferase Reporter Gene Assay kit (Beyotime) was employed for measuring firefly and Renilla luciferase activities. The

relative TOP/FOP activity ratio was calculated by normalizing firefly luciferase activity to Renilla luciferase activity to assess the transcriptional activity of TCF/LEF.

Inductively coupled plasma mass spectrometry (ICP-MS). As previously described, intracellular platinum levels were quantified using ICP-MS to assess the drug efflux capacity of cells [36]. Following transfection, DDP-resistant GC cell lines were exposed to 100 μM DDP for 6 h in the presence or absence of CP21R7 and IWR-1. Cells were subsequently harvested, counted, and resuspended in 500 μl of concentrated nitric acid for overnight digestion at 80 °C. After digestion, an internal standard (cadmium, 40 μg/l) was added to each sample, which was then diluted with ultrapure water prior to analysis. The platinum concentration in the samples was subsequently quantified using ICP-MS.

Statistical analysis. Three biological replicates were set in each assay. GraphPad Prism 8 software was adopted for statistical analysis. Between-group difference was compared by an unpaired t-test, while multi-group one by one-way ANOVA plus Tukey's post hoc test in sequence. $P < 0.05$ suggested statistical significance.

Results

Wnt/β-catenin pathway exhibits aberrant activation within DDP-resistant GC cells. Silencing CDH17 gene not only inhibits Wnt/β-catenin pathway activation within DDP-resistant GC cells, but also reverses their resistance to DDP, suggesting that this pathway may represent a key molecular mechanism through which CDH17 regulates DDP resistance in GC [33]. For better elucidating that Wnt/β-catenin pathway is involved in CDH17-induced DDP resistance, this study first assessed the pathway activation status within DDP-resistant GC cells. As discovered, compared with parental cells, total β-catenin and corresponding downstream targets, Cyclin D1 and c-Myc, had markedly elevated levels inside DDP-resistant cell lines (Figure 1A). Furthermore, the cytoplasmic and nuclear total β-catenin level significantly increased within these DDP-resistant cells (Figure 1B). Active β-catenin was further analyzed for its subcellular localization and expression, which significantly accumulated within nuclei in DDP-resistant GC cells (Figure 1C). The above results indicate aberrant Wnt/β-catenin pathway activation in DDP-resistant GC cell lines.

CDH17 modulates β-catenin expression and nuclear translocation in DDP- resistant GC cells. Next, we further investigated whether CDH17 is involved in the activation of the Wnt/β-catenin pathway inside DDP-resistant GC cells through loss-of- function and gain-of-function experiments. First, CDH17 was silenced within HGC-27/DDP cells but overexpressed in AGS/DDP cells (Figures 2A, 2B). Subsequently, silencing CDH17 reduced total β-catenin and p-β-catenin (Ser552) (phosphorylation at this site facilitates β-catenin nuclear transport) protein levels in HGC-27/DDP

cells, whereas CDH17 overexpression up-regulated their levels inside AGS/DDP cells (Figure 2C). Furthermore, in the nuclei of CDH17-silenced HGC-27/ DDP cells, total β-catenin level decreased (Figure 2D), accompanied by the significantly reduced active β-catenin accumulation and markedly reduced TCF/LEF transcriptional activity (Figures 2E-2G). In contrast, CDH17-overexpressing AGS/DDP cells exhibited the opposite pattern, as evidenced by higher total β-catenin levels, enhanced active β-catenin nuclear transport, and significantly elevated TCF/LEF transcriptional activities. Together, these findings clearly demonstrate that CDH17 promotes β-catenin nuclear transport, thus leading to Wnt/β-catenin pathway activation in DDP-resistant GC cells.

CDH17 up-regulates *ABCB1* expression by activating Wnt/β-catenin pathway within DDP-resistant GC cells. ABC transporters are membrane-bound proteins widely expressed in mammals that facilitate the efflux of various endogenous substrates via transmembrane transport [37]. This activity prevents the intracellular or tissue accumulation of drugs, thereby potentially reducing their therapeutic efficacy [38]. *ABCB1*/P-gp, ATP binding cassette subfamily C member 1 (*ABCC1*/MRP1), ATP binding cassette subfamily C member 2 (*ABCC2*/MRP2), and ATP binding cassette subfamily G member 2 (*ABCG2*/BCRP) are key members belonging to ABC transporter family, which have important effects on MDR occurrence within tumor cells [39]. To investigate the regulatory mechanism of CDH17 in regulating DDP resistance inside GC cells, these four key ABC transporters were analyzed for their levels. The results demonstrated that they were remarkably upregulated in DDP-resistant cell lines compared to their parental counterparts. Silencing of CDH17 led to a marked reduction in *ABCB1*/P-gp expression in HGC-27/DDP cells, whereas CDH17 overexpression further enhanced *ABCB1*/P-gp levels in AGS/DDP cells. In contrast, neither CDH17 knockdown nor its overexpression exerted significant effects on the expression of the other three ABC transporters (Figures 3A, 3B). As CDH17 enhances Wnt/β-catenin pathway activation within DDP-resistant GC cells, besides, *ABCB1* serves as β-catenin's target gene [24, 40], we hypothesize that CDH17 may upregulate *ABCB1* expression through modulation of Wnt/β-catenin pathway, thereby promoting chemoresistance of GC cells. Subsequently, according to our additional findings, activating Wnt pathway via CP21R7, the Wnt signaling pathway agonist, upregulated P-gp protein levels within HGC-27/DDP cells and reversed the CDH17 silencing-mediated P-gp down-regulation. Conversely, inhibiting the Wnt signaling pathway using IWR-1, a Wnt signaling pathway inhibitor, reduced P-gp protein expression in AGS/DDP cells and mitigated the enhancement of P-gp level by CDH17 overexpression (Figure 3C). From the above findings, CDH17 up-regulates *ABCB1* within DDP-resistant GC cells through modulating Wnt/β-catenin pathway.

CDH17 enhances the DDP efflux capability and drug resistance of GC cells through

modulation of the Wnt/β-catenin signaling pathway. To further validate the scientific validity of the aforementioned hypothesis, intracellular platinum levels were measured using ICP-MS. The results demonstrated that silencing of CDH17 led to increased platinum accumulation in HGC-27/DDP cells. Conversely, activation of the Wnt signaling pathway via the agonist CP21R7 reduced platinum content and counteracted the DDP accumulation induced by CDH17 silencing. In contrast, overexpression of CDH17 in AGS/DDP cells resulted in decreased intracellular platinum levels. Notably, inhibition of the Wnt signaling pathway using IWR-1 not only enhanced intracellular platinum concentration but also reversed the DDP efflux caused by CDH17 upregulation (Figure 4A). Furthermore, we conducted an additional evaluation of cellular drug resistance using the MTT assay. The results demonstrated that silencing CDH17 enhanced the sensitivity of HGC-27/ DDP cells to DDP, with an IC_{50} value of 5.035. Treatment with CP21R7 increased cellular resistance to DDP (IC_{50}=21.555) and counteracted the suppressive effect of CDH17 silencing on drug resistance in HGC-27/DDP cells (IC_{50}=8.033). Conversely, upregulation of CDH17 reduced the sensitivity of AGS/DDP cells to DDP, yielding an IC_{50} value of 29.848. Importantly, IWR-1 treatment attenuated cellular drug resistance (IC_{50}=9.274) and reversed the enhancing effect of CDH17 overexpression on drug resistance in AGS/DDP cells (IC_{50}=12.270) (Figure 4B). In short, the aforementioned results demonstrate that CDH17 enhances the efflux capacity of GC cells to DDP by modulating the Wnt/β-catenin signaling pathway, thereby contributing to increased cellular drug resistance.

Discussion

With the growing emphasis on individualized and precision medicine, targeted therapies and immunotherapies have emerged as promising treatment modalities for patients with advanced or refractory GC [41, 42]. Currently, numerous studies have confirmed that certain molecular targets are involved in the regulation of chemotherapy resistance in GC [43-45]. However, effective targeted therapies to overcome clinical chemotherapy resistance have not yet been established. Our previous research demonstrated that CDH17 mediates the Warburg effect through activation of the Wnt/β-catenin signaling pathway, thereby contributing to the development of chemotherapy resistance in GC [33]. This study further demonstrated that CDH17 promotes the nuclear translocation of β-catenin in DDP-resistant GC cells, resulting in the upregulation of *ABCB1*/P-gp expression and enhancing the efflux of DDP, thereby reducing cellular drug sensitivity. These findings provide additional evidence supporting CDH17 as a potential target for adjuvant therapeutic strategies in GC chemotherapy.

β-catenin serves as the central signal transduction molecule in the Wnt/β-catenin signaling pathway,

and its stability determined by either degradation via the destruction complex or cytoplasmic accumulation is critical for regulating pathway activity. In cancer cells, multiple mechanisms contribute to the abnormal accumulation of β-catenin in the cytoplasm and its subsequent translocation into the nucleus, where it activates the transcription of target genes that would otherwise be subject to degradation under normal physiological conditions. E-cadherin is capable of binding β-catenin, thereby anchoring it to the cell membrane and preventing its involvement in signal transduction [46]. Research has demonstrated that the expression level of *CDH1*, the gene encoding E-cadherin, is significantly downregulated in intestinal-type gastric cancer tissues [47]. The loss of E-cadherin expression results in the release of substantial amounts of β-catenin into the cytoplasm, followed by its nuclear translocation and subsequent activation of downstream target genes, including *c-Myc* and *Cyclin D1*, which promote tumor progression [48]. Additionally, *APC* gene mutations or *CTNNB1* (the gene encoding β-catenin) activating mutations, also facilitate β-catenin stable accumulation [49]. Consequently, Wnt/β-catenin pathway within GC remains an activated state [50, 51]. The findings of Wang et al. indicated that activating Wnt/β-catenin pathway was important for mediating decreased chemosensitivity in GC cells [52]. According to our observations, β-catenin expression and nuclear activation levels markedly elevated within DDP-resistant GC cells compared with those in the parental cell line. Furthermore, c-Myc and Cyclin D1 levels also markedly increased. These findings indicate enhanced Wnt/β-catenin pathway activation within drug-resistant cells relative to their parental counterparts, suggesting that hyperactivation of this pathway probably mediates chemoresistance of GC. Therefore, therapeutic strategies targeting the above pathway represents the candidate approach for overcoming chemoresistance of GC.

Numerous studies have demonstrated that CDH17 exerts a regulatory influence on the Wnt/β-catenin signaling pathway. Targeting CDH17 has been shown to inhibit the activation of this pathway, thereby suppressing the malignant progression in GC [32], hepatocellular carcinoma [53, 54], and colorectal cancer [55]. However, the precise molecular mechanisms through which CDH17 modulates the Wnt/β-catenin signaling pathway remain incompletely understood. Phosphorylation of β-catenin acts as a critical molecular switch governing its stability and degradation. The destruction complex mediates sequential phosphorylation (Ser45) of the N-terminal region of β-catenin, facilitating its recognition by E3 ubiquitin ligases, which leads to ubiquitination and degradation [56]. In contrast, phosphorylation at C-terminal residues, including Ser552 and Ser675, mediated by protein kinases such as PKA and AKT, promotes β-catenin stabilization and enhances its transcriptional activity [57, 58]. Studies have verified that the silencing of CDH17 can decrease AKT activation in pancreatic cancer, melanoma, and breast cancer cells [59, 60]. This study

discovered that CDH17 promotes AKT phosphorylation in DDP-resistant GC cells (Supplementary Figure S1A) and upregulates the expression of β-catenin as well as its phosphorylation at Ser552, thus facilitating the nuclear translocation of β-catenin. It is well established that the interaction between β-catenin and the TCF/LEF family of transcription factors constitutes a central mechanism through which β-catenin regulates the expression of target genes [18]. In this study, CDH17 was found to significantly enhance β-catenin- mediated TCF/LEF transcriptional activity in DDP-resistant GC cells. These findings indicate that CDH17 promotes the transcriptional activity of β-catenin by upregulating its expression and inducing phosphorylation at Ser552. This study verified via the Co-IP experiment that in HGC-27/DDP and AGS/DDP cells, no significant physical binding between CDH17 and β-catenin was observed (Supplementary Figure S1B). Nevertheless, it is yet to be determined whether this interaction is direct or indirect. It is hypothesized that CDH17, serving as an upstream regulatory factor, may regulate the stability, phosphorylation, and nuclear translocation of β-catenin via indirect mechanisms (such as disrupting the E-cadherin complex [61], activating kinase pathways like EGFR/PI3K/AKT, and regulating the desmosomal cadherin DSC1 [62]). In summary, CDH17 is a crucial factor in regulating the activation of the Wnt/β-catenin signaling pathway in drug-resistant GC cells.

Study has demonstrated that silencing CDH17 downregulates the expression of drug resistance-associated transporters in colorectal cancer cells, thereby enhancing the sensitivity of these cells to chemotherapeutic agents [55]. Furthermore, CDH17 knockdown has been shown to suppress the tumorigenic potential of lung cancer cells and increase their responsiveness to DDP [63]. Wang et al. developed an antibody- drug conjugate targeting CDH17, designated 7MW4911, which demonstrated favorable efficacy and safety profiles in overcoming multidrug resistance in gastrointestinal cancers [64]. Collectively, these findings indicate that targeting CDH17 holds significant promise for mitigating chemotherapy resistance in cancer. In this study, we observed that CDH17 promotes the expression of *ABCB1*/P-gp, a member of the ABC transporter protein family, in DDP-resistant GC cells, without affecting the expression of other transporters such as *ABCC1*/MRP1, *ABCG2*/BCRP, and *ABCC2*/MRP2. *ABCB1* is a downstream target of β-catenin, and its expression is regulated by the Wnt/β-catenin signaling pathway [24, 40]. Our experimental results further confirmed that CDH17 promotes the expression of *ABCB1*/P-gp through activation of the Wnt/β-catenin signaling pathway. Silencing CDH17 suppresses the expression of *ABCB1*/P-gp and diminishes the efflux capacity of DDP-resistant GC cells toward DDP.

In conclusion, this study has further elucidated the molecular mechanism through which CDH17 regulates chemotherapy resistance in GC. Specifically, CDH17 promotes the expression and nuclear translocation of β-catenin, leading to activation of the Wnt/β-catenin signaling pathway, which in

turn upregulates the expression of *ABCB1*/P-gp and enhances cellular DDP efflux, thereby reducing drug chemosensitivity. By progressively uncovering the role of CDH17 in mediating chemotherapy resistance, this work provides essential preclinical evidence supporting the potential clinical application of CDH17-targeted inhibitors to overcome drug resistance in GC. Nevertheless, the absence of in vivo validation constitutes a key limitation of the present study and represents an important focus for future research. In addition, copper-transporting ATPase α polypeptide (ATP7A) and β polypeptide (ATP7B) are regarded as contributors to the efflux of DDP [65]. A decrease in the expression of ATP7A and ATP7B can reverse the resistance of cancer cells to DDP [66, 67]. It is possible that CDH17 may influence the efflux of DDP in drug-resistant gastric cancer cells by regulating the expression of ATP7A and ATP7B; however, this still necessitates more experimental data for investigation.

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Supplementary data are available in the online version of the paper.

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Figure Legends

Figure 1. The Wnt/β-catenin signaling pathway is abnormally activated in DDP- resistant GC cell lines. A) Western blot analysis was employed to assess the expression levels of total β-catenin as well as its downstream targets, c-Myc and Cyclin D1 in GC cells. B) Subcellular fractionation followed by Western blot analysis was performed to examine the expression of total β-catenin in the cytoplasm and nucleus of GC cells. C) Immunofluorescence staining was employed to assess the subcellular localization and expression levels of active β-catenin in GC cells. Scale bar=10 μm. *p < 0.05, **p < 0.01, ***p < 0.001

Figure 2. CDH17 promotes the expression and nuclear translocation of β-catenin in DDP-resistant GC cell lines. CDH17 silencing was performed in HGC-27/DDP cells, and CDH17 overexpression was conducted in AGS/DDP cells. A, B) The mRNA and protein expression levels of CDH17 were assessed by qRT-PCR and Western blot analyses, respectively. C, D) The cellular levels of total β-catenin, p-β-catenin (Ser552), and nuclear total β-catenin were detected using Western blot analysis. E, F) The expression of activated β-catenin in the nucleus was evaluated using immunofluorescence staining. Scale bar=10 μm. G) Following transfection with TOPFlash and FOPFlash reporter plasmids, TCF/LEF transcriptional activity was measured using luciferase

reporter analysis. *p < 0.05, ** p < 0.01, ***p < 0.001

Figure 3. CDH17 promotes *ABCB1* expression through activation of the Wnt/ β-catenin signaling pathway in DDP-resistant GC cell lines. CDH17 silencing was performed in HGC-27/DDP cells, and CDH17 overexpression was conducted in AGS/DDP cells. A, B) The mRNA and protein expression levels of *ABCB1*/P-gp, *ABCC1*/MRP1, *ABCG2*/BCRP, and *ABCC2*/MRP2 in the GC cell lines and DDP-resistant GC cell lines were assessed by qRT-PCR and Western blot analyses. C) Following intervention with the Wnt signaling pathway agonist CP21R7 or the inhibitor IWR-1, the expression level of P-gp in DDP-resistant GC cells was assessed via Western blot analysis. *p < 0.05, **p < 0.01, ***p < 0.001

Figure 4. CDH17 enhances the DDP efflux capability and drug resistance of GC cells through modulation of the Wnt/β-catenin signaling pathway. Following silencing or overexpression of CHD17 in DDP-resistant GC cells, the cells were treated with either the Wnt signaling pathway agonist CP21R7 or the inhibitor IWR-1. A) Intracellular platinum level was quantified using ICP-MS to assess cellular drug efflux capacity. B) Cell proliferation following treatment with varying concentrations (0, 0.5, 1, 2, 4, 8, and 16 μg/ml) of DDP was evaluated via MTT assay to determine cellular sensitivity to DDP. *p < 0.05, **p < 0.01, ***p < 0.001

631 **Table 1.** The sequences of the primers used in qRT-PCR.

Gene	Primer sequences
CDH17	Forward primer: 5'-AGGCCAAGAACCGAGTCAAAT-3'
	Reverse primer: 5'-GCAACCTGGAGATTGTGAGTAGA-3'
ABCB1	Forward primer: 5'-GGCCTAATGCCGAACACATT-3'
	Reverse primer: 5'-CAGCGTCTGGCCCTTCTTC-3'
ABCC1	Forward primer: 5'- GGTGGACGAGAACCAGAAGG-3'
	Reverse primer: 5'-TCAAGTACGTGGTGACCTGC-3'
ABCG2	Forward primer: 5'-CAGGTGGAGGCAAATCTTCGT-3'
	Reverse primer: 5'-ACACACCACGGATAAACTGA-3'
ABCC2	Forward primer: 5'-CCAAAGACAACAGCTGAAA-3'
	Reverse primer: 5'-TACTTGGTGGCACATAAAC-3'
β-actin	Forward primer: 5'-CATGTACGTTGCTATCCAGGC-3'
	Reverse primer: 5'-CTCCTTAATGTCACGCACGAT-3'

632 Abbreviations: CDH17-Cadherin-17; ABCB1-ATP binding cassette subfamily B member 1;
633 ABCC1-ATP binding cassette subfamily C member 1; ABCG2-ATP binding cassette subfamily G
634 member 2; ABCC2-ATP binding cassette subfamily C member 2

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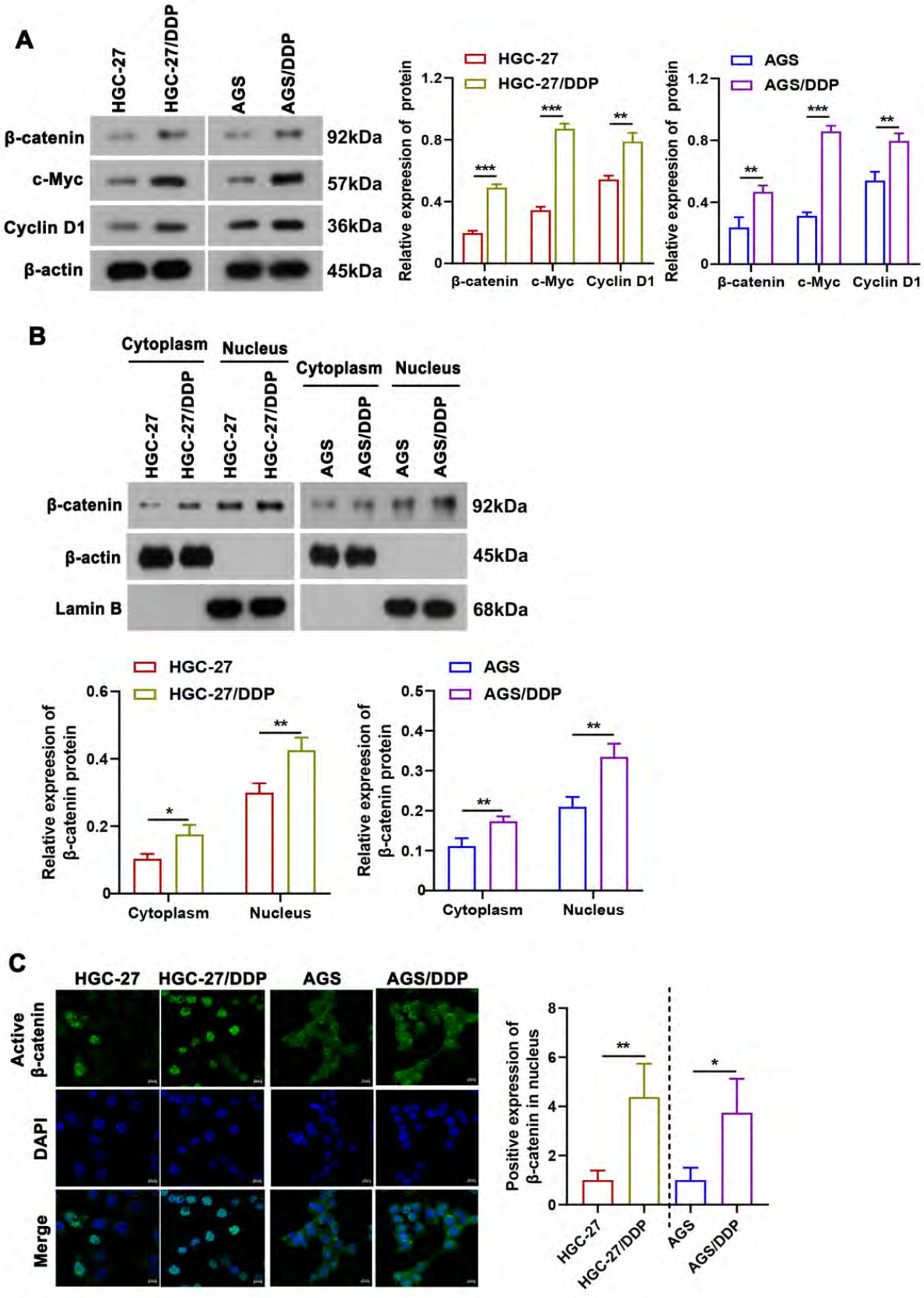

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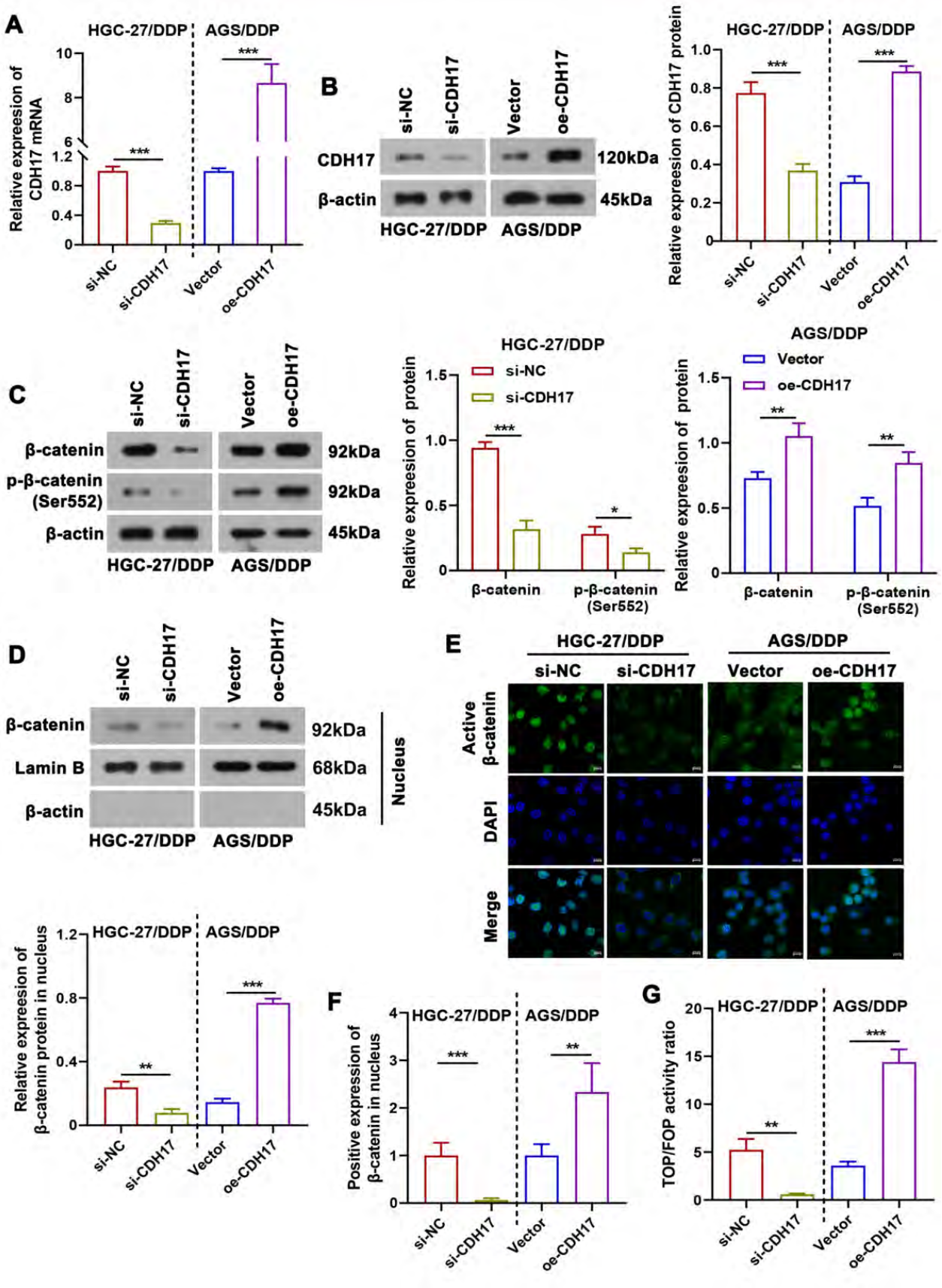

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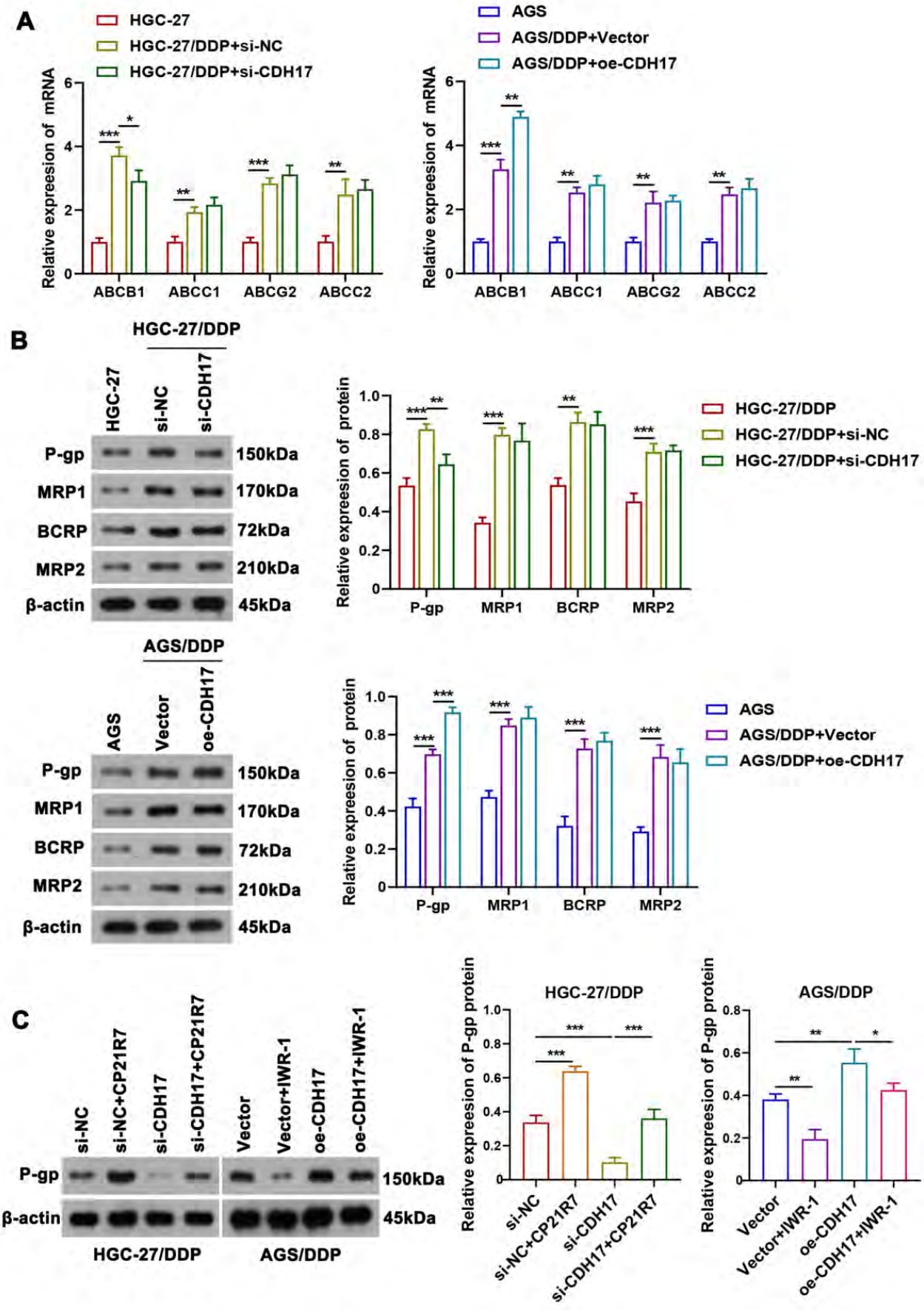

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