WITHDRAWN:
Tripartite Motif Containing 37 Activates the Wnt/β-catenin Signalling Pathway and Confers Cisplatin Resistance to Ovarian Carcinoma

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Research

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EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

Background

Emerging evidence shows that the deregulation of tripartite motif (TRIM) family proteins have various functions in cellular processes and play important role in innate immunity, nervous system diseases, protein quality control and carcinogenesis. However, the precise biological function and molecular mechanism of TRIM family proteins in ovarian cancer chemo-resistance remain unclear.

Methods

The protein and mRNA expression of TRIM37 in ovarian cancer cell lines and patient tissues were determined using Real-time PCR and Western blot and IHC respectively. Functional assays, such as MTT, FACS, and Tunel assay used to determine the oncogenic role of TRIM37 in human ovarian cancer progression. Furthermore, western blotting and luciferase assay were used to determine the mechanism of TRIM37 promotes chemoresistance in ovarian cancer cells.

Results

Herein, we found that the protein and mRNA expression of TRIM37 were markedly overexpressed in ovarian cancer tissues which shown partially responded to cisplatin chemotherapy. Moreover, TRIM37 expression was inversely correlated with patient survival in our cohort HCC tissue samples and public HCC database. Overexpression of TRIM37 confers cisplatin resistance on ovarian cancer cells; but, inhibition of TRIM37 sensitized ovarian cancer cell lines to cisplatin cytotoxicity both *in vitro* and *in vivo*. Additionally, TRIM37 upregulated the levels of nuclear β-catenin, thereby activating canonical wnt/β-catenin signaling.

Conclusions

our results demonstrate that targeting TRIM37/β-catenin axis may represent a promising strategy to enhance cisplatin response in patients with chemo-resistant ovarian cancer.

Background

Epithelial ovarian cancer is the most common cause of gynecological cancer death in the world and the majority of patients diagnosed at an advanced stage[1, 2]. Treatment approach of ovarian cancer mainly involves in combination with surgery and platinum/taxane chemotherapy ccurrently[3, 4]. Despite advances in treatment of ovarian cancer, overall survival for patients with advanced ovarian cancer still remain declining due to lacking of new diagnostic molecular marker[5]. Furthermore, the recommended management of ovarian cancer is primary cytoreductive followed by a platinum-based combination

chemotherapy; but, more than 75% of treated patients experience chemo-resistance and ultimately bearded tumor relapse[6]. Therefore, ovarian cancer was confronted with a critical research challenge due to poorly understanding of the mechanism of chemo-resistance and illustrate the molecular mechanisms underlying ovarian cancer chemo-resistance is urgent to improve clinical outcomes.

The Wnt/β-catenin signaling is a key developmental pathway and it has been reported that survival cascades initiated by Wnt/β-catenin signaling pathway is a key component of cellular apoptotic resistance[7, 8]. Reportedly, miR-128-3p induces mesenchymal and stemness-like properties then confers chemo-resistance-associated metastasis via simultaneous over-activation of Wnt/β-catenin and TGFβ signaling pathway in NSCLC[9]. Wickström and colleges shown that the DNA repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) regulated by Wnt/β-catenin pathway confers chemo-resistant to colon cancer[10]. SOX8 confers chemo-resistance and cancer stem-like properties, thereby mediates EMT processes in chemo-resistant tongue squamous cell carcinoma by acting on the FZD7-mediated Wnt/β-catenin pathway[11]. Zhang et al. found that Pygo2-mediated MDR1 overexpression in breast cancer play a clinical chemo-resistance via the Wnt/β-catenin pathway[12]. Blocking Wnt/β-catenin signaling using a natural compound Jatrophone inhibits proliferation and EMT in human triple-negative breast cancer[13]. Moreover, ICG-001, a small molecule binds CREB-binding protein (CBP) to disrupt its interaction with β-catenin, suppresses gastric cancer cell line growth, metastasis and reduces its stem cell-like properties and chemo-resistance[14]. The above studies suggest that Wnt/β-catenin signaling play an important role in cancer progression and inhibition β-catenin signaling may prevent recurrence and chemo-resistance in cancer. Therefore, the discovery of novel molecules capable of regulating aberrant activation of the Wnt/β-catenin signaling pathway may facilitate the treatment of chemo-resistant cancers.

Tripartite motif (TRIM) family proteins, most of which containing a RING-finger domain, could be defined as E3 ubiquitin ligases[15, 16]. Most of TRIM family proteins are involved in a broad range of biological processes range from regulation of immune, viral restriction and cell stress responses to proliferation, apoptosis, differentiation, transcription, DNA repair and carcinogenesis[17–19]. For example, deletion-mediated *TRIM3* downregulation led to NF-κB constitutive activation through disruption of the NF-κB-IκB-α negative feedback loop and promoted lymphatic metastasis of esophageal squamous cell carcinoma cells[20]. Another report demonstrated that TRIM14 stabilized dishevelled (Dvl2) and subsequently activated the canonical Wnt signaling and promoted chemo-resistance in gliomas[21]. Wang and colleges also that the ubiquitin E3 ligase TRIM31 regulates chronic inflammation via NF-κB signal pathway to promote invasion and metastasis in colorectal cancer [22]. These findings suggest that TRIM family proteins contributes to malignant development of tumor. Therefore, it would be worthy to enunciate the biological effects and molecular mechanisms of TRIM family proteins in ovarian cancer chemo-resistant.

MATERIALS and METHODS

Cell lines and cell culture.

The ovarian cancer cell lines, including A2780(parental) and A2780/cis (Cisplatin-resistant) was purchased from The European Collection of Authenticated Cell Cultures (ECACC), A2780 was grown in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen), at 37 °C in a 5% CO2 atmosphere in a humidified incubator. A2780/cis was grown in RPMI 1640 + 2 mM Glutamine + 1 μM cisplatin + 10% Foetal Bovine Serum (FBS), at 37 °C in a 5% CO2 atmosphere in a humidified incubator. All cell lines were authenticated by short tandem repeat (STR) fingerprinting at Medicine Lab of Forensic Medicine Department of Sun Yat-Sen University (Guangzhou, China).

Patient information and tissue specimens.

A total of 145 paraffin-embedded and archived ovarian cancer samples, which were histopathologically and clinically diagnosed at the Longgang District Central Hospital of Shenzhen from 2005 to 2010, were examined in this study. All of the patients received standardized platinum-based chemotherapy. Platinum resistance or sensitivity was defined as relapse or progression within 6 months or after 6 months from the last platinum-based chemotherapy, respectively. Clinical information on the samples is summarized in Supplementary Table 1. All tumours were staged according to the International Federation of Gynaecology and Obstetrics standards (FIGO). Prior patient consent and approval from the Institutional Research Ethics Committee were obtained for the use of these clinical materials for research purposes.

Vectors, retroviral infection and transfection.

A TRIM37 expression construct was generated by subcloning PCR-amplied full-length human TRIM37 cDNA into the pMSCV retrovirus plasmid, and human TRIM37 targeting short hairpin RNA (shRNA) oligonucleotides sequences were cloned into pSuper-retro-puro to generate pSuper-retro- TRIM37-shRNA(s). The shRNA sequences were: shRNA #1, TTCGAGAATATGATGCTGTGG; and shRNA #2, TTTGCGAGTAAGTCCAAACGG (synthesized by Invitrogen). β-catenin siRNA: TAAACTGGGCCTTGACCAGGT (synthesized by Invitrogen). Transfection of siRNA or plasmids was performed using the Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. Stable cell lines expressing TRIM37or TRIM37-shRNA were selected for 10 days with 0.5 μg/ml puromycin 48 h after infection.

Western blot analysis.

Western blot was performed using anti-TRIM37 (Abcam), anti-β-catenin, anti-cleaved caspase 3, anti-p84 antibodies (Cell Signaling Technology). The membranes were stripped and re-probed with an anti-α-tubulin antibody (Sigma, Saint Louis, MI) as a loading control.

Cytotoxicity assay.

The sensitivity to cisplatin of ovarian cancer cells was determined using the IC50 assay[23]. Briefly, 2×10^3 cells were seeded onto 96-well plates and incubated at 37 °C overnight. Cells were then transfected

with different concentrations of cisplatin (0-100 μM). After incubation for 72 hours, 50 μl of the MTT solution (0.15%) was added to each well, and the plates were further incubated for 2 hours. One hundred microliters of DMSO was added to solubilize the MTT formazan product. Absorbance at 540 nm was measured with a Falcon microplate reader (BD-Labware). Dose-response curves were plotted on a semilog scale as the percentage of the control cell number, which was obtained from the sample with no drug exposure. IC50 was determined by the intersection of the cisplatin concentration.

Annexin V assay.

For evaluation of apoptosis, PE Annexin V Apoptosis Detection Kit I (BD Pharmingen) was used. Briefly, 1 × 10^6 ovarian cancer cells were plated in 10-cm plates and incubated for 24 hours. Treatment was started with cisplatin (10 μM) for 24 hours. Cell morphology was assessed by phase-contrast microscopy. Then, cells were removed from plate by trypsin-EDTA, washed twice with PBS, and suspended with binding buffer at 10^6 cells/ml. FITC Annexin V and propidium iodide were added (each at 5 μl/10^5 cells). Cells were incubated for 15 minutes at room temperature in the dark. Percentage of apoptosis was analysed with an EPICS XL flow cytometer (Beckman-Coulter). Each sample was analysed in triplicate.

Transient Luciferase Assay

Cells (1 × 10^4) were seeded in triplicate in 48-well plates and allowed to settle for 24 h. For each transfection, one hundred nanograms of luciferase reporter plasmids pGL-3-TRIM37 or vector and 5 ng of pRL-TK, expressing Renilla luciferase as an internal control, were transfected into cells using the Lipofectamine 3000 reagent (Invitrogen) according to the manufacturer's instruction. 48 h after transfection, cells were harvested and Luciferase and renilla signals were measured using the Dual Luciferase Reporter Assay Kit (Promega) according to a protocol provided by the manufacturer. The luciferase activity was normalized by the Renilla luciferase activity of each transfection to normalize the transfection efficiency.

Nuclear and Cytoplasmic Extraction assay.

Nuclear fractions were prepared by using the nuclear extraction kit (Active Motif, Carlsbad, CA). Briefly, after CDDP treatment, cells were pelleted and lysed by vigorous vortex in hypotonic buffer for 15 min. The samples were then centrifuged at 14,000 × g for 1 min; the supernatant was considered cytoplasmic. Insoluble pellets were further lysed in complete lysis buffer for 30 min, and nuclear extracts (supernatant) were collected after a 10-min centrifugation at 14,000 × g. Both cytoplasmic and nuclear fractions were quantified and subjected to Western blot analysis.

Statistical analysis.

Statistical tests for data analysis included Fisher's exact test, log-rank test, Chi-square test, and Student's 2-tailed t test. Multivariate statistical analysis was performed using a Cox regression model. Statistical

analyses were performed using the SPSS 11.0 statistical software package. Data represent mean ± SD. $P < 0.05$ was considered statistically significant.

Microarray data process and visualization.

Microarray data were downloaded from the GEO database and The Cancer Genome Atlas datasets.

GSEA was performed using GSEA 2.0.9 :(http://www.broadinstitute.org/gsea/).

Results

TRIM37 was overexpression in chemo-resistance ovarian cancer tissues.

It has been reported that tripartite motif (TRIM) family proteins have various functions in cellular processes including cell stress responses, signal transduction, immune system diseases and carcinogenesis[15, 24, 25]. However, the biological function of TRIM family proteins in ovarian cancer chemo-resistance remain unclear. The real-time PCR assay showed that among 64 genes of TRIM family proteins, TRIM37 were markedly overexpressed in chemo-resistance ovarian cancer tissues compared to chemo-sensitive ovarian cancer tissues (Fig. 1A). Western blotting analyses revealed that the protein level of TRIM37 was markedly overexpressed in four chemo-resistance ovarian cancer tissues, compared with four chemo-sensitive ovarian cancer tissues (Fig. 1B). Furthermore, IHC assay showed that TRIM37 expression was markedly overexpressed in 145 paraffin-embedded, archived ovarian cancer tissues which received standardized platinum-based chemotherapy (Fig. 1C). Collectively, these results indicate that TRIM37 expression is upregulation in chemotherapy resistant ovarian cancer.

TRIM37 was correlates with progression and poor prognosis in human ovarian cancer.

To determine the clinical relevance of TRIM37 in ovarian cancer, TRIM37 expression was examined and analysed in 145 paraffin-embedded, archived ovarian cancer tissues. As showed in Supplementary Table 1–2, TRIM37 levels were correlated with the FIGO stage ($P < 0.001$), and chemo-resistance ($P = 0.008$) in patients with ovarian cancer. Importantly, statistical analysis showed that ovarian cancer patients with high TRIM37 expression had significantly worse overall and disease-free survival than those with low TRIM37 expression (Fig. 2A-B). Consistent with our results, we found that TRIM37 overexpression was correlated with overall survival and progression-free survival of ovarian cancer patients with platin-resistance in multiple public GEO datasets (Fig. 2C-D). These results suggest that TRIM37 has potential clinical value as a predictive biomarker for ovarian cancer chemo-resistant.

Upregulation of TRIM37 contributes cytotoxicity of ovarian cancer cells in vitro.

GSEA analysis revealed that TRIM37 overexpression was strongly correlated with gene signatures associated with CDDP-based chemotherapy signatures, suggesting that TRIM37 overexpression may contribute to CDDP-resistance in ovarian cancer (Fig. 3A). To investigate the chemo-resistance role of TRIM37 in ovarian cancer, A2780 that stably expressed TRIM37 and A2780/cis that silencing TRIM37 cell lines were established (Fig. 3B). IC50 assay shown that A2780 that stably expressed TRIM37 enhanced

cisplatin resistance compared with the vector control (IC50 values were 5.24 μM and 21.3 μM, respectively) (Fig. 3C). However, A2780/cis that depleted of TRIM37 were less resistant to cisplatin than control (IC50 values were 1.67 μM, 2.01 μM, and 16.85 μM, respectively) (Fig. 3C).

TRIM37 confers CDDP resistance in ovarian cancer in vitro.

Furthermore, the Annexin V and Tunel staining assay show that the percentage of apoptotic cells in A2780/TRIM37 ovarian cancer cells treated with CDDP was much lower compared than that in control cells, but much higher in A2780/cis TRIM37-shRNA cancer cells (Fig. 4A-B). Furthermore, the protein level of cleaved caspase 3, an apoptosis relative gene, was significantly decrease in A2780/TRIM37 overexpression ovarian cancer cells but increase in A2780/cis TRIM37-shRNA cancer cells (Fig. 4C). Interestingly, we also found that overexpressing TRIM37 or silencing TRIM37 only resulted in slightly change of apoptotic rate of ovarian cancer cells without any treatment (Fig. 4D). The above results indicating that deregulation of TRIM37 is involved in CDDP resistance of ovarian cancer cells.

Upregulation of TRIM37 activates the Wnt/β-catenin signalling pathway in ovarian cancer.

In order to better understand the mechanism of TRIM37 induced chemo-resistance, mRNA microarray (GEO-GSE8057) was performed. Gene ontology (GO) enrichment analysis showed that Wnt/β-catenin signalling pathway was enriched in TRIM37 up-regulated genes (Fig. 5A). GSEA analysis show that TRIM37 mRNA expression levels was positively correlated with Wnt/β-catenin signalling gene signatures (Fig. 5B). These results suggest that TRIM37 may play crucial roles in Wnt/β-catenin signalling regulation. As expected, overexpression of TRIM37 significantly enhanced, whereas silencing of TRIM37 reduced, the activity of TOP/FOP luciferase reporter activity in ovarian cancer cells (Fig. 5C). Moreover, western blotting revealed that the levels of nuclear β-catenin were dramatically upregulated in TRIM37-overexpressing cells but were downregulated in TRIM37-silenced cells (Fig. 5D). Furthermore, the expression levels of numerous well-characterized Wnt/β-catenin signalling downstream genes were showed to be increased in TRIM37 overexpressing cells, but were lower in TRIM37-silenced cells (Fig. 5E). These results suggesting that TRIM37 plays an important role in activating the Wnt/β-catenin signalling pathway in ovarian cancer.

Wnt/β-catenin signalling pathway is required for TRIM37 induced chemo-resistance.

Next, we investigated whether TRIM37 mediated ovarian cancer chemo-resistance through Wnt/β-catenin activation. The chemo-resistant effect of TRIM37 on ovarian cancer through Wnt/β-catenin activation was determined by Annexin V and Tunel staining assay. Strikingly, we found that blockade of the Wnt/β-catenin pathway by β-catenin siRNA significantly abrogates the effect of TRIM37 on ovarian cancer aggressiveness in both *in vitro* (Fig. 6A-B). Similar to the effect of β-catenin silencing in TRIM37 overexpression cells, treatment with ICG-001, a specific inhibitor of β-catenin signalling via blockage of β-catenin/CBP interaction also significantly decreased the effect of TRIM37 on ovarian cancer chemo-resistance (Fig. 6A-B). Taken together, these results indicate that activation of the Wnt/β-catenin signalling pathway exerted functional effects of TRIM37 on ovarian cancer chemo-resistance.

Discussion

In the current study, we provide evidence that TRIM37 confers cisplatin resistance to ovarian cancer and actives the Wnt/β-catenin signalling pathway. TRIM37 was significant increase in cisplatin-resistant ovarian cancer tissues and TRIM37 overexpression enhanced cisplatin resistance, but TRIM37 silencing restored the sensitivity of ovarian cancer cells to cisplatin. Moreover, we found that TRIM37 enhanced cisplatin resistance by upregulating downstream target genes that regulate the anti-apoptosis effect of the Wnt/β-catenin signaling pathway. These findings identify TRIM37/β-catenin axis may be a potential target for overcoming cisplatin resistance in patients with ovarian cancer.

It has been reported that the biological functions of TRIM family proteins in cancer in the context of their function as E3 ubiquitin ligases, which containing amino-terminal tripartite domain arrangement — RING–B box1/2–coiled coil (RBCC)[26–28]. For example, it is reported that the ubiquitin E3 ligase TRIM31 promotes aggregation and activation of the signaling adaptor MAVS through Lys63-linked polyubiquitination[29]. Jain et al. demonstrate that TRIM24 undergoes ATM-mediated phosphorylation and autodegradation during DNA damage via p53-induced E3-ubiquitin ligase[30]. Consistent with other family members, TRIM37 act as oncogenic gene via it's E3-ubiquitin ligase function. Bhatnagar and collages reported that TRIM37 is overexpressed in a subset of breast cancers and promotes transformation by facilitating silencing of tumor suppressors via acting as an oncogenic H2A ubiquitin ligase[31]. Moreover, TRIM37 bound to TRAF2 and promoted K63-linked ubiquitination of TRAF2, sustaining the activation of the NF-κB pathway then promotes the aggressiveness of non-small-cell lung cancer cells[32]. Herein, our results show that TRIM37 confers cisplatin resistance to ovarian cancer and actives the Wnt/β-catenin signalling pathway. However, the underlying mechanism by which TRIM37 activates Wnt/β-catenin signalling pathway remains unclear. It is likely that TRIM37 activates Wnt/β-catenin signalling pathway via it's E3 ubiquitin ligases function in ovarian cancer cells. Therefore, the underlying mechanism by which TRIM37 confers chemo-resistance by activates Wnt/β-catenin signalling requires further investigation.

Multiple studies reported that TRIM37 is overexpressed in cancers, including colorectal cancer, pediatric osteosarcoma, pancreatic cancer cells and hepatocellular carcinoma, however, the mechanism of TRIM37 upregulation in cancer remains unknown[33–36]. Interestingly, we found that TRIM37 exhibited a high amplification rate of 21.3% in ovarian cancer according to copy number variation analysis of TCGA datasets (https://www.cureline.com/the-cancer-genome-atlas.html), suggesting that the overexpression of TRIM37 in ovarian cancer is associated with genomic amplification. Furthermore, by using the chromatin immunoprecipitation sequencing tracks in the University of California Santa Cruz Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway), we found that large amounts of β-catenin were recruited to the promoter region of TRIM37. Therefore, it would be worthy to determine whether TRIM37 upregulation in ovarian cancer is attributable to genomic amplification or β-catenin-mediated transcriptional upregulation.

Conclusion

TRIM37 was markedly upregulated in clinical ovarian cancer tissues with chemo-resistance and a positive correlation was evident between TRIM37 expression and the recurrence-free survival of ovarian cancer patients. Overexpression of TRIM37 confers cisplatin resistance of ovarian cancer by activating downstream gene of Wnt/β-catenin signaling pathway. Illustrates the biologic function and molecular mechanism of TRIM37 in ovarian cancer chemo-resistance will advance our knowledge of ovarian cancer chemo-resistance. Our study suggests that TRIM37 may be a potential therapeutic target for overcoming drug resistance in patients with ovarian cancer.

Abbreviations

tripartite motif (TRIM); methyltransferase (MGMT); CREB-binding protein (CBP); dishevelled (Dvl2); short hairpin RNA (shRNA); RING
B box1/2–coiled coil (RBCC);

Declarations

Authors' contributions:

Jinxin Liu and Yizhi Chen carried out the experiments and drafted the manuscript; Dapeng Ding contributed to the immunohistochemistry experiments ; Feiye Liu was involved in the statistical analysis; Yizhi Chen managed the experimental design, reviewed the manuscript and provided funding support. All authors read and approved the final version of the manuscript.

Conflicts of interest:

The authors declare that they have no competing interests.

Consent for publication:

Not applicable.

Funding

Not applicable.

Availability of data and material

The datasets used and analyzed in the current study are available from the corresponding author in response to reasonable requests.

Acknowledgments:

Not applicable.

Ethics approval and consent to participate:

The collection and use of the ovarian cancer tissue samples were reviewed and approved by the Institutional Research Ethics Committee of Longgang District Central Hospital of Shenzhen (China).

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Figures

Figure 1

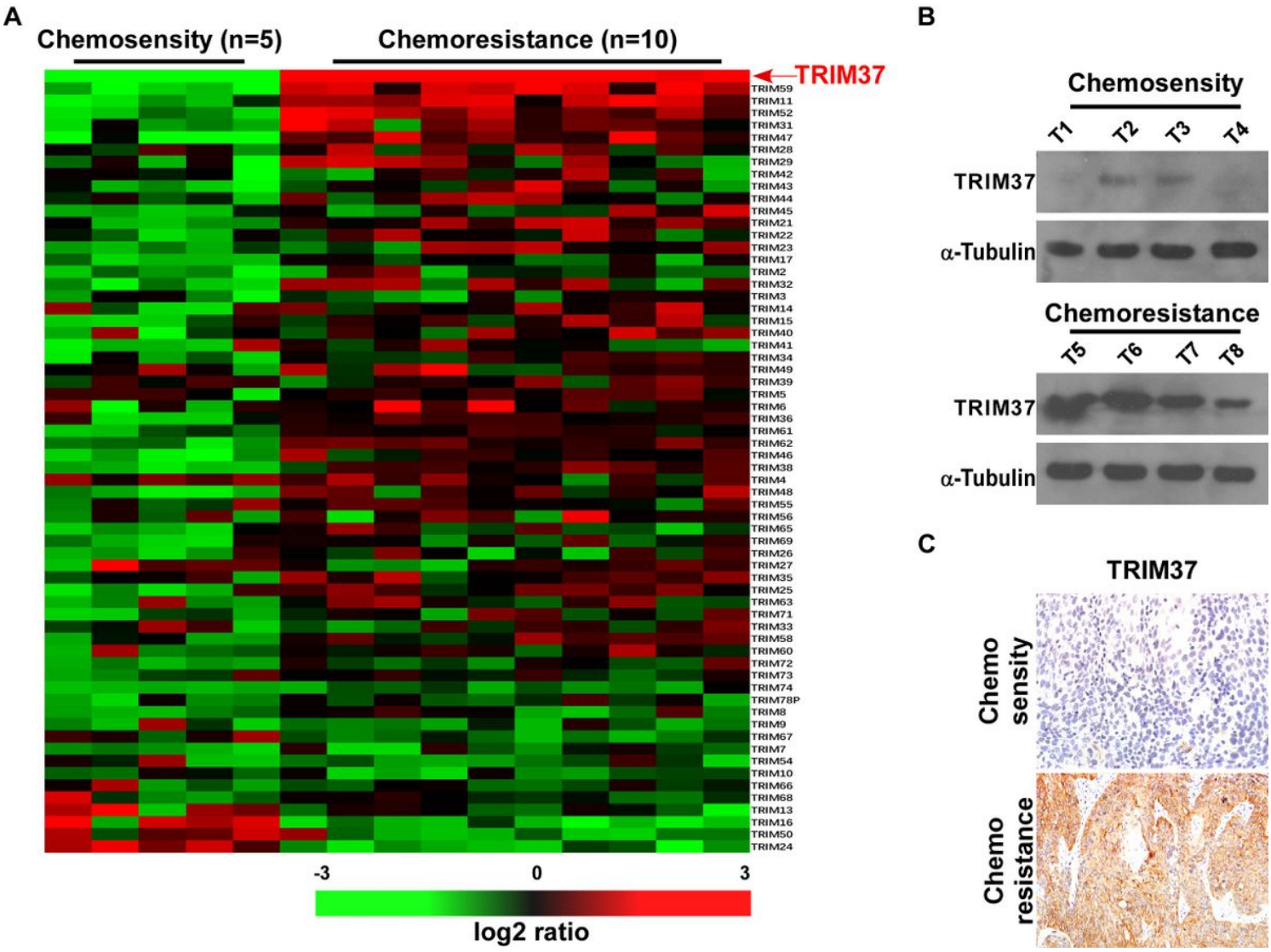

Figure 1

TRIM37 was overexpression in chemo-resistant ovarian cancer. (A). Expression profiling of mRNAs showing that TRIM37 were markedly overexpressed in chemo-resistant ovarian cancer tissues. (B) Western blotting analysis of TRIM37 expression in four chemo-sensitive ovarian cancer tissues and four chemo-resistant ovarian cancer tissues, α-Tubulin was used as a loading control. (C) IHC staining indicating the TRIM37 protein expression in chemotherapy ovarian cancer tissues (n = 145).

Figure 2

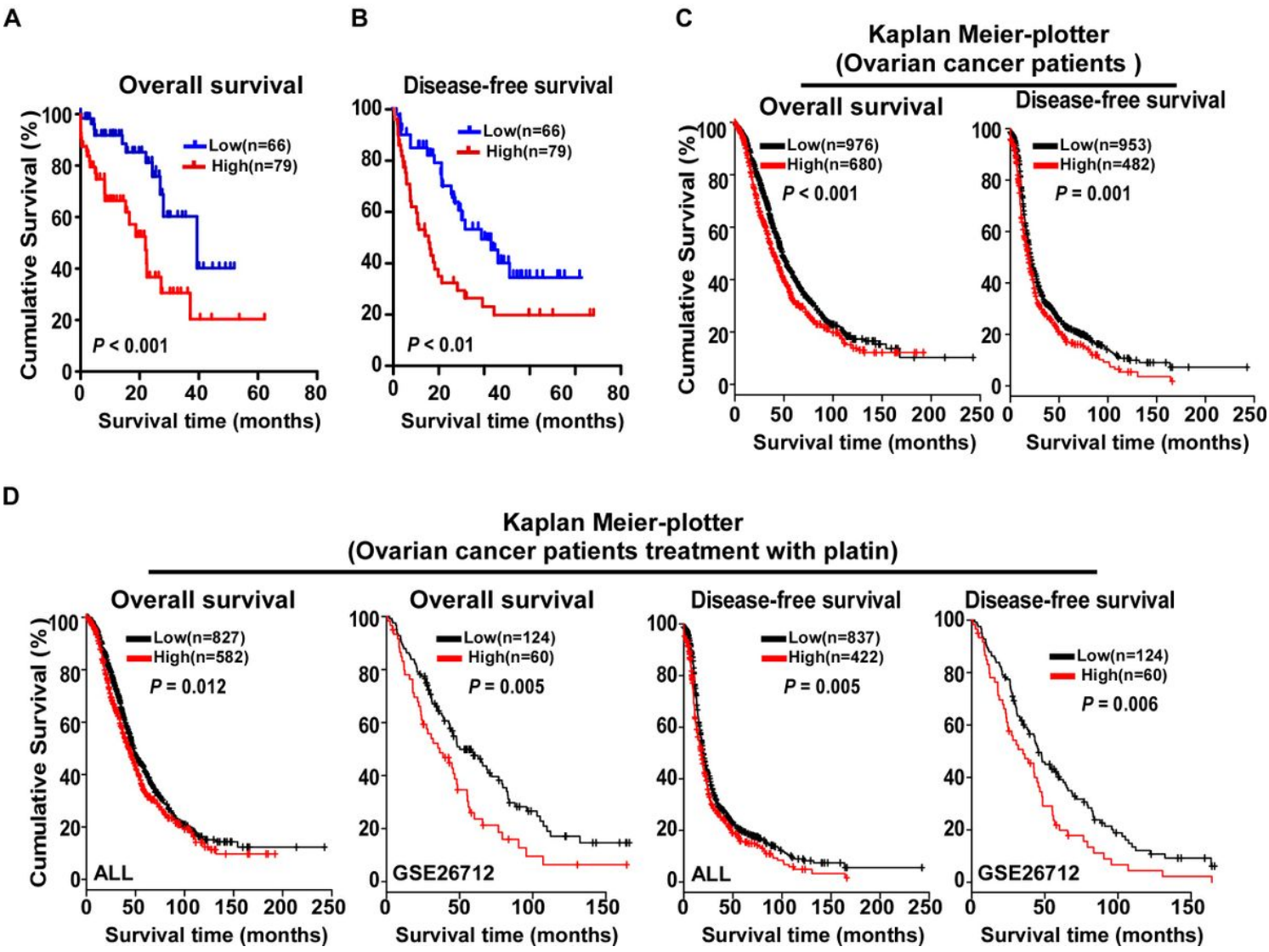

Figure 2

Overexpression of TRIM37 correlates with ovarian cancer progression and poor prognosis. (A-B) Survival curves compare ovarian cancer patients with low and high TRIM37 expression levels (n = 145; P < 0.01). (C) The Kaplan-Meier survival curves compare ovarian cancer patients with low and high TRIM37 expression levels (published datasets, P < 0.01). (D) The Kaplan-Meier survival curves compare ovarian cancer patients (treatment with platin) with low and high TRIM37 expression levels (published datasets, P < 0.01).

Figure 3

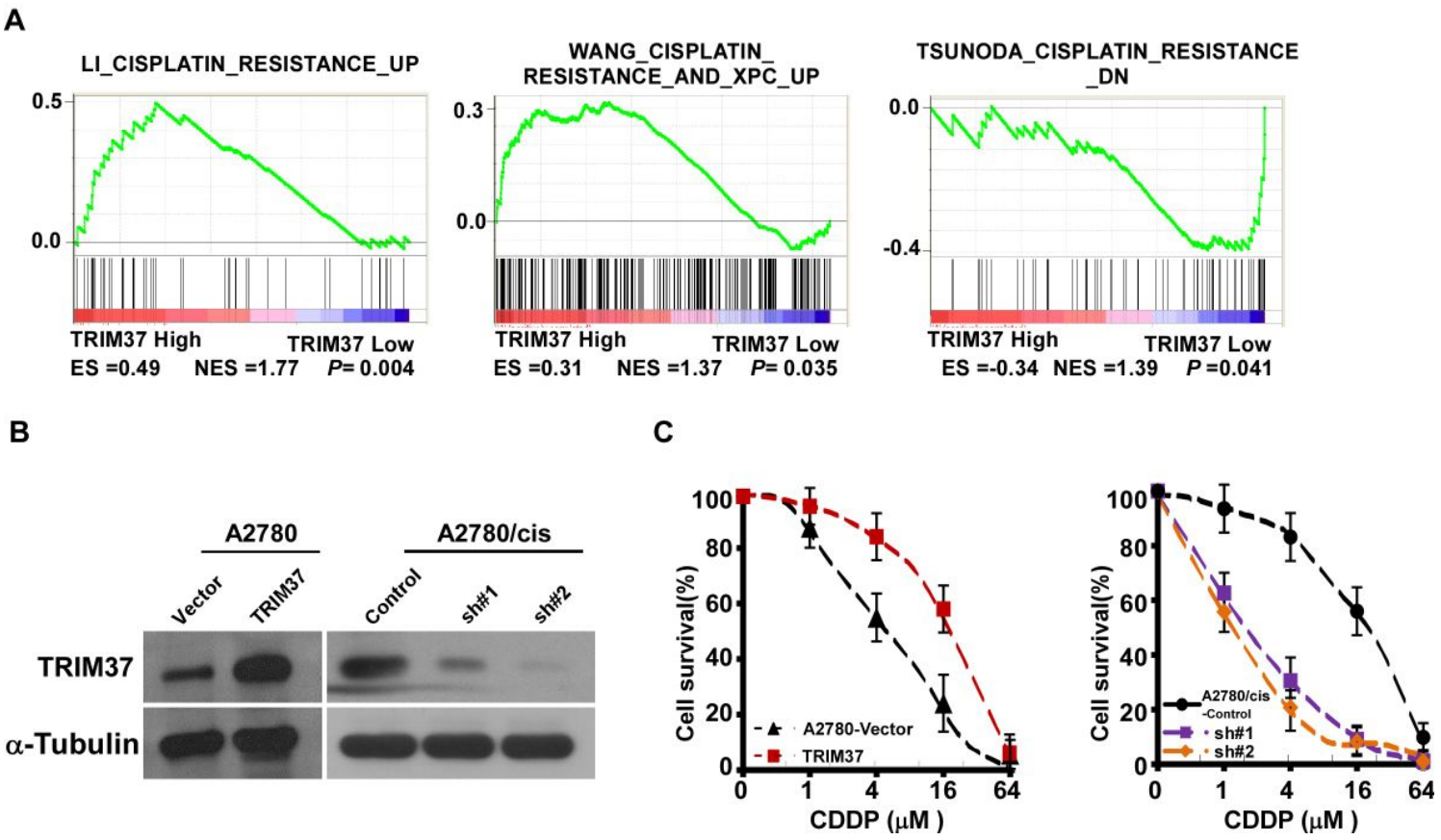

Figure 3

Upregulation of TRIM37 contributes cytotoxicity of ovarian cancer cells in vitro. (A) GSEA plot, indicating a significant correlation between the mRNA levels of TRIM37 expression in ovarian cancer and the CDDP resistance gene signatures in published datasets. (B) Western blotting analysis of the expression levels of TRIM37 proteins in the indicated cells. -tubulin was used as a loading control. (C) IC50 of CDDP in the indicated cells.

Figure 4

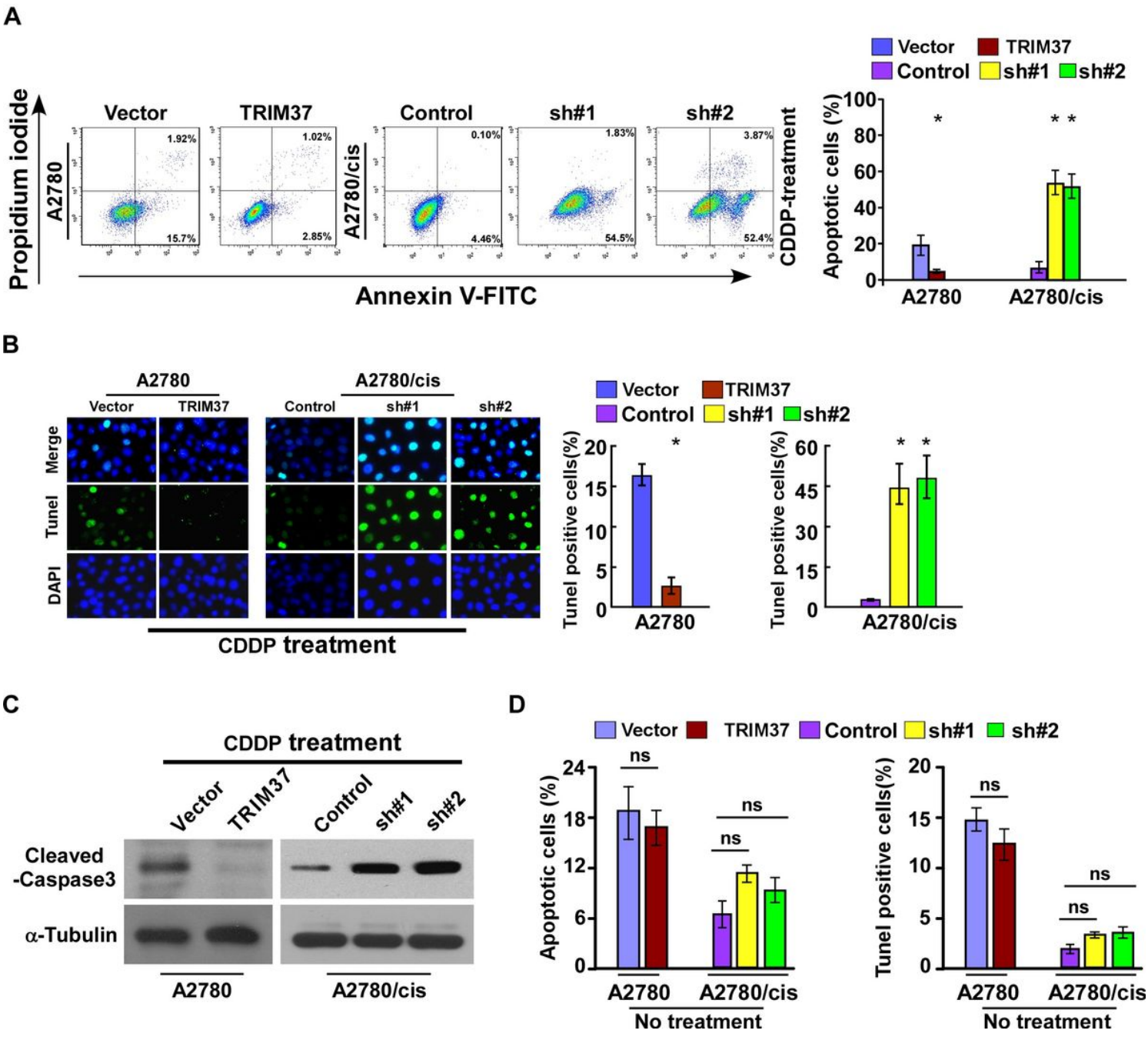

Figure 4

Upregulation of TRIM37 conferred ovarian cancer to CDDP resistance in vitro. (A) Annexin V-FITC and PI staining of the indicated cells treated with cisplatin (10 μM) for 24 h. Each bar represents the mean ± SD of three independent experiments. (C). Representative micrographs (left) and quantification of Tunel positive signalling in the indicated assay. * P <0.05. (d). Western blotting analysis of Cleaved caspase3 in the indicated cells. α-tubulin was used as a loading control. (D) Annexin V-FITC and PI staining of the indicated cells with no treatment. Each bar represents the mean ± SD of three independent experiments(left). Representative micrographs and quantification of Tunel signalling in the indicated assay. (right).

Figure 5

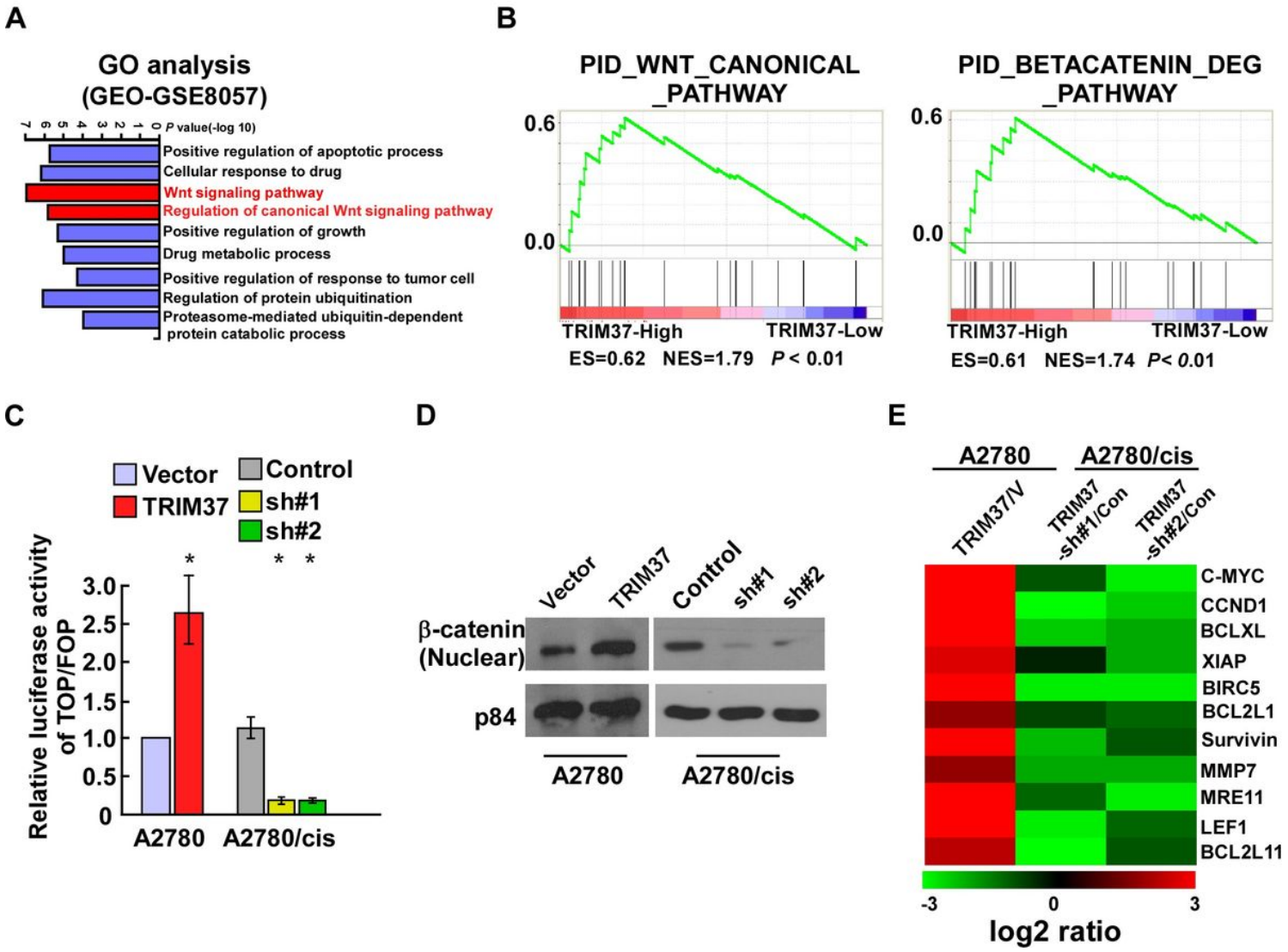

Figure 5

TRIM37 up-regulation activates the Wnt/β-catenin signalling pathway in ovarian cancer. (A). Enrichment of transcripts which were up- or downregulated (P < 0.05) by TRIM37 was determined by their Gene Ontology (GO) terms. (B) GSEA plot, indicating a significant correlation between the mRNA levels of TRIM37 expression in ovarian cancer and the Wnt/β-catenin activated gene signatures in published datasets. (C) Analysis of luciferase reporter activity in the indicated cells. (D) Western blotting analysis of the expression levels of nuclear β-catenin proteins in the indicated cells. p84 was used as a loading control. (E) Real-time PCR analysis demonstrating an apparent overlap between Wnt/β-catenin–dependent gene expression and TRIM37-regulated gene expression. The pseudo colour represents an intensity scale for TRIM37 versus vector or TRIM37 siRNA versus control siRNA, calculated by log2 transformation. P <0.05.

Figure 6

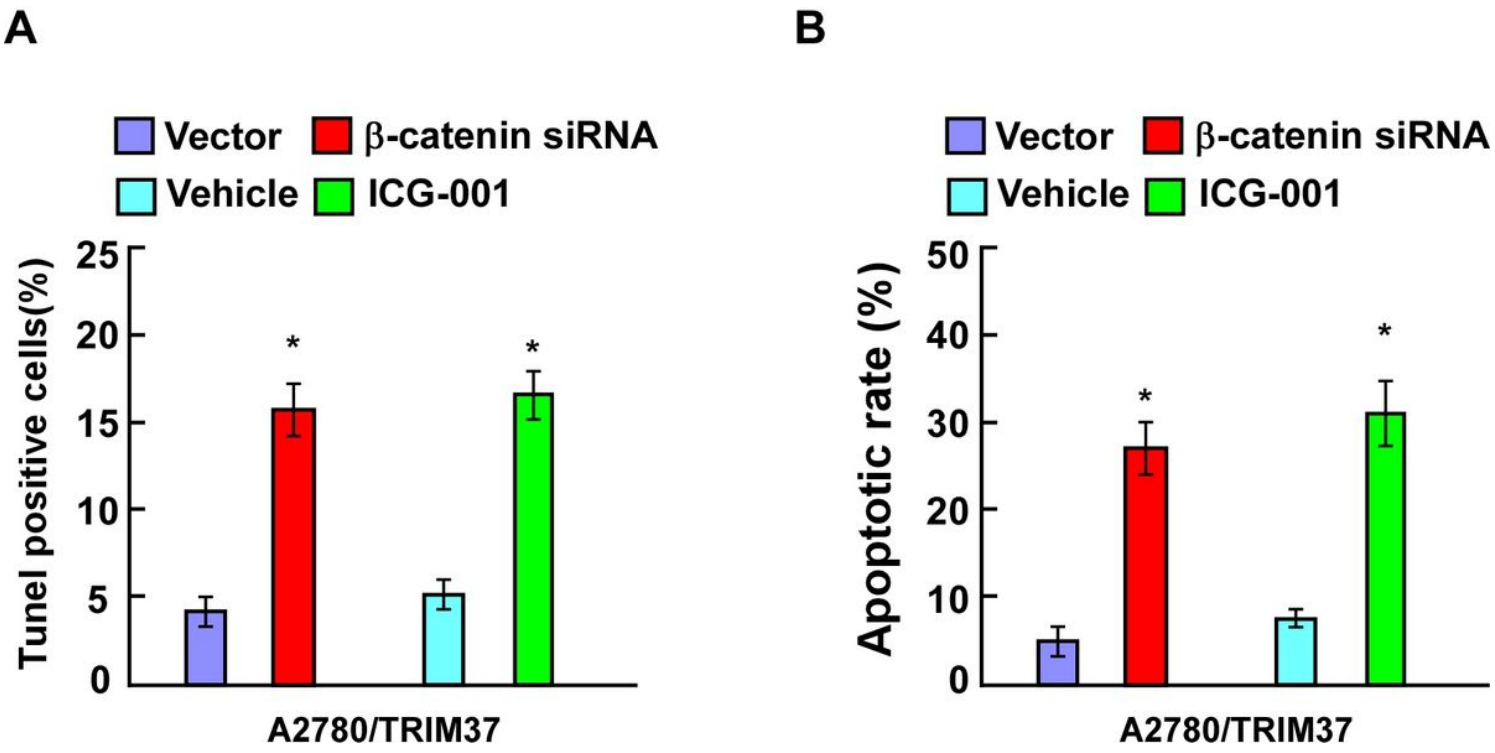

Figure 6

Wnt/β-catenin signaling pathway is required for TRIM37-induced chemo-resistance. (A) Quantification of cisplatin-induced TUNEL-positive cells in ovarian cancer cells transfected with vector, β-catenin siRNA or treated with the β-catenin inhibitor. Each bar represents the mean ± SD of three independent experiments. (B) Annexin V-FITC and PI staining of the indicated cells transfected with vector, β-catenin siRNA or treated with the β-catenin inhibitor (ICG-001).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformation.doc