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# β-Catenin expression is regulated by an IRES-dependent mechanism and stimulated by paclitaxel in human ovarian cancer cells

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ABSTRACT

Paclitaxel (PTX) is commonly used in the chemotherapy of ovarian cancer, but resistance occurs in most cases, allowing cancer progression. The Wnt/β-catenin pathway has been associated with this resistance, but there are no reports on the regulation of β-catenin expression at the translational level. In the present study, we found that PTX induced different transcription and translation levels of β-catenin in the human ovarian cancer cell lines A2780 and SKOV3. We also demonstrated that β-catenin mRNA contained an internal ribosome entry segment (IRES) that regulated its translation. Using gene transfection and reporter assays, we revealed that the entire CTNNB1 5′-untranslated region (UTR) contributed to IRES activity. Interestingly, we found that c-myc and cyclin D1 increased significantly in transfected cells with increasing PTX concentration, and cell-survival rates remained at 60% while the PTX concentration increased. Suppressing β-catenin resulted in decreased expression of c-myc and cyclin D1 and made these cells less resistant. These results indicate that β-catenin translation is initiated *via* the IRES and this is regulated by PTX, suggesting that regulation of the IRES-dependent translation of β-catenin may be involved in the cancer cell response to PTX treatment.



## 1. Introduction

Ovarian cancer (OC) is the deadliest gynecological malignancy and the second leading cause of cancer-related deaths among women worldwide [1]. It is hardly detectable at the early stage, largely due to the lack of specific symptoms and reliable screening [2], so, surgical resection and subsequent chemotherapy are still the major therapeutic strategies [3]. However, the efficacy of chemotherapy is limited by chemoresistance [4]. Paclitaxel (PTX) is a first-line chemotherapeutic agent that is effective in epithelial ovarian cancer by stabilizing microtubules and activating pro-apoptotic signaling [5], while the prognosis of advanced or recurrent cases remains poor since mortality is mostly the result of metastasis that is refractory to conventional chemotherapy [6]. Therefore, definition of the key molecules involved in the development of OC is important for understanding the detailed process.

Canonical Wnt signaling plays a pivotal role in intracellular signal transduction [7] and it has been reported that the Wnt pathway is associated with OC. However, the role of the Wnt pathway in the various OC subtypes remains undefined, partly because of its diverse mechanisms of activation in OC [8]. Indeed, β-catenin mutation is mainly confined to endometrioid adenocarcinomas [9] where it is predominantly expressed in the nucleus; it is at the “heart” of the Wnt/β-catenin pathway and activates the expression of many important proteins responsible for the cell cycle, proliferation, and survival, such as cyclin D1 and c-myc [10]. So far, there are no reports on the regulation of β-catenin expression at the translational level.

Translation is predominantly regulated during initiation, either globally or through sequences in the mRNA that allow for message-specific regulation. Translational control plays an important role in differentiation, cell growth, mitosis, angiogenesis, and apoptosis. Therefore, dysregulation of translation initiation makes a significant contribution to tumor cell growth, survival, invasion, and metastasis [11–14]. Initiation of translation can occur by two mechanisms, cap-dependent scanning and internal ribosome entry (IRES) [15]. Many cellular messages that encode the proteins required for tumor genesis use an IRES-mediated mechanism of initiating protein synthesis. IRES is defined by a functional assay that establishes that an RNA element or sequence is capable of

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recruiting a ribosome internally to the message [16]. The proteins encoded by these messages are generally expressed in low amounts but can have a significant effect on the cell program, such as transcription factors. It is believed that translation is generally initiated in eukaryotic cells by the 5′ end of capped mRNA, which is used for proteins required in bulk, such as β-actin. However, when the cell suffers stressful conditions, such as starvation, hypoxia, or apoptosis, protein synthesis through cap-dependent translation decreases dramatically due to its high energy demand. In order to cope with the stress or to enter into programmed cell death, the cell requires the synthesis of certain proteins. These proteins may be encoded by mRNAs that contain IRESs. Now, 10–15% of cellular mRNAs are estimated to contain IRESs [12]. Indeed, numerous reports have described IRESs in genes encoding key regulatory proteins such as VEGF, HIF-1a, c-myc, XIAP, and various others [17–20].

An assay commonly used to detect IRES activity is to clone the putative IRES sequence into a DNA dicistronic reporter and assay for expression of the second cistron. The cistrons can be any protein that can be easily quantified; commonly used reporters are *Renilla* and firefly luciferase, and β-galactoside. It has been reported that optimal translation of the dicistronic reporter occurs at 24 h and up to 48 h after transient transfection of the DNA plasmid into tissue culture cells. The expression of the first cistron is cap-dependent while expression of the second cistron may be IRES-dependent [21].

We found that CTNNB1 protein expression increased under stress without corresponding changes in transcript abundance. Thus, we propose the possibility that CTNNB1 translation is regulated by an IRES-dependent mechanism. The results have clinical significance for the OC therapy, since we found a link between IRES-regulated β-catenin translation and resistance to PTX chemotherapy.

## 2. Materials and methods

### 2.1. Cells and cell culture

All cells were from the American Type Culture Collection. A2780, SKOV3, and HEK293 cells were cultured in Dulbecco's modified Eagle's medium, while the others were maintained in RPMI 1640. All cells were cultured with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin, and were incubated at 37 °C in 5% $CO_2$ humidified air.

### 2.2. Antibodies, reagents and plasmids

The primary antibodies anti-β-catenin (ab6301), anti-c-myc (ab32072), and anti-cyclin D1 (ab40754) were from Abcam (Cambridge, UK), and anti-β-actin (AA128) was from Beyotime Biotechnology (Shanghai, China). The secondary antibodies fluorophore-labeled donkey anti-rabbit IgG (A11374) and fluorophore-labeled donkey anti-mouse IgG (A-21202) used in immunofluorescence experiments were from Invitrogen (Carlsbad, CA). Paclitaxel and Navelbine (NVB) used in chemotherapeutic drug tests were from Sigma. β-catenin-siRNA was from GenePharma (Shanghai, China) (sequence: sense strand, CCC AAG CUU UAG UAA AUA UTT; antisense strand, AUA UUU ACU AAA GCU UGG GTT). The plasmids used in transfection were constructed in our laboratory. Control experiments were performed using one set of cells, and then repeated at least three times.

### 2.3. RT-PCR

Total RNA was extracted using the Trizol protocol and cDNA was synthesized from mRNA using a Super Script first-strand synthesis system (Invitrogen) for RT-PCR. PCR was performed with 30 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 45 s. The data were analyzed with ImageJ software (NIH, Bethesda, MD). Actin was used as an endogenous control. The primer sequences were: actin: sense 5′-TGGAGTCCACTGGCGTCTTC-3′ and antisense 5′-GCTTGACAAAGTGGTCGTTGAG-3′; β-catenin: sense 5′-CTCAGGACAAGGAAGCTGCAGAAGC-3′ and antisense 5′-CAAGGCATCCTGGCCATATCCA-3′

### 2.4. Western blot

Whole-cell protein was obtained using radioimmunoprecipitation assay buffer (Sigma–Aldrich, Steinheim, Germany) containing 1 mM phenylmethanesulfonyl fluoride, and equal protein loading of the lysates was achieved by standardization with a BCA protein assay kit (Beyotime). Samples were separated by electrophoresis on SDS-PAGE gel and transferred to polyvinylidene fluoride membranes (GE Healthcare, Barrington, IL). Membranes were blocked at room temperature for 1 h in Tris-buffered saline containing 0.1% Tween-20 and 5% fat-free powdered milk, and incubated overnight with primary antibodies (anti-β-catenin 1:1000 and anti-β-actin 1:1000) at 4 °C, followed by horseradish peroxidase-conjugated secondary antibody (1:1000) for 2 h at room temperature. Protein bands were analyzed using the Gel Doc XR$^+$ system (Bio-Rad, California, USA).

### 2.5. Immunofluorescence

For immunofluorescence analysis, cells were plated in chamber slides then fixed in 2% paraformaldehyde for 10 min at room temperature, permeabilized with 0.1% Triton X-100 in 0.01 M phosphate-buffered saline (PBS) pH 7.4, and incubated for 30 min with 5% bovine serum albumin in PBS. Cells were then exposed to primary antibodies (anti-c-myc 1:100 and anti-cyclin D1 1:100, and anti-β-catenin 1:100) diluted in PBS containing 5% bovine serum albumin overnight at 4 °C. For negative controls, the primary antibody was omitted. After washing three times with PBS for 15 min, secondary antibody (Alexa Fluor 488-donkey anti-mouse 1:200 or Alexa Fluor568 donkey anti-rabbit 1:200) diluted in PBS was added and incubated for 1 h at room temperature. Cells were then washed in PBS and mounted using 4′,6-diamidino-2-phenylindole to counterstain DNA. Images were collected using a confocal microscope (Leica TCS SP8, Solms, Germany).

### 2.6. Plasmid construction

The plasmids pR-F, pRemcvF, and pRxiapF have been described previously [19,20,22]. To construct pRctnnb1F, the entire 268 bp of the CTNNB1 5′-UTR was cloned into pR-F between the *Nde*I and *Eco*RI sites. To generate dicistronic plasmids without promoter (generically called pR-utr-FDprom), the SV40 promoter was deleted to generate pRF-ΔSV40 and pRctnnb1F-ΔSV40. The CTNNB1 5′-UTR fragments −228 to −1, −188 to −1, −108 to −1, and −68 to −1 were subcloned into pR-F between the *Nde*I and *Eco*RI sites.

### 2.7. Gene transfection and reporter assay

Transient transfections were performed to identify the presence of IRES activity in the CTNNB1 5′-UTR. Cells were transfected with plasmids using TurboFect transfection reagent (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's protocol. Cells were seeded for 24 h in 24-well plates before transfection, generating monolayers that were 50–80% confluent. One microgram of each dicistronic construct was transfected into cells using the

TurboFect reagent. Cells were incubated with transfection complexes for 24 h and extracts were prepared with 1× lysis buffer, then 20-ml aliquots of the supernatant were mixed first with 100 ml of Luciferase Assay Reagent II (Promega, Wisconsin Madison, USA) to measure the firefly luciferase (FL) activity, and next, the *Renilla* luciferase (RL) activity was determined by adding Stop & Glo® reagent to the same sample. These luciferase activities were analyzed using Microplate Instrumentation (BioTek, Vermont, USA).

## 3. Results

### 3.1. Different levels of transcription and translation of beta-catenin in human ovarian cancer cell lines induced by paclitaxel

To determine whether β-catenin is associated with PTX in human OC, we collected A2780 and SKOV3 cells cultured in different doses of PTX and analyzed the transcription and translation levels of β-catenin by western blot and reverse-transcription PCR (RT-PCR). No change was found in the transcription of β-catenin, but a significant dose-dependent increase in its translation level was found (Fig. 1A and B). In addition, immunocytochemistry showed that increasing concentrations of PTX led to significantly increased nuclear translocation of β-catenin (Fig. 1C and D). To explore whether the effects of PTX on β-catenin expression were specific for this compound or any microtubular inhibitor would have the same effect, we exposed A2780 cells to increasing concentrations (0.01, 0.05, or 0.25 μg/ml) of Navelbine (NVB). We found that the β-catenin expression was also increased but not as strongly as with PTX (Fig. 1E). These results imply that microtubular inhibitors like PTX or NVB can induce β-catenin expression at the translational level.

### 3.2. CTNNB1 5′-UTR mediates internal ribosome entry in different cell lines

To investigate the possibility that the CTNNB1 5′-UTR contains an IRES, we cloned the entire 268 bp of the CTNNB1 5′-UTR into an empty dicistronic vector pR-F [20] between the *Nde*I and *Eco*RI sites, thereby creating pRctnnb1F (Fig. 2A). This vector contained the coding region for the RL gene followed by an intercistronic region that contained a multiple cloning site and an additional downstream cistron that encoded FL. To indicate whether the inserted CTNNB1 5′-UTR had cryptic promoter activity, we deleted the SV40 promoter that drives the transcription of the dicistronic mRNA to generate the promoter-less dicistronic vectors pRF-ΔSV40 and pRctnnb1F-ΔSV40.

A2780 cells were transfected with the plasmids pR-F, pRctnnb1 F, pRF-ΔSV40, and pRctnnb1F-ΔSV40. All the RL and FL activities were determined using the stop and Glo™ reagents (Promega) 24 h post-transfection and are expressed as normalized values. In the absence of the SV40 promoter, the RL and FL activities were both eliminated, suggesting that the CTNNB1 5′-UTR does not have cryptic promoter activity (data not shown). To investigate how widely the IRES is used in a range of cell types, cell lines derived from different tissues (A2780, SKOV3, HEK293, Bel-7402, Hep-2, and HCT-8) were each transfected with the plasmids pR-F and pRctnnb1F, and positive controls pRemcvF and pRxiapF containing EMCV and XIAP IRESs [19,22] (Fig. 2C). RL and FL activities were determined 24 h post-transfection and are

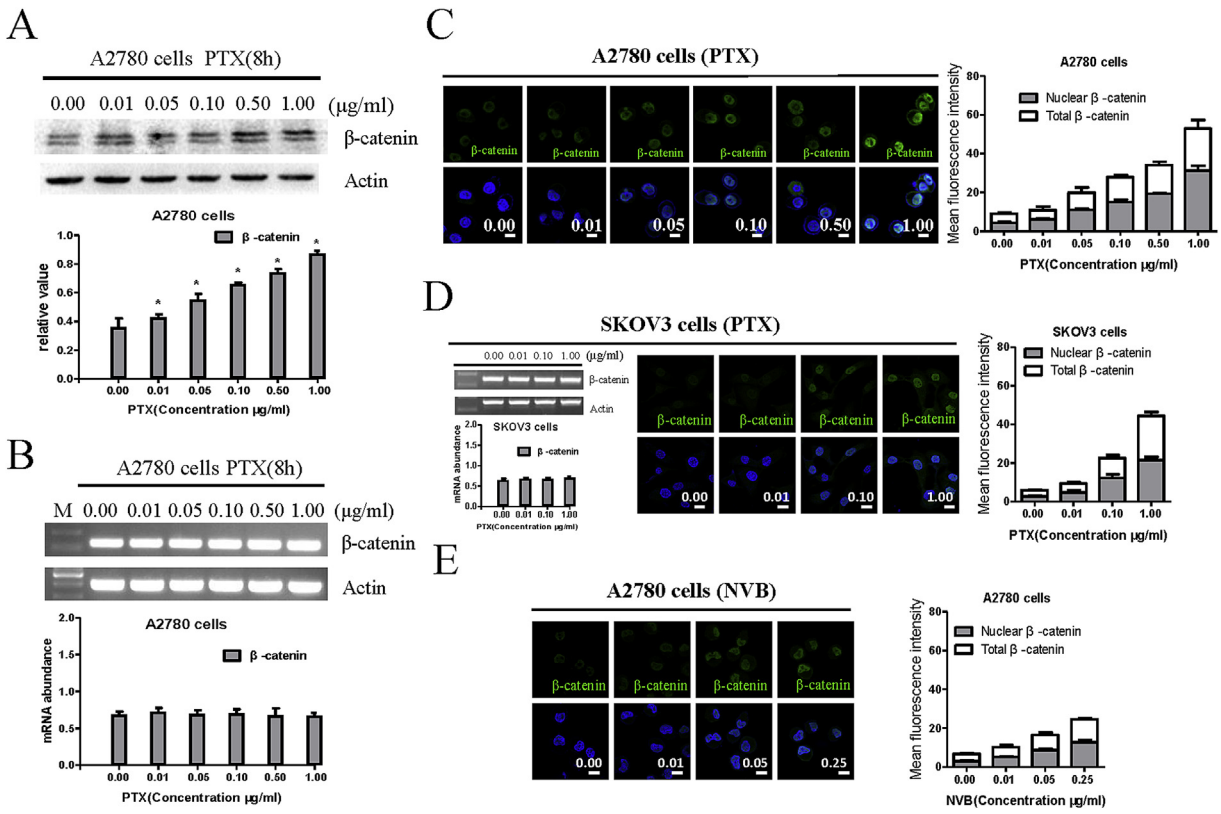


**Fig. 1.** Analysis of western blots of the levels of transcription and translation (A) and mRNA expression levels (B) of β-catenin in A2780 cells with increasing concentrations of Paclitaxel (PTX). (C) Immunofluorescence showed greater nuclear expression of β-catenin in A2780 cells. (D) RT-PCR and immunofluorescence showed different levels of transcription and translation of beta-catenin in SKOV3 cells. (E) Expression of β-catenin with increasing concentrations of NVB (0, 0.01, 0.05 or 0.25 μg/ml) as determined by immunofluorescence analysis. Values are means ± SEM *$p < 0.05$; Student's t-test). Scale bars, 20 μm.

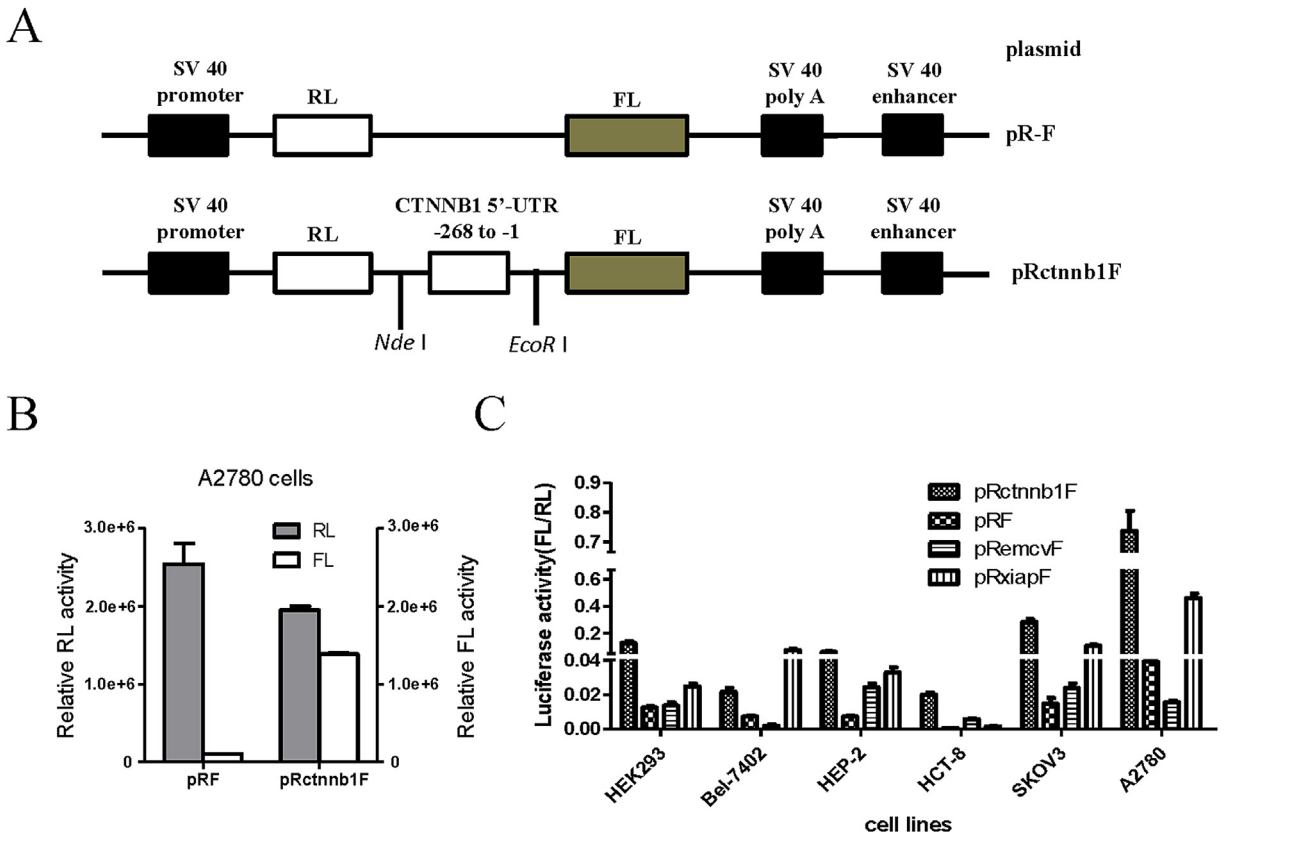


**Fig. 2.** Analysis of IRES activity of the 5′-UTR of CTNNB1. (A) Insertion of the CTNNB1 5′-UTR sequence (268 bp) into the intercistronic region of the empty dicistronic vector pR-F between the *Nde*I and *Eco*RI sites (pRctnnb1F). (B) *Renilla* (RL) and firefly (FL) luciferase activity analyses following transfection of A2780 cells with 1 μg of each plasmid. (C) CTNNB1 5′-UTR IRES activity in different cell lines. Data are expressed as FL/RL ratios.

expressed as FL/RL ratios. The data (Fig. 2C) showed that the translation of the downstream FL cistron mediated by the CTNNB1 5′-UTR varied widely among cell lines. The highest activity was found in A2780, followed by SKOV3, HEK293, Hep-2, Bel-7402, and HCT-8. The reason for the different ratios is not clear but could be due to differential expression of the additional IRES-trans activating factors that are required for CTNNB1 5′-UTR IRES activity [23].

### 3.3. Mapping the CTNNB1 IRES

Taken together, the above data indicated that the CTNNB1 5′-UTR might direct internal initiation on a dicistronic mRNA and therefore contains an IRES. To define the boundaries of the CTNNB1 IRES, a series of plasmid constructs was generated containing decreasing lengths of the sequence coding for the 5′-UTR. The ability of these truncated sequences to promote internal ribosome entry on a dicistronic mRNA was compared to the full-length 5′-UTR. Based on the online simulation of the secondary structure of the CTNNB1 5′-UTR, we generated dicistronic plasmids containing a series of 5′–3′–deleted CTNNB1 5′-UTR fragments. Each of these plasmids was transfected into A2780. RL and FL activities were determined and are expressed as FL/RL ratios. The results showed that removal of 40 nt from the 5′ end of the CTNNB1 5′-UTR (deleted fragment 41–268) resulted in a 37% increase compared with the whole length in A2780 cells; the reason for this variation is not yet clear. After deleting 80, 160, and 200 nt from the 5′-UTR, the FL/RL ratio gradually decreased to 66%, 24%, and 11%, respectively, in A2780 cells (Fig. 3C), consistent with the decreasing trend in HEK293 cells (data not shown). This suggests that the entire CTNNB1 5′-UTR contributes to IRES activity.

### 3.4. Induction of CTNNB1 IRES activity and downstream factor protein expression by paclitaxel

We performed gene transfections and reporter assays to determine whether PTX induced CTNNB1 5′-UTR IRES activity. Both A2780 and SKOV3 cells were transfected with the pRctnnb1F plasmid and then treated with PTX. The FL activity increased significantly along with the increasing dose of PTX (Fig. 4A). More nuclear expression of β-catenin was found, while the nuclear-cytoplasmic ratio did not change (Fig. 1C), which indicated that the increase of total β-catenin expression led to the nuclear accumulation of β-catenin rather than inducing the phosphorylation of PTEN, leading to activation of PI3K/Akt signaling, resulting in the stabilization and nuclear translocation of β-catenin [24]. It has been reported that β-catenin translocates to and accumulates in the nucleus, where it activates the expression of T-cell factor/lymphoid enhancer factor (TCF/LEF), and promotes the expression of downstream target genes, such as c-myc, MMP-7, COX-2, MRP1, and cyclin D1 [25]. To test this possibility in our model, we assessed the protein expression of c-myc and cyclin D1 while treating transfected cells with PTX, and found that they increased with increasing PTX concentration (Fig. 4B). To better understand whether the maintenance of high β-catenin expression explains the PTX resistance of A2780 and SKOV3 cells, we analyzed the effect of decreasing β-catenin using β-catenin-specific siRNA. The results showed that suppressing β-catenin in these cells decreased the expression of c-myc and cyclin D1 (Fig. 4C).

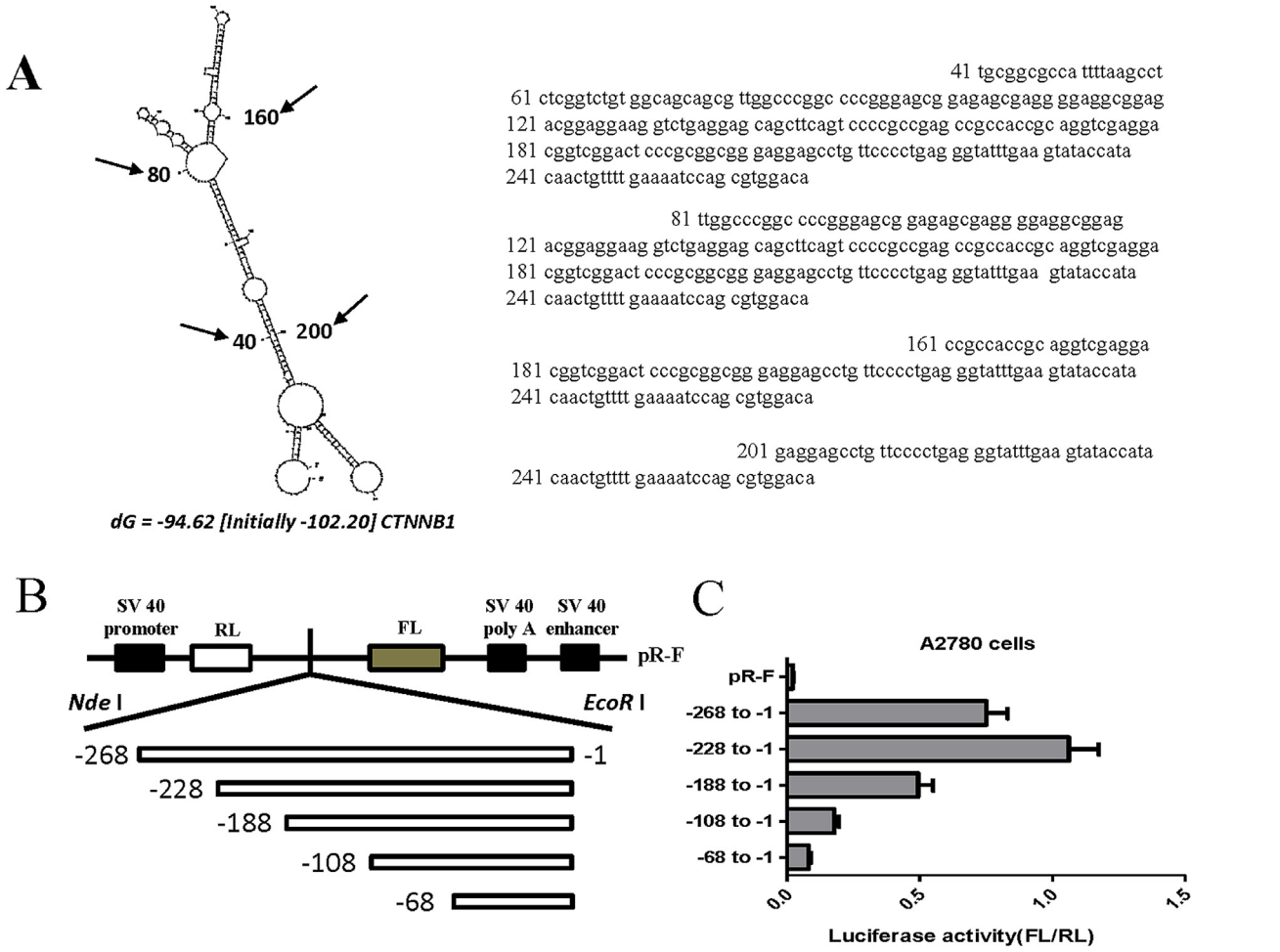


**Fig. 3.** Deletion analysis of CTNNB1 IRES. (A) Model of the secondary structure of the CTNNB1 5′-UTR. Energy minimization analysis was performed using Zuker's 'mfold' package. Arrows indicate deletion positions. The sequences on the right are those of the 4 deleted fragments. (B) Schematic representation of dicistronic pR-F constructs containing a series of 5′—3′ deleted CTNNB1 5′-UTR fragments. (C) The constructs pR-F and pRctnnb1F (CTNNB1), and the above deletion constructs were co-transfected into A2780 cells. *Renilla* (RL) and firefly (FL) luciferase activities were determined and expressed as FL/RL ratios.

Furthermore, we tested whether PTX induced CTNNB1 5′-UTR IRES activity that mediated growth-inhibition and cell death. We measured cell growth with different doses of PTX in 24 h, and found that A2780 and SKOV3 cell-survival rates remained at 60% while the PTX concentration increased (Fig. 4D). However, β-catenin knockdown made these cells less resistant. These results showed that PTX induces CTNNB1 IRES activity in A2780 and SKOV3 cells, and this regulates the expression of downstream factors (c-myc and cyclin D1), reducing PTX sensitivity.

## 4. Discussion

Currently, PTX-based chemotherapy plays an irreplaceable role in the treatment of OC; however, chemoresistance remains a major therapeutic barrier [26,27]. A thorough understanding of the mechanisms is essential for improving therapeutic agents. The findings described here shed light on the unconventional and poorly-understood mechanism of IRES-dependent translation in the regulation of β-catenin expression. In particular, we have shown that PTX induces CTNNB1 5′-UTR IRES activity while also activating the Wnt/β-catenin pathway in OC.

We first found that in A2780 and SKOV3 cells under the stress of chemotherapy with PTX, both western blot and confocal assays demonstrated that β-catenin expression increased in a dose-dependent manner, while the mRNA remained at the same level, raising the question of why the protein level change was not consistent with the mRNA level. By searching the Human Genome database, we discovered that CTNNB1 mRNA contains a 268-nt 5′-UTR, and the sequence is ~63% G + C rich. This suggested that the CTNNB1 5′-UTR may be involved in regulating translation initiation when A2780 cells are exposed to PTX [28]. Second, we constructed a pRctnnb1F dicistronic reporter vector and transfected it into A2780 cells; this resulted in a higher FL/RL ratio than control, indicating that the translation of the downstream FL cistron is mediated by the CTNNB1 5′-UTR. We also showed that the CTNNB1 5′-UTR is widely used in a range of cell types with varying efficiency. Since expression of the second cistron (FL) in the dicistronic reporter was found, the next step was to rule out other, non-IRES-dependent causes, such as cryptic promoter activity, activation of a splicing event, read-through, or ribosomal shunting. The most common false-positives are caused by cryptic promoter activity [16], so we deleted the SV40 promoter to construct promoter-less dicistronic vectors and showed that the CTNNB1 5′-UTR does not have cryptic promoter activity. So far, the evidence suggests that the CTNNB1 5′-UTR contains an IRES to regulate translation initiation when A2780 cells are stressed by PTX.

It has recently been reported that β-catenin acts as a transcriptional switch, as its presence reduces the association of TLE

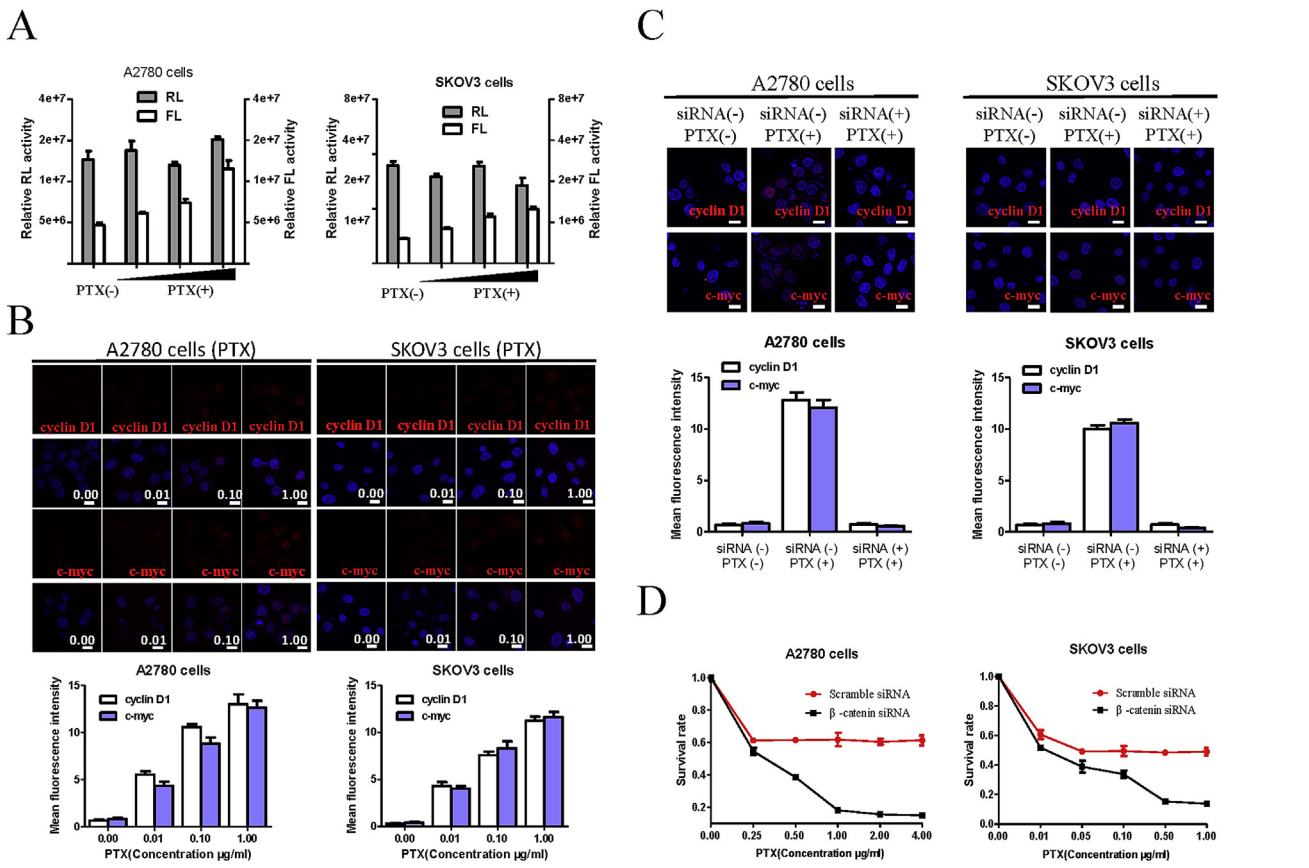


**Fig. 4.** Induction of CTNNB1 IRES activity and downstream factor protein expression by Paclitaxel (PTX). (A) Transfection and reporter assays for the effect of PTX on CTNNB1 5′-UTR IRES activity. A2780 and SKOV3 cells were transfected with pRctnnb1F plasmid containing the entire CTNNB1 5′-UTR and treated with or without increasing concentrations (0.01, 0.1, or 1 μg/ml) of PTX. (B) Immunofluorescence analysis of cyclin D1 and c-myc expression with increasing PTX concentrations in A2780 and SKOV3 cells. (C) Immunofluorescence analysis showed low expression of cyclin D1 and c-myc in A2780 and SKOV3 cells after inhibition of β-catenin with siRNA. (D) Cell viability assays in A2780 and SKOV3 cells transfected with β-catenin-specific siRNA with a range of concentrations of PTX. n = 4–6 in each group; values are mean ± SEM; $^{*}p < 0.05$ compared to control. Scale bars, 20 μm.

(transducin-like enhancer) with TCF/LEF, while recruiting various transcriptional cofactors [29]. Here, we found that A2780 and SKOV3 cells transfected with a plasmid containing the CTNNB1 5′-UTR sequence, when exposed to PTX, showed increasing β-catenin expression, a nuclear distribution of β-catenin, and cyclin D1 and c-myc expression. Importantly, we found that A2780 and SKOV3 cell-survival rates remained at 60% with increasing PTX concentration. Furthermore, suppressing β-catenin resulted in decreased expression of c-myc and cyclin D1 and made these cells less resistant, indicating that PTX induces CTNNB1 IRES activity and regulates the expression of downstream factors (c-myc and cyclin D1), reducing PTX sensitivity.

Collectively, our data suggest that IRES-regulated β-catenin translation is activated during the treatment of OC cells with the chemotherapeutic drug PTX. Exposure to PTX significantly increased the expression of β-catenin protein, which translocated into the nucleus. Notably, we found an association between the Wnt/β-catenin pathway and sensitivity to PTX in A2780 and SKOV3 cells. Although there is no reason to think these effects are limited to IRES-regulated β-catenin translation, we, for the first time, have demonstrated that the initiation of translation by β-catenin is regulated by an IRES-dependent mechanism, providing a new clue for the treatment of drug-resistant OC.

## Conflict of interest

None.

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## Transparency document

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## References

[1] A. Jemal, R. Siegel, J. Xu, E. Ward, Cancer statistics, 2010, CA Cancer J. Clin. 60 (2010) 277–300.
[2] H. Liu, Z.Q. Yan, B. Li, S.Y. Yin, Q. Sun, J.J. Kou, D. Ye, K. Ferns, H.Y. Liu, S.L. Liu, Reduced expression of SOX7 in ovarian cancer: a novel tumor suppressor through the Wnt/beta-catenin signaling pathway, J. Ovarian Res. 7 (2014) 87.
[3] L. Chen, T. Liu, J. Zhou, Y. Wang, X. Wang, W. Di, S. Zhang, Citrate synthase expression affects tumor phenotype and drug resistance in human ovarian carcinoma, PLoS One 9 (2014) e115708.
[4] F. Du, X. Wu, Y. Liu, T. Wang, X. Qi, Y. Mao, L. Jiang, Y. Zhu, Y. Chen, R. Zhu, X. Han, J. Jin, X. Ma, D. Hua, Acquisition of paclitaxel resistance via PI3Kde-pendent epithelialmesenchymal transition in A2780 human ovarian cancer cells, Oncol. Rep. 30 (2013) 1113–1118.

[5] H. Kajiyama, K. Shibata, M. Terauchi, M. Yamashita, K. Ino, A. Nawa, F. Kikkawa, Chemoresistance to paclitaxel induces epithelial-mesenchymal transition and enhances metastatic potential for epithelial ovarian carcinoma cells, Int. J. Oncol. 31 (2007) 277–283.
[6] L. Jia, S. Zhang, Y. Ye, X. Li, I. Mercado-Uribe, R.C. Bast Jr., J. Liu, Paclitaxel inhibits ovarian tumor growth by inducing epithelial cancer cells to benign fibroblast-like cells, Cancer Lett. 326 (2012) 176–182.
[7] W. Kildal, B. Risberg, V.M. Abeler, G.B. Kristensen, J. Sudbo, J.M. Nesland, H.E. Danielsen, beta-catenin expression, DNA ploidy and clinicopathological features in ovarian cancer: a study in 253 patients, Eur. J. Cancer 41 (2005) 1127–1134.
[8] M. Asad, M.K. Wong, T.Z. Tan, M. Choolani, J. Low, S. Mori, D. Virshup, J.P. Thiery, R.Y. Huang, FZD7 drives in vitro aggressiveness in Stem-A subtype of ovarian cancer via regulation of non-canonical Wnt/PCP pathway, Cell. Death Dis. 5 (2014) e1346.
[9] J. Palacios, C. Gamallo, Mutations in the beta-catenin gene (CTNNB1) in endometrioid ovarian carcinomas, Cancer Res. 58 (1998) 1344–1347.
[10] L. Bodnar, A. Stanczak, S. Cierniak, M. Smoter, M. Cichowicz, W. Kozlowski, C. Szczylik, M. Wieczorek, M. Lamparska-Przybysz, Wnt/beta-catenin pathway as a potential prognostic and predictive marker in patients with advanced ovarian cancer, J. Ovarian Res. 7 (2014) 16.
[11] C.R. Stumpf, D. Ruggero, The cancerous translation apparatus, Curr. Opin. Genet. Dev. 21 (2011) 474–483.
[12] K.A. Spriggs, M. Stoneley, M. Bushell, A.E. Willis, Re-programming of translation following cell stress allows IRES-mediated translation to predominate, Biol. Cell. 100 (2008) 27–38.
[13] J.P. Le Quesne, K.A. Spriggs, M. Bushell, A.E. Willis, Dysregulation of protein synthesis and disease, J. Pathol. 220 (2010) 140–151.
[14] M. Holcik, Targeting translation for treatment of cancer–a novel role for IRES? Curr. Cancer Drug Targets 4 (2004) 299–311.
[15] S.A. Chappell, J.P. LeQuesne, F.E. Paulin, M.L. deSchoolmeester, M. Stoneley, R.L. Soutar, S.H. Ralston, M.H. Helfrich, A.E. Willis, A mutation in the c-myc-IRES leads to enhanced internal ribosome entry in multiple myeloma: a novel mechanism of oncogene de-regulation, Oncogene 19 (2000) 4437–4440.
[16] S.R. Thompson, So you want to know if your message has an IRES? Wiley Interdiscip. Rev. RNA 3 (2012) 697–705.
[17] G. Akiri, D. Nahari, Y. Finkelstein, S.Y. Le, O. Elroy-Stein, B.Z. Levi, Regulation of vascular endothelial growth factor (VEGF) expression is mediated by internal initiation of translation and alternative initiation of transcription, Oncogene 17 (1998) 227–236.
[18] M. Stoneley, F.E. Paulin, J.P. Le Quesne, S.A. Chappell, A.E. Willis, C-Myc 5′ untranslated region contains an internal ribosome entry segment, Oncogene 16 (1998) 423–428.
[19] M. Holcik, C. Lefebvre, C. Yeh, T. Chow, R.G. Korneluk, A new internal-ribosome-entry-site motif potentiates XIAP-mediated cytoprotection, Nat. Cell. Biol. 1 (1999) 190–192.
[20] K.J. Lang, A. Kappel, G.J. Goodall, Hypoxia-inducible factor-1alpha mRNA contains an internal ribosome entry site that allows efficient translation during normoxia and hypoxia, Mol. Biol. Cell. 13 (2002) 1792–1801.
[21] M.S. Carter, P. Sarnow, Distinct mRNAs that encode La autoantigen are differentially expressed and contain internal ribosome entry sites, J. Biol. Chem. 275 (2000) 28301–28307.
[22] A.V. Borovjagin, M.V. Ezrokhi, V.M. Rostapshov, T. Ugarova, T.F. Bystrova, I.N. Shatsky, RNA–protein interactions within the internal translation initiation region of encephalomyocarditis virus RNA, Nucleic Acids Res. 19 (1991) 4999–5005.
[23] K.A. Spriggs, L.C. Cobbold, S.H. Ridley, M. Coldwell, A. Bottley, M. Bushell, A.E. Willis, K. Siddle, The human insulin receptor mRNA contains a functional internal ribosome entry segment, Nucleic Acids Res. 37 (2009) 5881–5893.
[24] D. Li, W. Liu, X. Wang, J. Wu, W. Quan, Y. Yao, R. Bals, S. Ji, K. Wu, J. Guo, H. Wan, Cathelicidin, an antimicrobial peptide produced by macrophages, promotes colon cancer by activating the Wnt/beta-catenin pathway, Oncotarget 6 (2015) 2939–2950.
[25] T. Wang, Z. Chen, Y. Zhu, Q. Pan, Y. Liu, X. Qi, L. Jin, J. Jin, X. Ma, D. Hua, Inhibition of transient receptor potential channel 5 reverses 5-Fluorouracil resistance in human colorectal cancer cells, J. Biol. Chem. 290 (2015) 448–456.
[26] L. Poulain, H. Lincet, F. Duigou, E. Deslandes, F. Sichel, P. Gauduchon, C. Staedel, Acquisition of chemoresistance in a human ovarian carcinoma cell is linked to a defect in cell cycle control, Int. J. Cancer 78 (1998) 454–463.
[27] L. Rosano, R. Cianfrocca, F. Spinella, V. Di Castro, M.R. Nicotra, A. Lucidi, G. Ferrandina, P.G. Natali, A. Bagnato, Acquisition of chemoresistance and EMT phenotype is linked with activation of the endothelin A receptor pathway in ovarian carcinoma cells, Clin. Cancer Res. 17 (2011) 2350–2360.
[28] N.K. Gray, M. Wickens, Control of translation initiation in animals, Annu Rev. Cell. Dev. Biol. 14 (1998) 399–458.
[29] S. Taurin, N. Sandbo, Y. Qin, D. Browning, N.O. Dulin, Phosphorylation of beta-catenin by cyclic AMP-dependent protein kinase, J. Biol. Chem. 281 (2006) 9971–9976.