

# PCDH10 inhibits cell proliferation of multiple myeloma via the negative regulation of the Wnt/ $\beta$ -catenin/BCL-9 signaling pathway

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**Abstract.** The tumor suppressor protocadherin-10 (*PCDH10*) gene is important in cell proliferation, survival, apoptosis and migration. Inactivation of *PCDH10* by promoter methylation is a frequent pathogenetic event in multiple myeloma (MM). The Wnt/ $\beta$ -catenin pathway is known to be involved in the cell growth of various types of cancer, including MM. However, the relationship between *PCDH10* and Wnt signaling in MM remains unclear. In this study, we found that *PCDH10* deficiency highly enhanced MM cell proliferation, Wnt signaling and the expression of BCL-9, an essential coactivator of Wnt transcriptional activity that is correlated with cell growth, survival and drug resistance. Restoration of *PCDH10* suppressed nuclear localization of  $\beta$ -catenin, the activity of LEF/TCF, the expression of BCL-9 and AKT, whereas the expression of GSK3 $\beta$  was increased. The antagonistic effect of *PCDH10* was associated with G1-phase blockage. Collectively, *PCDH10* antagonized MM cell proliferation via the downregulation of Wnt/ $\beta$ -catenin/BCL-9 signaling, whereas *PCDH10* repressed the expression of AKT to promote the expression of GSK3 $\beta$  and then to restrain the activation of  $\beta$ -catenin. Thus, the results offer a novel preclinical rationale in order to explore *PCDH10* as an effective and selective therapeutic strategy to eradicate MM cells.

## Introduction

Multiple myeloma (MM) is a plasma cell malignancy and the second most common hematologic malignancy. Although numerous promising new drugs are currently being tested

in MM, the disease remains incurable because most patients eventually relapse or become refractory to treatments. A comprehensive knowledge of the tumor-suppressor genes and signaling pathways should pinpoint additional molecular targets. Additionally, the development of new therapeutic agents is required (1-3).

Protocadherin-10 (*PCDH10*) belongs to the  $\delta 2$  subgroup of the protocadherin subfamily (4,5). Cadherins are important in calcium-dependent homophilic cell-cell adhesion and are involved in the establishment of cell polarity, cell-sorting, cell differentiation, proliferation, survival and migration (6-8). The human *PCDH10* gene is located at 4q28.3 and is involved as a tumor-suppressor gene. The promoter methylation and down-regulation of *PCDH10* gene expression has been demonstrated in various human cancer types including lymphoma, as well as gastric, prostate, bladder, colorectal and cervical cancer (9-12). In previous studies, we found that *PCDH10* is broadly expressed in normal adults, but almost undetectable in MM tissues and cell lines due to the promoter methylation of *PCDH10*. The ectopic expression of the *PCDH10* gene suppressed tumor cell growth, survival, invasion and migration (13,14). *PCDH10* has been closely correlated with poor prognosis of colorectal, gastric, prostate and bladder cancer (15-17).

However, little is known regarding the specific mechanism of *PCDH10* which function as an important tumor suppressor, although it has been reported that the *PCDH-X/Y* gene and *PCDHGC3*, which also belong to the protocadherin families, are closely connected with the Wnt/ $\beta$ -catenin pathway and influence the progression of cancers including prostate cancer, Wilms tumor and colon cancer (18-20). However, the relationship between *PCDH10* and Wnt/ $\beta$ -catenin pathway in MM remains unclear.

The Wnt/ $\beta$ -catenin signaling pathway is known to promote cell proliferation, survival and invasion through  $\beta$ -catenin/TCF-mediated transcription in various types of cancer (21,22). The molecular genetics underlying Wnt/ $\beta$ -catenin activation in cancer centers on mutations in genes of the Wnt/ $\beta$ -catenin pathway that enable  $\beta$ -catenin nuclear translocation and drive oncogenic Wnt transcription. However, coactivators for  $\beta$ -catenin activation have been identified as an alternate pathway in MM, which lacks known mutations of the Wnt pathway genes. Of note, the human *BCL-9* gene, which was first identified by cloning the t(1;14)(q21;q32)

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translocation from a patient with B-cell acute lymphoblastic leukemia, has been identified as a critical coactivator of  $\beta$ -catenin activation in association with LEF/TCF family members. It has been confirmed that BCL-9 possesses a potent transcription activation domain, which is crucial for BCL-9 to promote  $\beta$ -catenin translocation and aberrant transcription of Wnt target genes, which in turn promotes tumor cell proliferation, disease progression and drug resistance (23-25). Those findings emphasize the importance of this pathway and BCL-9 for identification of appropriate target drugs.

In the present study, it was found that PCDH10 suppressed MM cell proliferation and cell cycle progression via the negative modulation of Wnt/ $\beta$ -catenin/BCL-9 signaling. As a result, we provide a proof-of-concept for the potential translation of PCDH10 as a novel therapeutic agent to target the oncogenic Wnt/ $\beta$ -catenin/BCL-9 pathway in MM and other cancer types with deregulated Wnt activity.

## Materials and methods

**Construction of the expression plasmids.** The plasmid pcDNA3.1(+)/TP53 was constructed by subcloning the full-length wild-type copy of the tumor protein 53 gene (*TP53*) from the plasmid pC53-SN (a gift from Bert Vogelstein) into the pcDNA3.1(+) vector. pcDNA3.1(+)/PCDH10 was constructed by subcloning into the same vector the full-length *PCDH10* gene, amplified by PCR from the clone KIAA1400 (a gift from the Kazusa DNA Research Institute, Japan) using the AccuPrime Pfx DNA polymerase (Life Technologies, Grand Island, NY, USA). The plasmid sequences and the orientation of the cloned fragments were confirmed by sequencing.

**Cell cultures and transfection.** KM3 and RPMI-8226 MM cell lines were kindly provided by Dr Jian Hou (The Second Military Medical University, Shanghai, China). The cell lines were routinely maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (both from Gibco BRL, Rockville, MD, USA), in 5% CO<sub>2</sub> in humidified air at 37°C. For stable transfection, the cells were plated into 6-well plates and kept in antibiotic-free medium for 24 h prior to transfection. The cells were then transfected with the pcDNA3.1(+)/PCDH10 plasmid or the empty vector (2  $\mu$ g each) using Lipofectamine 2000 (Invitrogen-Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. After 48 h, the cells were transferred to new plates selected with G418 (Sigma-Aldrich, St. Louis, MO, USA) (0.4 mg/ml) for 21 days. The expression of PCDH10 in the resistant cells was confirmed by RT-PCR and western blot analysis.

**Determination of appropriate concentrations of LiCl.** To determine the appropriate concentration of LiCl, RPMI-8226 and KM3 cells (10<sup>5</sup>/ml) were cultured in serum-free medium for 12 h prior to treatment with LiCl at doses of 0, 5, 10, 15 and 20  $\mu$ M/ml. After 48 h, RT-PCR was used to detect the expression of  $\beta$ -catenin in cells with different concentrations of LiCl and determine the applicable concentration of LiCl.

**Semi-quantitative reverse transcription PCR (RT-PCR) and quantitative RT-PCR (RT-qPCR).** Total RNA was isolated from cells using TRIzol reagent and reverse transcribed using

an RT reagent kit (Takara Bio, Inc., Shiga, Japan) according to the manufacturer's instructions. RT-PCR was performed as described previously to amplify the mRNA expression level of PCDH10. RT-qPCR was performed to specify the expression level of the relative target genes according to the manufacturer's instructions which was amplified with SYBR-Green real-time PCR master mix (Takara). Relative expression level of target genes was normalized according to  $\beta$ -actin. The primer sequences are shown in Table I.

**Protein extraction and western blot analysis.** The cells were harvested in lysis buffer supplemented with protease and phosphatase inhibitors, following the manufacturer's instructions. The total protein was extracted using the M-PER mammalian protein extraction reagent (Pierce, Rockford, IL, USA). Extraction of nuclear proteins was performed using BeyoECL Plus nuclear and cytoplasmic protein extraction kits (Beyotime Institute of Biotechnology, Jiangsu, China). Protein concentrations were determined by the bicinchoninic acid (BCA) method using the BCA protein assay reagent kit (Pierce). The gel-separated proteins (50-80  $\mu$ g of protein/lane) were then electrophoretically transferred onto polyvinylidene fluoride (PVDF) membranes (Bio-Rad Laboratories, Hercules, CA, USA). After blocking with 5% BSA for 1.5 h, the membranes were incubated overnight at 4°C with the respective primary antibodies including anti-PCDH10 (1:2000), anti-TBP (1:10000), anti-c-Myc (1:10000), anti-BCL-9 (1:1000) (all from Abcam, Cambridge, MA, USA), anti- $\beta$ -catenin, anti-GSK3 $\beta$ , anti-p-GSK3 $\beta$  (Tyr216), anti-cyclin D1, anti-AKT and anti- $\beta$ -actin (1:1000; Cell Signaling Technology, Danvers, MA, USA). The secondary horseradish peroxidase-conjugated antibody was then incubated at room temperature for 1-2 h. The bands were visualized using enhanced chemiluminescence (ECL; Beyotime Institute of Biotechnology).

**Cell proliferation assay.** Stably transfected clones of RPMI-8226 and KM3 cells expressing PCDH10 were selected and multiplied as previously described (16). Cell proliferation was analyzed by using the Cell Counting Assay Kit-8 (CCK-8) (Sigma-Aldrich) according to the manufacturer's instructions. Briefly, the cells were seeded in 96-well plates and incubated in 10% CCK-8 diluted in normal culture media at 37°C for 2 h. Proliferation rates were determined at 0, 24, 48, 72 and 96 h, respectively. The absorbance (A) at 450 nm was measured using a spectrophotometer (Bio-Rad, Richmond, CA, USA). For Wnt treatment, the cells were pretreated with LiCl for 48 h to activate Wnt/ $\beta$ -catenin signaling (27). Experiments were performed at least three times with representative data presented.

**Cell cycle analysis.** The cells were cultured in RPMI-1640 medium and 10% FBS with or without LiCl. These cells were collected and fixed in ice-cold 70% ethanol for 5 h. The cell cycle profiles were assayed using an Elite ESP flow cytometer and data were analyzed with the Cell Quest software (BD Biosciences, Bedford, MA, USA).

**Luciferase assay.** The cells were initially cultured in serum-free medium for 3 h and seeded in 24-well plates at a density of 2x10<sup>5</sup> cells and transfected with TOPflash or FOPflash reporter plasmids (Millipore, Temecula, CA, USA)

Table I. The relative gene primer sequences.

Gene	Forward primer	Reverse primer
<i>PCDH10</i>	ACTGCTATCAGGTT GCCTG	GTCTGT CAACTAGAT AGCTG
<i>β-catenin</i>	TGGTGACAGGGAAGACATCA	CCATAGTGAAGGCGAACTGC
<i>c-Myc</i>	GAGACAGATCAGCAACAACCGA	CTGCTTGGACGGACAGGATG
<i>Cyclin D1</i>	TTCGTTGCCCTCTGTGCCA	GAAGCGTGTGAGGCGGTTAGTAG
<i>BCL-9</i>	CCAACTTGCCATCAATGAATAA	GGCATCTGATTGGAGTGAGAA
<i>β-actin</i>	CCACGAACTACCTTCAACTCC	GTGATCTCCTTCTGCATCCTGT

as well as pRL-SV40 to normalize for transfection efficiency. FOPflash is a negative control for TOPflash containing mutated TCF-binding sites. Transfection was achieved by using Lipofectamine 2000 (Invitrogen-Life Technologies) according to the manufacturer's instructions. Luciferase samples were assayed after 48 h using a Dual Luciferase Reporter Assay system (Promega, Madison, WI, USA). Experiments were performed at least three times in triplicate.

**Immunofluorescence assay.** RPMI-8226 cells were applied onto ice-cold microscope slides, fixed with 10% paraformaldehyde solution at room temperature for 30 min and washed gently with PBS. The cells were permeabilized in 1% Triton X-100 and followed by incubation in 10% normal goat serum for 1 h at room temperature for 1 h. After gently removing the blocking solution, the cells were incubated with anti-β-cat (1:100) followed by staining with phalloidin dye Alexa Flour-488 goat anti-rabbit anti-IgG (1:200; Proteintech, Chicago, USA) for 1.5 h. Nuclear staining with propidium iodide (PI) for 5 min was performed before the cells were imaged for the localization of β-catenin (28). Stained slides were viewed under a fluorescence microscope at a magnification of x400 (Carl Zeiss Micro Imagine, Axio Observer ZI, Germany).

**Statistical analysis.** Data were presented as the mean ± standard deviation (SD) from three independent experiments. Statistical analysis was conducted using the Student's t-tests.  $P < 0.05$  was considered to indicate statistically significant differences. Data quantification and statistical analysis were performed using the SPSS 18.0 software (IBM, Armonk, NY, USA) and GraphPad Prism 5 software (San Diego, CA, USA).

## Results

**Restoration of PCDH10 successfully inhibits MM cell proliferation.** To assess the effect of PCDH10 on MM cell growth, the RPMI-8226 and KM3 cell lines were transfected with the full-length PCDH10 or empty vector. After selection in G418-supplemented medium for three weeks, the stable expression of PCDH10 was confirmed by RT-PCR and western blot analysis (Fig. 1A).

To identify the effect of PCDH10 on cell proliferation, we used LiCl, an inhibitor of GSK3β to stimulate the activation of Wnt signaling. It was observed that the expression of β-catenin was increased in a dose-dependent manner. β-catenin was increased significantly at the dose of 15 and

20 μM/ml in RPMI-8226 and KM3 cells, respectively. Thus, 15 and 20 μM/ml were used as the appropriate concentrations of LiCl and applied to stimulate RPMI-8226 and KM3 cells, respectively, in the subsequent investigations (Fig. 1B).

Using the CCK-8 assay, the cell proliferation capacity was determined in RPMI-8226 and KM3 cells transfected with PCDH10 or empty vector, with non-transfected cells as control. We found that the growth of cells transfected with PCDH10 was significantly suppressed even when the cells were treated with LiCl compared to the control groups ( $P < 0.05$ , Fig. 1C and D), indicating that PCDH10 can functionally antagonize MM cell proliferation.

**PCDH10 induces cell cycle arrest at the G1 phase in MM cells.** To examine the mechanism regarding how PCDH10 blocks the cell proliferation capacity, we investigated the cell cycle distribution by flow cytometry in RPMI-8226 cells. It was shown that G1 phase was markedly increased in the cells transfected with PCDH10, while G2 phase was decreased compared with the control groups ( $P < 0.05$ , Fig. 2A and C). The result was more notable when the cells were exposed to LiCl ( $P < 0.05$ , Fig. 2B and D). There was no statistical difference of the alteration in S phase in cells without LiCl, but increased after the treatment of LiCl ( $P < 0.05$ ). Collectively, our results demonstrated that PCDH10 exerted its inhibitory activity by the G1 phase retardant.

**PCDH10 evidently hampers the Wnt signaling in MM cells.** The Wnt/β-catenin pathway is frequently activated in various types of cancer and is involved in cancer cell proliferation, survival and invasion (29,30). To determine whether the inhibitory effect of PCDH10 is connected with Wnt signaling, a luciferase assay was utilized to detect the Wnt activity in MM cells transfected with or without PCDH10. It was revealed that LEF/TCF activity (TOP-Flash) was obviously suppressed by PCDH10. Moreover, the stimulated TOP-Flash activity of LiCl was also impaired by PCDH10 re-expression ( $P < 0.05$ ) (Fig. 3A and B), confirming that PCDH10 can effectively inhibit the activity of LEF/TCF.

To verify this, the expression of relative genes in the Wnt/β-catenin signaling pathway, including GSK3β, pGSK-3β, β-catenin, cyclin D1 and c-Myc, were determined in RPMI-8226 cells and KM3 cells. We found that PCDH10 obviously downregulated the mRNA expression of cyclin D1 and c-Myc ( $P < 0.05$ ) (Fig. 3C and D). For the protein analysis, the pGSK3β, cyclin D1, and c-Myc were blocked by PCDH10 compared with the control groups. Conversely, GSK3β

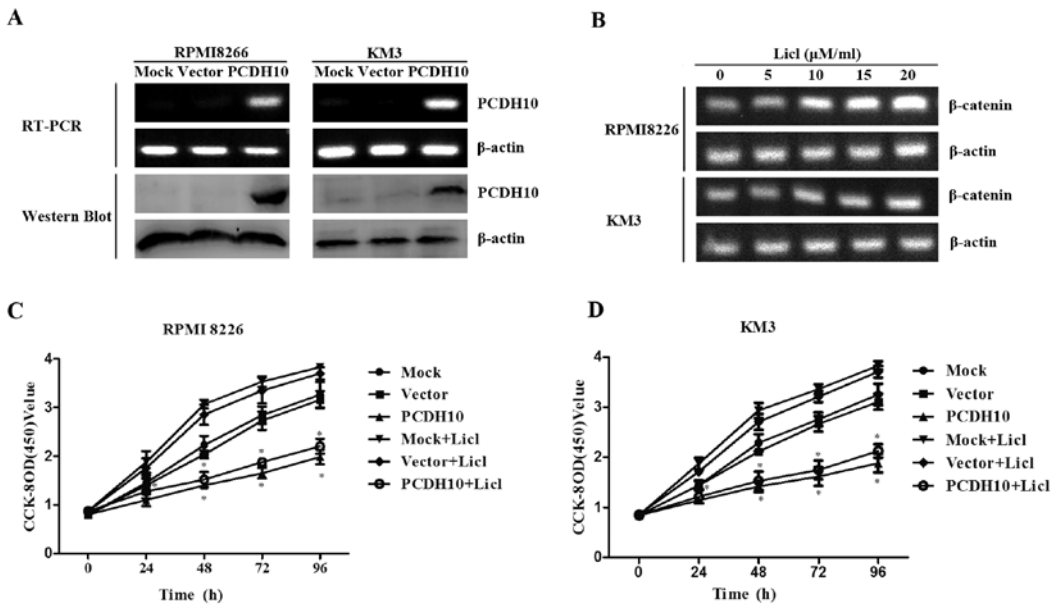


Figure 1. PCDH10 suppresses MM cell proliferation. (A) Ectopic expression of PCDH10 was confirmed by RT-PCR and western blot analysis in RPMI-8226 and KM3 cells. (B) RPMI-8226 and KM3 cells were treated with LiCl at the doses of 0, 5, 10, 15 and 20  $\mu$ M/ml for 48 h, the expression of  $\beta$ -catenin was increased in a dose-dependent manner.  $\beta$ -catenin was increased significantly at the doses of 15 and 20  $\mu$ M/ml in RPMI-8226 and KM3 cells. (C and D) Cell proliferation capacity was quantified by CCK-8 assay. Cell proliferation was suppressed by PCDH10 overexpression, even when cells were exposed to LiCl. \* $P$ <0.05 compared to the control groups. Mock, non-transfected cells; Vector, empty vector-transfected cells; PCDH10, PCDH10-transfected cells.

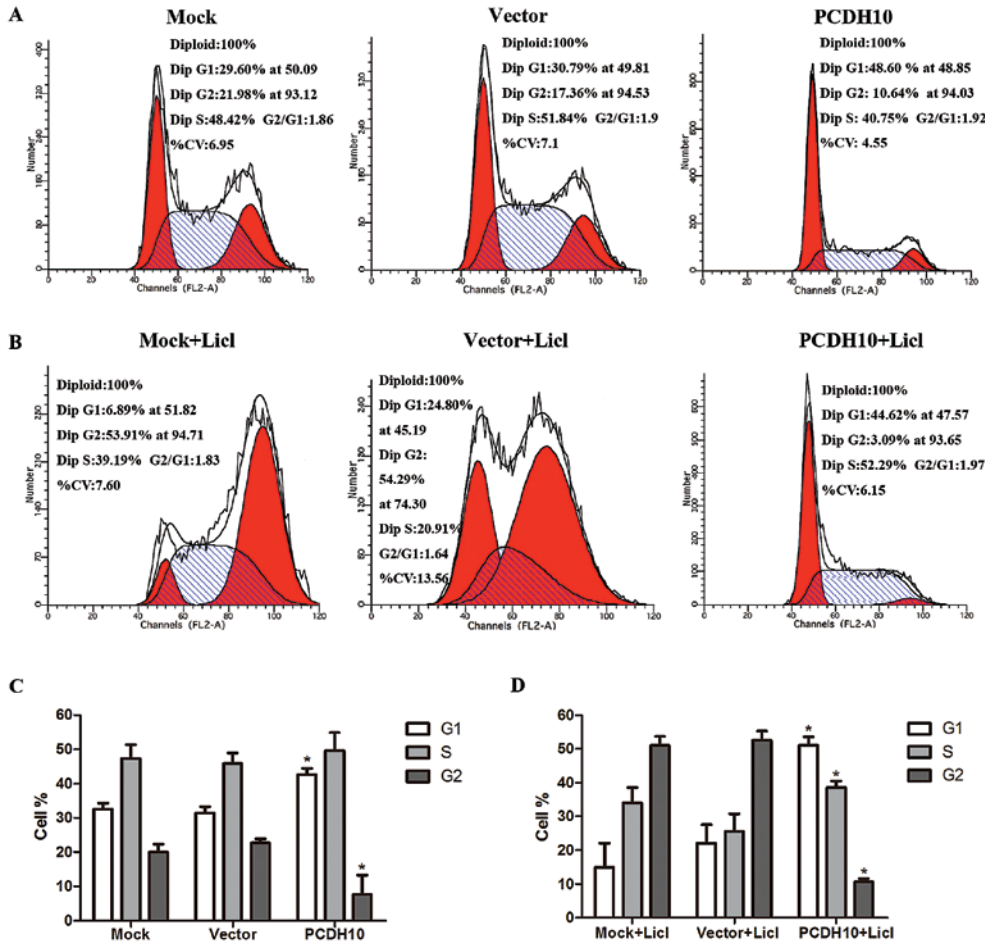


Figure 2. PCDH10 induces cell cycle arrest at the G1 phase. (A) Representative distribution of cell cycles in RPMI-8226 cells transfected with or without PCDH10 by flow cytometry. (B) Representative distribution of cell cycles in RPMI-8226 cells transfected with or without PCDH10. Cells were treated with LiCl for 48 h prior to examination by flow cytometry. (C and D) Analysis of the distribution of RPMI-8226 cells transfected with or without PCDH10 and treated with or without LiCl in different phases of cells. \* $P$ <0.05 compared to the control groups. Mock, non-transfected cells; Vector, empty vector-transfected cells; PCDH10, PCDH10-transfected cells.

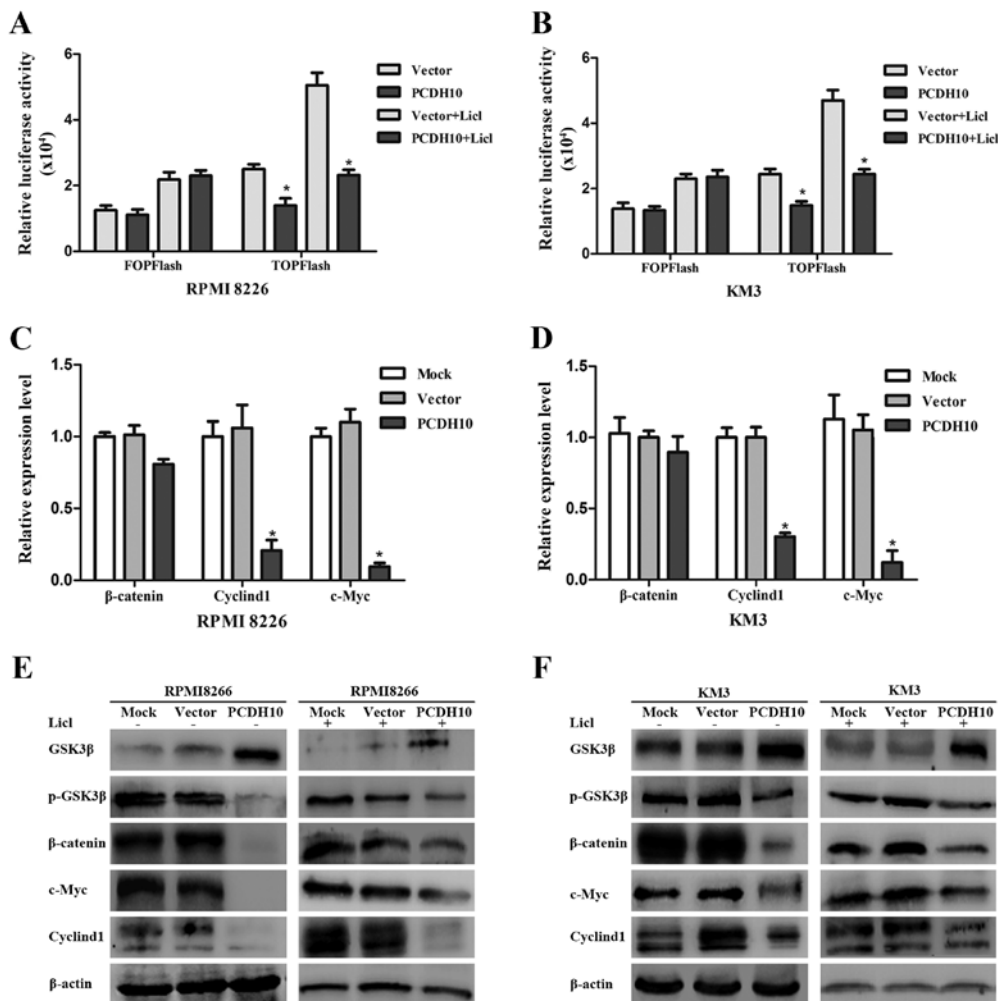


Figure 3. PCDH10 hampers Wnt signaling in MM cells. (A and B) The activity of Wnt luciferase reporter TOPFlash was determined when cells were stably transfected with PCDH10 or empty vector, supplemented with or without Licl. FOPFlash served as the negative control, \* $P < 0.05$  compared to the vector group. (C and D) The expression of  $\beta$ -catenin, Cyclin D1 and c-Myc was examined by RT-qPCR. \* $P < 0.05$  compared to the control group. (E and F) Total protein was extracted from the cells under the same conditions as in (A and B) and subjected to western blot analysis. Mock, non-transfected cells; Vector, empty vector-transfected cells; PCDH10, PCDH10-transfected cells.

was enhanced by PCDH10 overexpression, even in the cells which were supplemented with Licl (Fig. 3E and F). Of note, the mRNA level of  $\beta$ -catenin was not inhibited by PCDH10, whereas its protein expression was blocked effectively. Thus, the forced expression of PCDH10 significantly hindered the Wnt/ $\beta$ -catenin signaling in the presence or absence of Licl.

*PCDH10 restrains the translocation of  $\beta$ -catenin and the expression of BCL-9.* The canonical Wnt signaling pathway is known to underlie the pathogenesis of MM by the accumulation and nuclear localization of  $\beta$ -catenin. Nuclear localization of the  $\beta$ -catenin is translocated from the cytoplasm into the nucleus to stimulate Wnt/ $\beta$ -catenin signaling and then to accelerate tumor cell proliferation (27,30). Since we have confirmed that PCDH10 suppresses MM cell growth by targeting Wnt signaling, we investigated whether the functional PCDH10 disturbed the nuclear translocation of  $\beta$ -catenin and its coactivator BCL-9.

Immunofluorescence was utilized in RPMI-8226 cells supplemented with or without Licl to evaluate the relationship between PCDH10 and the translocation of  $\beta$ -catenin.

Compared to the control groups, PCDH10 overexpression, not only obviously resulted in the reduction of nuclear  $\beta$ -catenin, but also arrested  $\beta$ -catenin in the cytoplasm (Fig. 4A). This result suggested that the enhanced expression of PCDH10 can hinder the translocation of  $\beta$ -catenin.

Subsequently, the expression of BCL-9 was evaluated by RT-qPCR. It was revealed that BCL-9 was highly expressed in RPMI-8226 and KM3 cells, but not in PCDH10-transfected cells ( $P < 0.05$ , Fig. 4B). The result suggested that PCDH10 was associated with the modulation of BCL-9 to achieve its function. To verify this, western blot analysis was performed to assess the nuclear localization of  $\beta$ -catenin and BCL-9. It was also observed that PCDH10 blocked the nuclear localization of  $\beta$ -catenin and BCL-9 (Fig. 4C and D). These results showed that PCDH10 exerted an antagonistic effect by negatively affecting the Wnt/ $\beta$ -catenin/ BCL-9 signaling pathway.

*PCDH10 suppresses the activity of AKT.* Since the expression of GSK3 $\beta$  is promoted by the re-expression of PCDH10, PCDH10 is more involved in intracellular signaling than adhesion ability (5) and GSK3 $\beta$  is frequently downregulated by

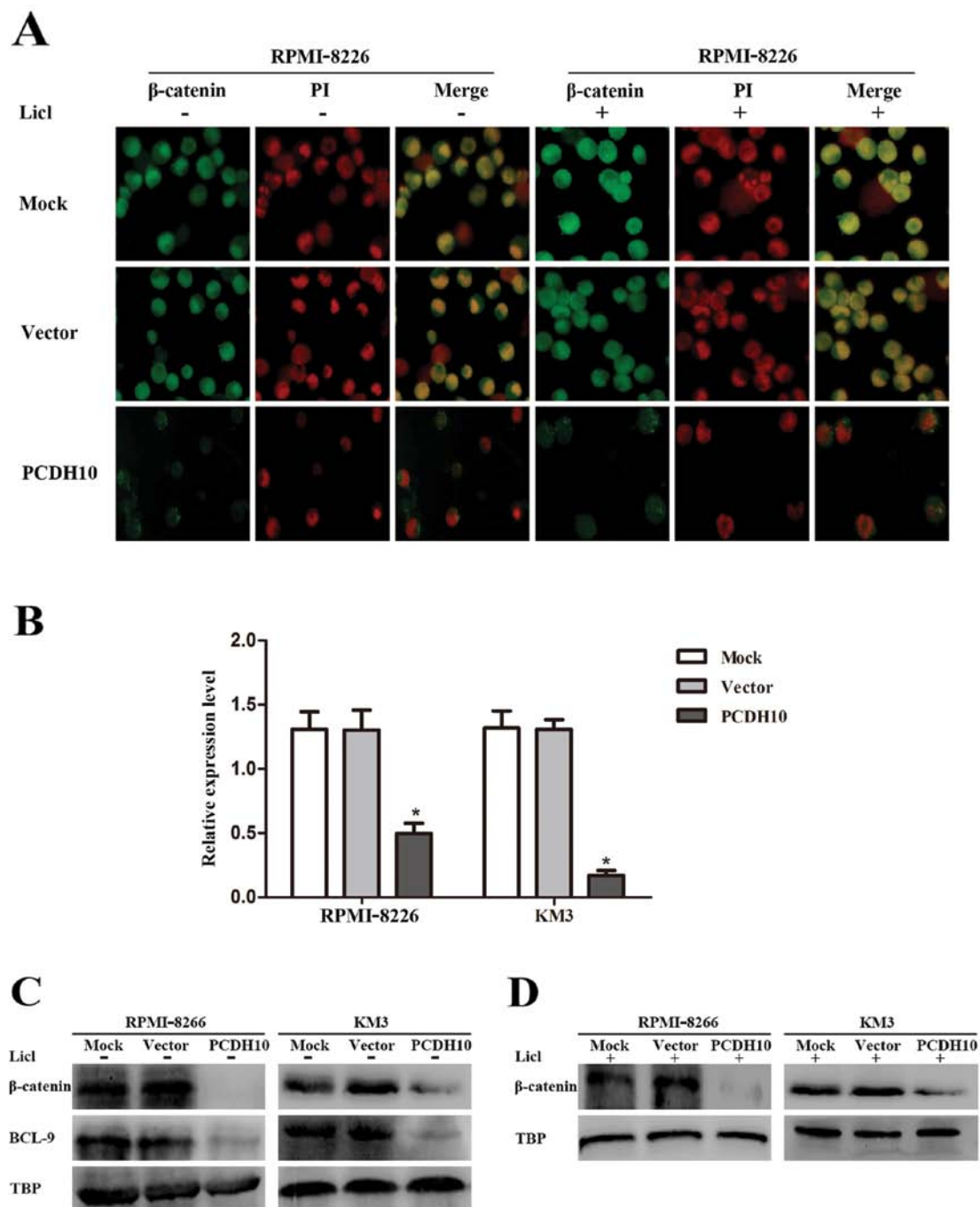


Figure 4. PCDH10 suppresses the nuclear accumulation of  $\beta$ -catenin and the expression of BCL-9. (A) Immunofluorescence was performed in RPMI-8226 cells supplemented with or without LiCl. It was shown that PCDH10 blocks  $\beta$ -catenin in the cytoplasm and downregulates the nuclear expression of  $\beta$ -catenin. (B) The expression of BCL-9 was examined by RT-qPCR in RPMI-8226 and KM3 cells. It was observed that BCL-9 is downregulated by PCDH10. (C and D) Nuclear protein was subjected to western blot analysis. It was shown that nuclear  $\beta$ -catenin and BCL-9 are successfully inhibited by the forced expression of PCDH10. \* $P < 0.05$  compared to the control groups. Mock, non-transfected cells; Vector, empty vector-transfected cells; PCDH10, PCDH10-transfected cells.

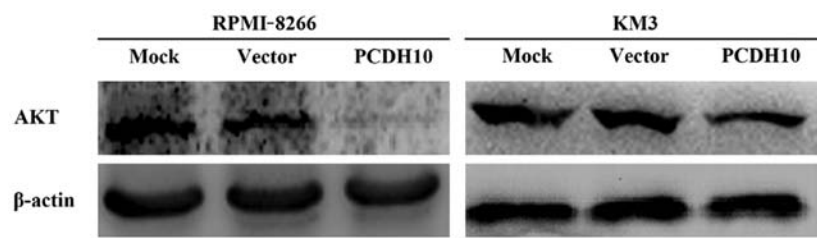


Figure 5. PCDH10 suppresses the activity of AKT. Western blot analysis was utilized in RPMI-8226 and KM3 cells transfected with or without PCDH10. It was observed that AKT is downregulated by PCDH10. Mock, non-transfected cells; Vector, empty vector-transfected cells; PCDH10, PCDH10-transfected cells.

the activity of PI3K/AKT (33,34). Thus, we hypothesized that PCDH10 increased the expression of GSK3 $\beta$  by obstructing the activity of AKT.

To confirm this, we detected the protein level of AKT in RPMI-8226 and KM3 cells. It was shown that PCDH10 successfully suppressed the expression of AKT (Fig. 5). This result suggested that PCDH10 was involved in PI3K/AKT signaling, allowing PCDH10 to upregulate the expression of GSK3 $\beta$ . However, the accurate mechanism involved in this process needs to be further elucidated.

## Discussion

In the present study, we identified a novel functional link between PCDH10 and Wnt signaling pathway in MM. It was confirmed that PCDH10 is a tumor suppressor and it can be a potential therapeutic tool by targeting Wnt/ $\beta$ -catenin/BCL-9. The constitutively active canonical Wnt/ $\beta$ -catenin signaling pathway has been documented in MM. However, MM remains incurable because of drug resistance, relapse or refractory (28-30). Therefore, further exploration of novel and more selective molecular targets is necessary.

In the present study, we determined that PCDH10 can successfully block MM cell growth, even in the situation of Wnt stimulation and the antagonistic effect was associated with cell cycle, which was achieved by G1-phase blockage. Additionally, the genes that associate with cell proliferation such as cyclin D1 and c-Myc were also downregulated by PCDH10 overexpression. We also revealed that the ectopic expression of PCDH10 highly contributed to the inactivation of LEF/TCF. This result shows that the ectopic expression of PCDH10 can significantly suppress the expression of cyclin D1 and c-Myc by arresting the activity of LEF/TCF and leading to the retardation of MM cell proliferation.

It is known that Wnt signaling results in the inhibition of GSK3 $\beta$  activity by promoting the phosphorylation of GSK3 $\beta$ , and then activates the accumulation of  $\beta$ -catenin, which translocates to the nucleus (31,32). Of note, we found that, PCDH10 restrained the phosphorylation of GSK3 $\beta$  and the protein expression of  $\beta$ -catenin, but there was no statistical difference of the alteration in the mRNA expression of  $\beta$ -catenin. Thus,  $\beta$ -catenin is modulated by PCDH10 mainly at the protein level rather than via mRNA expression. PCDH10 effectively obstructed the protein expression of AKT. Furthermore, it has been shown that the activation of AKT promotes the phosphorylation of GSK3 $\beta$  and accelerates the development of various types of cancer (33,34). This allows PCDH10 to upregulate GSK3 $\beta$  by blocking the activity of AKT, which in turn, stimulates the phosphorylation of  $\beta$ -catenin but decreases the protein expression of  $\beta$ -catenin in the cytoplasm and nucleus. Consequently, the  $\beta$ -catenin-TCF complex, which is crucial to the activation of Wnt signaling was also obstructed, together with the suppression of target genes such as cyclin D1 and c-Myc. However, the specific mechanism involved needs to be further investigated.

$\beta$ -catenin nuclear translocation has been identified as a key event that disturbs Wnt/ $\beta$ -catenin signaling and BCL-9, an extremely essential coactivator of the  $\beta$ -catenin-TCF complex and a novel therapeutic target (24,25). Our results show that, the ectopic expression of PCDH10 can hinder the protein expression of  $\beta$ -catenin and restrain its nuclear

translocation as well as the expression of BCL-9. This result suggests that PCDH10 is directly involved in the activation of Wnt/ $\beta$ -catenin transcriptional activity, which is mediated by the  $\beta$ -catenin-TCF complex and its coactivator BCL-9. BCL-9 is broadly associated with MM cell proliferation, survival, migration and drug resistance. More importantly, BCL-9 regulates Wnt target genes that control transition and stem cell-like behavior with a negligible effect on the homeostatic role of Wnt signaling in mammalian (24,27). These results suggest that the strategies employed on the restoration of PCDH10 can be a potential therapy to refractory and recurrent patients without a particular effect on normal tissues.

Recent findings have shown that, detection of PCDH10 methylation can identify high risk of biochemical recurrence and evaluate the prognosis of patients with gastric, colorectal and prostatic cancer (15-17). Additionally, c-Myc contributes to drug resistance in cancer chemotherapy (35,36) while PCDH10 markedly downregulated the expression of c-Myc. These findings suggest promising utilization of PCDH10 in the clinic. Therefore, the role of PCDH10 should be examined in clinical trials with regard to cancer progression, drug resistance and relapse in MM patients.

In conclusion, the deficiency of the tumor suppressor PCDH10 is a frequent pathogenetic event in MM, while restoration of PCDH10 successfully inhibits MM cell growth by obstructing Wnt signaling. In the present study, we provide solid evidence for the concept that PCDH10 restrains MM cell proliferation by the negative regulation of the Wnt/ $\beta$ -catenin/BCL-9 signaling pathway, which is broadly involved in cell proliferation, survival, drug resistance and relapse. Taken together, our results provide novel insights into the potential for clinical translation of strategies using PCDH10 as a novel selective therapeutic tool. Future studies should focus on the restoration of PCDH10 and identify its application in combination with therapeutic drugs.

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