WNT10A/β-catenin pathway in tumorigenesis of papillary thyroid carcinoma

TIANYI DONG[1,2*], ZHUN ZHANG[2*], WENHONG ZHOU[3], XIANGYU ZHOU[1], CHONG GENG[2], LAP KAM CHANG[1], XINGSONG TIAN[2] and SHILI LIU[1]

[1]School of Medicine, Shandong University, Jinan, Shandong 250012; [2]Department of Breast Thyroid Surgery, Shandong Provincial Hospital Affiliated to Shandong University; [3]Department of Nursing, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong 250021, P.R. China

Received January 4, 2017; Accepted May 31, 2017

DOI: 10.3892/or.2017.5777

Abstract. Papillary thyroid carcinoma is the most common thyroid cancer and the incidence is increasing. Aberrant activation of the WNT/β-catenin pathway plays an important role in carcinogenesis. In the present study, microarray analysis was employed to compare tissues from papillary thyroid cancer and adjacent normal tissues to determine candidate genes facilitating tumor invasion. The result demonstrated that genes involved in WNT/β-catenin signaling pathway were activated in papillary thyroid cancer, WNT10A expression was found to be upregulated >4-fold. The variations in gene expression were verified in tissues obtained from other papillary thyroid cancer patients. Molecular mechanism exploration in thyroid cells showed that enhanced WNT10A/β-catenin signaling pathway activation promoted cell proliferation and migration. The promotion was validated by RNA interference of WNT10A and LEF1 expression. Moreover, results from flow cytometry demonstrated that WNT/β-catenin signaling pathway activation reduced the percentage of late apoptotic thyroid cells. Conclusively, the results suggest for the first time that WNT10A/β-catenin signaling pathway plays a crucial role in human papillary thyroid cancer.

Correspondence to: Professor Xingsong Tian, Department of Breast Thyroid Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong 250021, P.R. China
E-mail: tianxs0509@163.com

Dr Shili Liu, Department of Medical Microbiology, School of Medicine, Shandong University, Jinan, Shandong 250012, P.R. China
E-mail: liushili@sdu.edu.cn

*Contributed equally

Key words: papillary thyroid carcinoma, WNT10A, β-catenin, late apoptosis

Introduction

Thyroid cancer is the most common endocrine malignancy, which affected ~63,000 persons in the USA, in 2014. Studies between 1975 and 2009 revealed an annual increase in incidence rate from 4.9 to 14.3 per 100,000 (1,2). Thyroid cancer can be categorized according to histopathological findings. Papillary thyroid carcinoma (PTC) is the most prevalent subtype that accounts for ~70-80% of all thyroid cancer cases. One report predicted that the incidence of PTC will double in the USA by 2019 (3,4). The current protocol for treating PTC involve the combination of thyroidectomy and radioiodine therapy. However, these are often not curative for cases that exhibit aggressive clinical characteristics. Hence, a better understanding in molecular pathogenesis and mechanism in PTC would enable us to develop more effective treatment targets and strategies.

WNT and its signaling pathways play a crucial role in human organogenesis (5). In human, WNT genes encode secretory signaling glycoproteins that are cysteine rich and are 350-400 amino acids in length (6). Its corresponding signaling pathways are classified according to the involvement of β-catenin: the canonical (WNT-β-catenin pathway) (7) and the non-canonical pathways (WNT/planar cell polarity (PCP) or WNT/Ca^{2+} pathway) (8).

In the canonical pathway, WNT ligands bind to Frizzled receptors that induce phosphoprotein Dishevelled for activation. This activation would inhibit β-catenin phosphorylation by glycogen synthase kinase-3β-adenomatous polyposis coli-axin complex. Consequently, β-catenin accumulates in the cytoplasm and eventually translocate into the nucleus, where it acts as a transcriptional coactivator alongside the T-cell factor and lymphoid enhancing factor (TCF/LEF). They form complexes that initiate the expression of downstream genes such as cyclin D1 and c-myc. Such activation seems to play an important role in cell proliferation, differentiation, cell-cell adhesion and cell migration (6,9,10). Hence, any disruption in this pathway would contribute to tumorigenesis.

WNT10A is one of the nineteen WNT signaling glycoproteins and it is the focus of this study. The WNT10A genes are encoded on human chromosome 17q21 (11). Previous studies indicated any aberration of WNT10A in human can induce

defects in tooth morphogenesis, dentinogenesis, odontoblast differentiation, hair follicle development, papillae of the tongue and sweat gland, nail formation, and regeneration of the epidermis (12-14). In addition, WNT10A mutation was demonstrated to play key roles in carcinoma of esophageal, gastric, kidney and colorectal as well as endometrioid carcinoma.

Further studies into cellular level revealed that mutation promotes tumor cell proliferation and migration, which may be linked to self-renewal of a subset of ESCC cells in esophageal squamous cell carcinoma by regulating β-catenin (15-18). In concurrence, the knockdown of WNT10A suppressed cell proliferation. It also induces S-phase cell cycle arrest in mouse embryonic palatal mesenchymal (MEPM) cells through WNT/β-catenin signaling pathway (19). However, the association of WNT ligands and its pathway in the pathogenesis of PTC and progression have not yet been determined. In this study, the WNT10A/β-catenin pathway was demonstrated to promote and play a role in the PTC development.

Materials and methods

Specimens. A total of 35 cases of primary papillary thyroid cancer and 35 cases of adjacent normal tissues were collected as fresh frozen tissues from Shandong Provincial Hospital. All samples were collected under the approved guidelines of Shandong Provincial Hospital's institutional review board. The protocols were reviewed and approved by the ethic committee of Shandong University (Jinan, Shandong, China).

Microarray analysis. The microarray chip consisted of 27,326 different human cDNAs (Angilent, Wilmington, DE, USA), in which house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal control. The cDNAs from 5 cases of papillary thyroid cancer were labeled with Cy5, and the cDNAs from 5 cases of adjacent normal tissues were labeled with Cy3. The labeled cDNAs were hybridized with microarray chip under standard conditions according to the manufacturer's instructions. The data were analyzed by Molecule annotation system 3.0.

Cell culture and transfection. Thyroid cancer cell lines GLAG-66 was used in this study. All thyroid cancer cell lines were maintained in our laboratory. GLAG-66 was maintained in Nutrient mixture F-12 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (2%) at 37˚C with 5% CO_2, as described previously (20). Cells were passaged 1:3 until 80% confluence was reached in a 75-cm^2 culture flask (Nest Biotechnology, Shanghai, China). The ORF human WNT10A cDNA expression plasmid and LEF1 cDNA expression plasmid were purchased from Biosune Co. (Shanghai, China). FuGENE HD transfection reagent (Roche Applied Science, Basel, Switzerland) was used for transfection. All transfections were performed according to the manufacturer's instructions.

SiRNA interference. Chemical modified Stealth siRNA (chemical modified stealth siRNA) targeting WNT10AsiRNA and LEF1siRNA were from RiboBio Co., Ltd. (Guangzhou, Guangdong, China). The sequence for WNT10A siRNA was 5'-CCACGAATGCCAACACCAA-3'. The sequence for LEF1 siRNA was 5'-GCTACATATGCAGCTTTAT-3'. Cells were transfected with siRNA by the Lipofectamine 3000 method (Life Technologies, CA, USA).

RNA isolation and quantitative real-time PCR. Total cellular or tissue RNA was extracted with TRIzol (Life Technologies) according to the manufacturer's instructions. First-strand cDNA was synthesized from 1 μg total cellular or tissue RNA using PrimeScript™ RT Master Mix (Takara) with random primers. Then cDNA was amplified for quantitative real-time PCR, and the specific primers used were as follows: for WNT10A, forward, 5'-TCCCATCTTCAGCAGAGGTTTC-3' and reverse, 5'-CACTGCCTGCCTCCCAACT-3'; for β-actin, forward, 5'-AGTTGCGTTACACCCTTTCTTG-3' and reverse, 5'-CACCTTCACCGTTCCAGTTTT-3'; for LEF1, forward, 5'-AGAGGAAGGCGATTTAGC-3' and reverse, 5'-ACCACG GGCACTTTATTT-3'; for CTNNB1, forward, 5'-GCAGCAA CAGTCTTA CCT-3' and reverse, 5'-ACAGGACTTGGGAGG TAT-3'; for CCND1 forward, 5'-GCGAGGAACAGAAGT GCG-3' and reverse, 5'-TGGAGTTGTCGGTGTAGATGC-3'; for MYC forward, 5'-TCCTGTCCGTCCAAGCAG-3' and reverse, 5'-ACGCACAAGAGTTCCGTAG -3'. The real-time PCR reactions were performed under the following conditions: denaturation at 95˚C for 10 sec, annealing at 55˚C for 30 sec, and extension at 72˚C for 30 sec, a total of 35 cycles. The real-time PCR reactions were performed in the Roche 480 Real-Time PCR system with SYBR Premix Ex Taq™ according to the manufacturer's instructions.

Wound healing assay. Confluent GLAG-66 cell monolayers on 6-well tissue culture plastic dishes were transfected with pEnter-WNT10A, pEnter-LEF1 and their control plasmid pEnter-MOCK, as well as WNT10A and LEF1 siRNA. GLAG-66 cells were grown to confluent monolayers on 6-well plates and a thin disposable tip was used to create linear scratch wounds. Cultures were rinsed with PBS and replaced with fresh quiescent medium containing 10% fetal bovine serum. Wound images were taken with a digital camera mounted on a light microscope at 0, 24, 36 and 72 h. The wound gap widths were measured using ImageJ software.

Protein extraction and western blot analysis. Total cellular or tissue protein extracts were obtained using PMSF with RIPA (the ratio of PMSF to RIPA is 1:100). Cells or tissues were washed twice with cold PBS and lysed in a buffer containing 100 μl RIPA and 1 μl PMSF. Protein concentration was determined by the BCA method using the BCA protein assay (Thermo Scientific/Pierce, Courtaboeuf, France). Cellular or tissue protein extracts were separated on 10% SDS-PAGE gel. Proteins were transferred to polyvinylidene difluoride microporous membranes (Millipore, MA, USA), and then blocked with 5% non-fat dry milk for 1 h at room temperature. The membranes were incubated overnight at 4˚C with the primary antibody specific to WNT10A (rabbit polyclonal, Abcam, 1:500, Abcam), β-catenin (rabbit monoclonal, 1:5,000, Abcam), LEF1 (rabbit monoclonal, 1:1,000, Abcam), MYC (rabbit monoclonal, 1:10,000, Abcam), cyclin D1 (rabbit monoclonal, 1:10,000, Abcam) or GAPDH (rabbit polyclonal, 1:500 Boster Co., Ltd., Wuhan, China) as the internal control. The membranes were washed and incubated with anti-rabbit antibody (rabbit polyclonal, 1:2,000 Boster) for

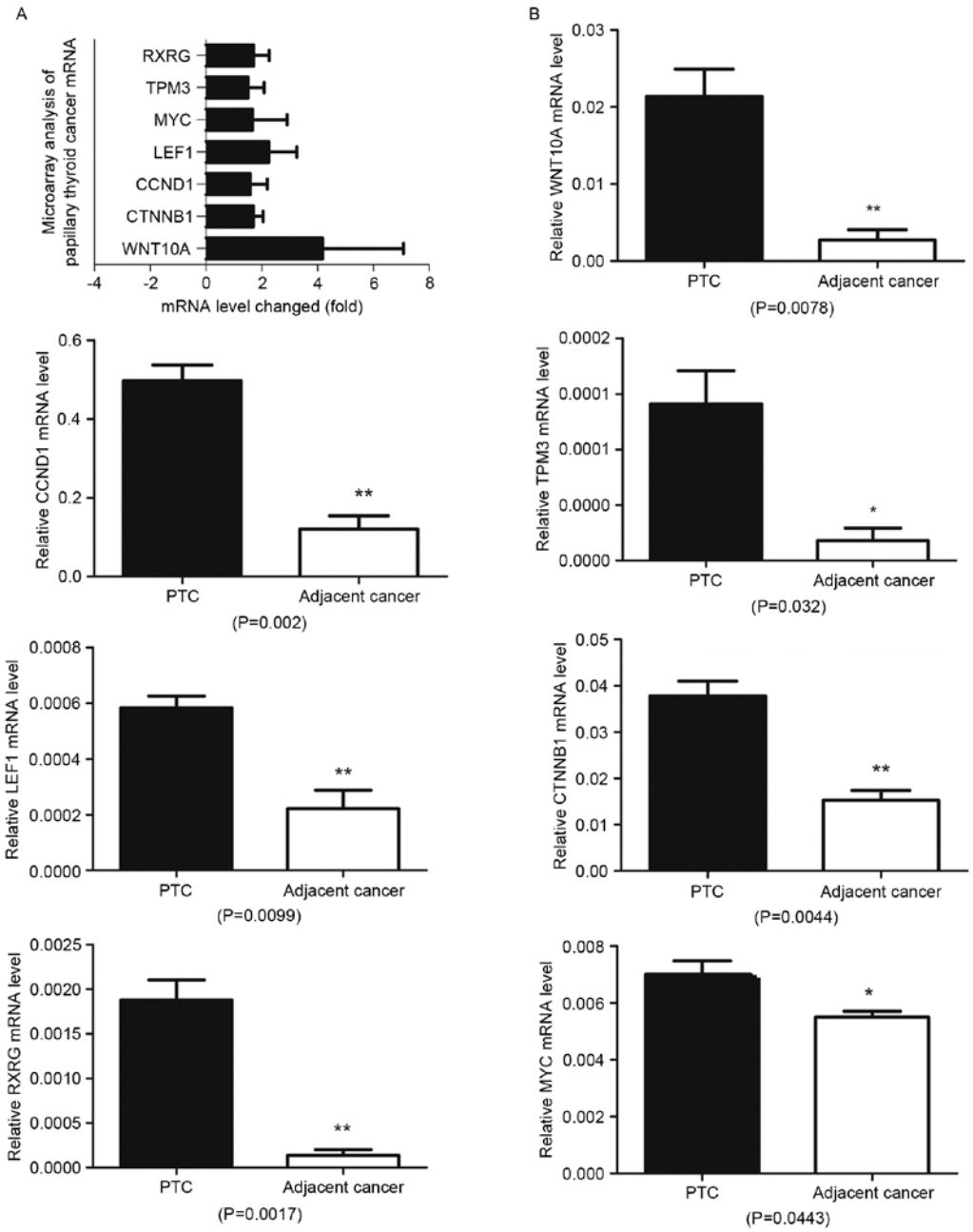

Figure 1. WNT10A/β-catenin signaling pathway is activated in microarray analysis data of papillary thyroid cancer and adjacent normal tissues. Microarray analysis showed that CCND1, RXRG, LEF1, MYC, CTNNB1, TPM3 expression increased >1.5-fold in papillary thyroid cancer tissues, all these molecules are involved in WNT10A/β-catenin signaling pathway. (A) Upregulated expression of the molecules involved in WNT10A/β-catenin signaling pathway was verified by QRT-PCR (B). QRT-PCR was performed as described in Materials and methods.

1 h at room temperature, which were subsequently washed and shown using chemiluminescence reagent.

Cell proliferation assay. A total of $2x10^3$ cells were cultured in 96-well plates. Cell proliferation was quantified using the Cell Counting Kit-8 assay (Roche, Penzberg, Germany) according to the manufacturer's instructions. Absorbance was measured on a microplate reader (Tecan Sunrise, Mannedorf, Switzerland) at a wavelength of 450 nm. Data represent the average values of three independent experiments.

Apoptosis analysis. $5x10^5$ GLAG-66 cells in 6-well plates were transfected with pEnter-WNT10A, pEnter-LEF1, control plasmid pEnter-mock, WNT10AsiRNA and LEF1siRNA, and incubating for varied time-points before the cells were digested and harvested by centrifugation. Then the cells were separately fixed gently (drop by drop) in 70% ethanol overnight at -20˚C and then re-suspended in 535 μl DDW containing 500 μl PI stain buffer, 10 μl RNaseA solution (50X) and 25 μl PI. After 30 min at 37˚C in the dark, the cells were analyzed with flow cytometry equipped with an argon laser at 488 nm. Then cell cycle was determined and analyzed. Analysis of apoptosis was quantified by Annexin V-FITC apoptosis detection kit according to the manufacturer's instructions (BD Biosciences, USA) (21,22). Apoptotic events were acquired and analyzed by BD-FACS calibur instrument (BD Biosciences).

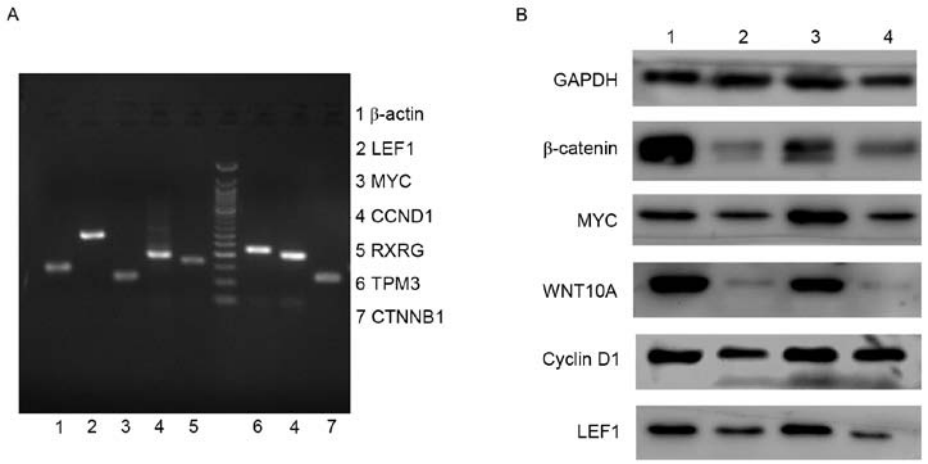

Figure 2. Upregulated expression of the molecules involved in WNT10A/β-catenin signaling pathway verified by electrophoresis (A) and western blot analysis (B). (B) Lane 1, papillary thyroid cancer tissues; lane 2, adjacent normal tissues; lane 3, papillary thyroid cancer tissues; lane 4, adjacent normal tissues. QRT-PCR and western blot analysis were performed as described in Materials and methods.

Results

WNT10A/β-catenin signaling pathway is activated in papillary thyroid cancer. To investigate the molecular mechanisms involved in tumorigenesis of papillary thyroid cancer, microarray analysis was performed to compare the variation of gene expressions within the PCT cell populations and their adjacent normal tissues. Up- or downregulation 1.5-fold was set as a cutoff value, and changes in gene expression were analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID). In the DAVID analysis, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway related to papillary thyroid cancer was hsa05216: thyroid cancer, six genes (CCND1, RXRG, LEF1, MYC, CTNNB1 and TPM3) were upregulated >1.5-fold in this pathway (Fig. 1A). All these genes were associated with WNT/β-catenin signaling pathway, among them LEF1 and CTNNB1 play critical roles in WNT/β-catenin signaling pathway. Of the signaling proteins, WNT10A was the only WNT ligand that was upregulated >1.5-fold and its upregulation was found to be >4-fold in the papillary thyroid cancer comparing to adjacent normal tissues.

Molecules involved in WNT10A/β-catenin signaling pathway are upregulated in papillary thyroid cancer tissues. Next, the expression of WNT10A, LEF1, MYC, β-catenin and cyclin D1 were verified by QRT-PCR in 15 cases of papillary thyroid cancer (Fig. 1B). The real-time PCR (QRT-PCR) products were further separated by agarose gel electrophoresis and visualized to compare the initial levels of WNT10A, LEF1, MYC, β-catenin and cyclin D1 mRNA in the tissues from papillary thyroid cancer and adjacent normal tissues (Fig. 2A). To confirm the results at mRNA level, WNT10A, LEF1, MYC, β-catenin and cyclin D1 expression was further detected by western blot analysis in 10 cases of papillary thyroid cancer and adjacent normal tissues, the results demonstrated upregulated expression in papillary thyroid cancer tissues comparing to adjacent normal ones (Fig. 2B). The results demonstrated that WNT10A/β-catenin signaling pathway was activated in papillary thyroid cancer tissues.

WNT10A/β-catenin signaling pathway activation promotes proliferation of thyroid cells. To investigate the role of WNT10A/β-catenin signaling pathway in mediating migration and invasion of thyroid cancer cells, cultured GLAG-66 cells were transfected with pEnter-WNT10A, pEnter-LEF1 and control plasmid pEnter-mock. WNT10A siRNA and LEF1siRNA were employed to knockdown the expression of the two genes, and the proliferations of the treated cells were quantified with the Cell Counting Kit-8 assay. The expression of WNT10A in the cell lines was confirmed by RT-PCR and western blot analysis (Fig. 3A), the levels of β-catenin, LEF1, MYC and cyclin D1 protein were induced (Fig. 3B), suggesting that WNT10A effectively activates β-catenin signaling pathway in thyroid cancer cells. As a response to the WNT/β-catenin signaling pathway activation, the CCK-8 assay results showed that the WNT10A and LEF1 overexpressing cells exhibited significant higher level of proliferation as compared with the empty vector controls and WNT10A siRNA (Fig. 3C).

WNT10A/β-catenin signaling pathway activation suppressed late apoptosis of thyroid cancer cells. GLAG-66 cells were then examined by flow cytometry to test whether cell cycle and cell apoptosis were modulated concomitant with WNT10A and LEF1 administration. Following transfection with the pEnter-WNT10A and pEnter-LEF1 plasmid, respectively. The results indicated a lower percentage of late apoptotic cells after WNT10A and LEF1 overexpression (Fig. 4A, Q2-4). As expected, higher percentage of late apoptotic cells were detected after reduction of WNT10A and LEF1 with siRNA (Fig. 4B, Q2-4). Quantification of the results confirmed the experimental conclusion (Fig. 4C).

WNT10A/β-catenin signaling pathway activation enhances migration of the thyroid cells. Next, the directional migration of GLAG-66 cells was examined with the wound-healing assay under WNT10A/β-catenin signaling pathway activation. The 'wound' was created in confluent cell cultures (0 h), and migration of cells into the gap was monitored after 72 h. As shown in Fig. 5A, GLAG-66 cells grew into the wound

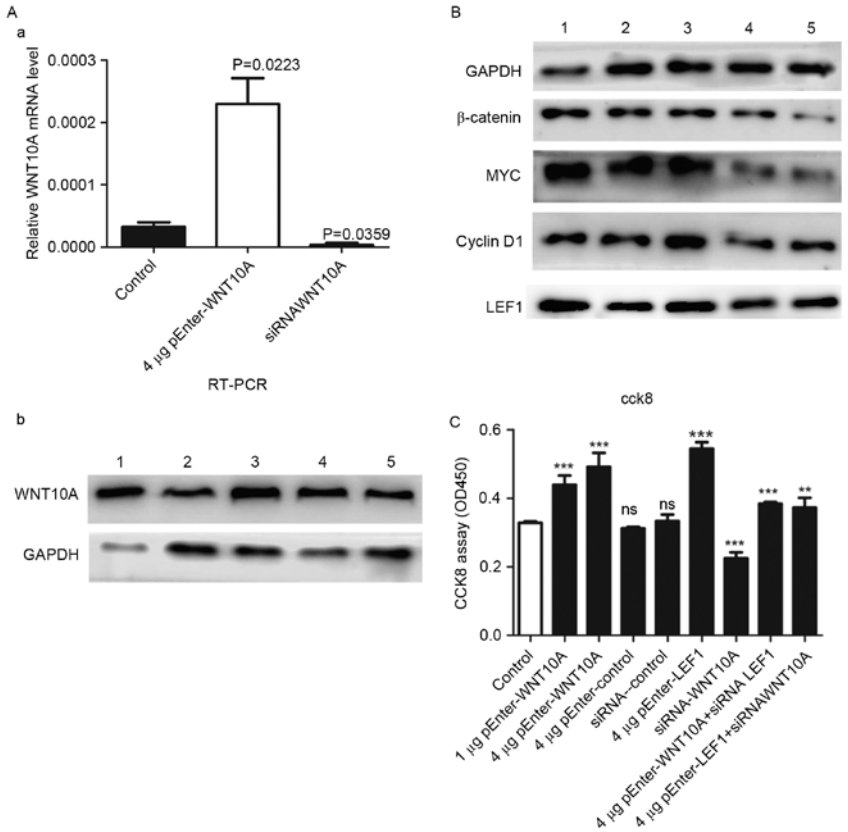

Figure 3. WNT10A/β-catenin signaling pathway activation promotes proliferation of thyroid cells. (A) QRT-PCR and western blot analysis of WNT10A expression under the overexpression plasmid pEnter-WNT10A and siRNA transfection in GLAG-66 cells. (B) Western blot analysis of β-catenin, MYC, cyclin D1 and LEF1 expression. Lane 1, 4 μg of pEnter-LEF1; lane 2, 4 μg of pEnter-LEF1 and WNT10A siRNA; lane 3, 4 μg of pEnter-WNT10A; lane 4, 4 μg of pEnter-WNT10A and LEF1siRNA; lane 5, control plasmid pEnter-mock. (C) Cell Counting Kit-8 assay of GLAG-66 cells (***P<0.0001, **P=0.0079; Student's t-test). QRT-PCR, western blot analysis and CCK-8 were performed as described in Materials and methods.

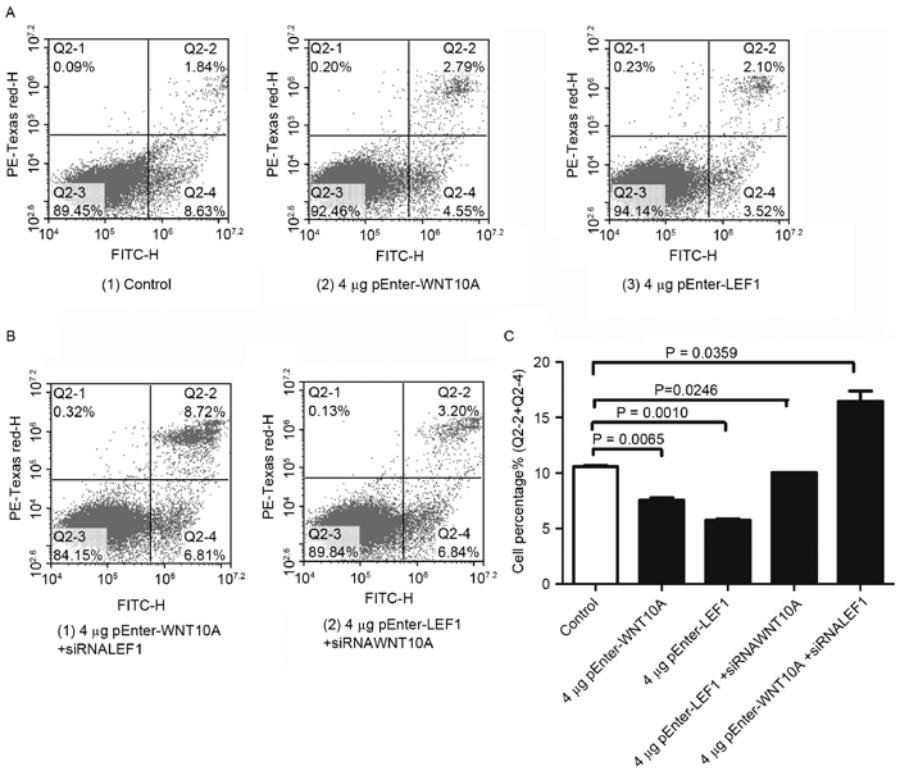

Figure 4. WNT10A/β-catenin signaling pathway activation suppresses late apoptosis of thyroid cancer cells. (A) Late apoptotic cells were reduced concomitant with pEnter-WNT10A and pEnter-LEF1 transfection. (B) Late apoptotic cells were restored by pEnter-WNT10A and pEnter-LEF1 siRNA. (C) Quantification of apoptosis analysis results (Student's t-test). RNA interference and apoptosis analysis were performed as described in Materials and methods.

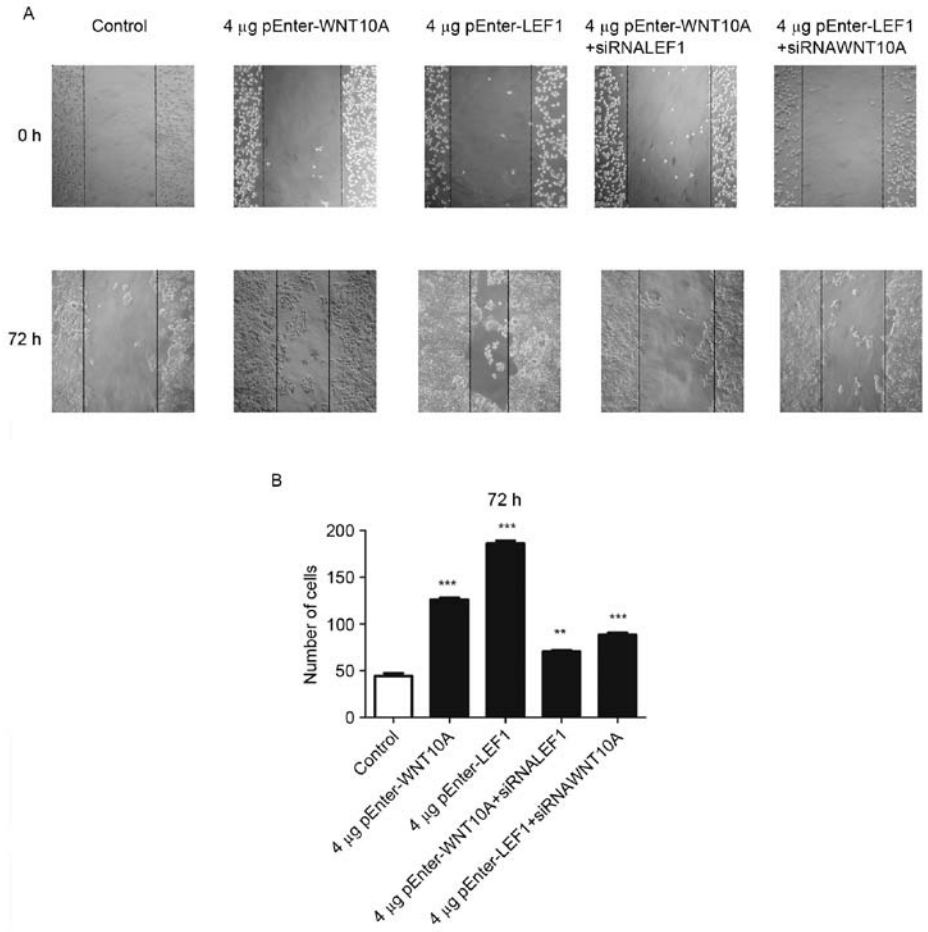

Figure 5. WNT10A/β-catenin signaling pathway activation enhanced migration of the thyroid cells. (A) WNT10A and LEF1 expression in GLAG-66 cells had significant effects on cell migration; cell migration was enhanced when WNT10A and LEF1 were overexpressed, whereas migration was reduced as WNT10A and LEF1 were downregulated. (B) Quantification of wound healing assay results (Student's t-test). Wound healing assays were performed as described in Materials and methods.

as time passed, and the speeds of the WNT10A and LEF1 overexpressing cells migrating into the wound were significantly increased compared to the control cells. In line with this result, knockdown of WNT10A and LEF1 expression with their siRNA reduced the migration ability of GLAG-66 cells, respectively. Quantification of wound healing assay confirmed this conclusion (Fig. 5B). These data showed that upregulation of WNT10A and LEF1 expression significantly increased migration of the GLAG-66 cells.

Discussion

WNT/β-catenin signaling is known to be an important factor in stem cell regulation of intestinal cancer (23,24), breast cancer (25-27) and skin cancer (28). Activation of canonical WNT signaling pathway is an important process both in the establishment and maintenance of cancer stem cells (29), as well as promoting self-renewal phenotype (18). Previous studies have indicated that DACT2 (30), PPFP (31), Dickkopf-1 (32), GPX3 (33), miR-146b-5p (34) and PROX1 (35) are methylated or disordered in thyroid cancer, leading to growth, metastasis and EMT of thyroid cancer cells by activating WNT signaling pathway. The WNT/β-catenin pathway is a direct and forward enhancer of the TTF-1 expression that can regulate serum thyroglobulin levels, which is intimately linked to follow-up of PTC patients (5,36).

In previous studies, WNT10A was shown to play an important role in the pathogenesis of idiopathic pulmonary fibrosis (37), agenesis of the maxillary permanent canines and dental agenesis (38,39), hypohidrotic ectodermal dysplasia (40) and keratoconus (41). Specifically, the aberration of WNT10A leads to malformation of ectodermal appendages during development. During hair follicle morphogenesis, expression of WNT10A is weakly upregulated in the placode compared with adjacent epidermis (42). Actually, WNT10A uniquely observed in basal and mammary stem cells may regulate the canonical WNT signaling pathway to maintain basal and mammary stem cell activity and was secreted by mammary stem cells (43). Consequently, it is believed that WNT10A may be necessary for epithelial migration and proliferation during normal development. However, the role of WNT10A in PTC is still unknown.

By employing microarray analysis of PTC, we found that WNT10A was upregulated in PTC tissues but limitedly expressed in adjacent normal tissues. WNT10A and WNT6 may be considered to be the key factors in human carcinogenesis through activation of WNT/β-catenin pathway (44). Previous studies have shown that WNT6 expression is not significantly different in primary gastric cancer. However,

another report claimed that WNT10A was upregulated in primary gastric cancer (45). It was further verified that overexpression or knockdown of WNT10A had direct influence on proliferation, migration and invasion of PTC cells by modulating WNT/β-catenin pathway in thyroid papillary cancer. In our study, it was found that overexpression of WNT10A led to increase in β-catenin, upregulated cyclin D1 and c-myc expression and it was the same with overexpression of LEF1. Forced WNT10A expression in GLAG-66 cells resulted in increased cell proliferation, migration, invasiveness, and cell transformation. Consistently, knockdown of WNT10A in GLAG-66 can decrease intracellular β-catenin accumulation, downregulate cyclin D1 and c-myc expression, and thus suppress cell proliferation, migration, invasiveness, and cell transformation. Combined with the designs of cell line models, our results suggested that WNT10A might exert an autocrine effect on PTC, resulting in activation of WNT/β-catenin signaling and promoting cell proliferation and migration.

These data indicated that WNT10A plays a crucial role in carcinogenesis and aggressiveness in papillary thyroid cancer by activating β-catenin-dependent pathway. This in turn would also increase β-catenin, LEF1, MYC and cyclin D1 expression. Similar results were obtained from esophageal squamous cell carcinoma, the upregulation of WNT10A has been proven to induce proliferation and migration and to promote a self-renewal phenotype of esophageal carcinoma cell lines (18). Several molecular components in Wnt/β-catenin signaling have been proposed as potential therapeutic targets of cancer treatment (46).Wnt/β-catenin signaling is associated with lymph node metastasis of PTC by regulating the expression of cyclin D1 (47). Dickkopf-1, a Wnt/β-catenin pathway inhibitor, suppressed proliferation and migration of PTC cells by modulating the Wnt/β-catenin pathway (48). Our study demonstrated the role of WNT10A/β-catenin pathway in thyroid tumorigenesis for the first time. WNT10A/β-catenin shows potential as a future therapeutic target for papillary thyroid cancer.

Acknowledgments

We gratefully acknowledge the financial support from The National Natural Science Foundation of China (81272351), The Fundamental Research Funds of Shandong University (2015JC010), The Project of Medical and Health Technology Development Program in Shandong Province (grant no. 2014ws0349), The Open Research Fund of State Key Laboratory of Environmental Chemistry and Ecotoxicology (KF2014-08) and The Primary Research and Development Plan of Shandong Province (2016GSF201137).

References

1. Liu S, Semenciw R, Ugnat AM and Mao Y: Increasing thyroid cancer incidence in Canada, 1970-1996: Time trends and age-period-cohort effects. Br J Cancer 85: 1335-1339, 2001.
2. Ferlay J, Parkin DM and Steliarova-Foucher E: Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 46: 765-781, 2010.
3. Davies L and Welch HG: Current thyroid cancer trends in the United States. JAMA Otolaryngol Head Neck Surg 140: 317-322, 2014.
4. Aschebrook-Kilfoy B, Schechter RB, Shih YC, Kaplan EL, Chiu BC, Angelos P and Grogan RH: The clinical and economic burden of a sustained increase in thyroid cancer incidence. Cancer Epidemiol Biomarkers Prev 22: 1252-1259, 2013.
5. Behrens J and Lustig B: The Wnt connection to tumorigenesis. Int J Dev Biol 48: 477-487, 2004.
6. Sastre-Perona A and Santisteban P: Role of the wnt pathway in thyroid cancer. Front Endocrinol (Lausanne) 3: 31, 2012.
7. Xing Y, Clements WK, Kimelman D and Xu W: Crystal structure of a beta-catenin/axin complex suggests a mechanism for the beta-catenin destruction complex. Genes Dev 17: 2753-2764, 2003.
8. James RG, Conrad WH and Moon RT: Beta-catenin-independent Wnt pathways: Signals, core proteins, and effectors. Methods Mol Biol 468: 131-144, 2008.
9. Polakis P: The many ways of Wnt in cancer. Curr Opin Genet Dev 17: 45-51, 2007.
10. Roberts DM, Slep KC and Peifer M: It takes more than two to tango: Dishevelled polymerization and Wnt signaling. Nat Struct Mol Biol 14: 463-465, 2007.
11. Katoh M: WNT and FGF gene clusters (Review). Int J Oncol 21: 1269-1273, 2002.
12. Bohring A, Stamm T, Spaich C, Haase C, Spree K, Hehr U, Hoffmann M, Ledig S, Sel S, Wieacker P, *et al*: WNT10A mutations are a frequent cause of a broad spectrum of ectodermal dysplasias with sex-biased manifestation pattern in heterozygotes. Am J Hum Genet 85: 97-105, 2009.
13. Cluzeau C, Hadj-Rabia S, Jambou M, Mansour S, Guigue P, Masmoudi S, Bal E, Chassaing N, Vincent MC, Viot G, *et al*: Only four genes (EDA1, EDAR, EDARADD, and WNT10A) account for 90% of hypohidrotic/anhidrotic ectodermal dysplasia cases. Hum Mutat 32: 70-72, 2011.
14. Nawaz S, Klar J, Wajid M, Aslam M, Tariq M, Schuster J, Baig SM and Dahl N: WNT10A missense mutation associated with a complete odonto-onycho-dermal dysplasia syndrome. Eur J Hum Genet 17: 1600-1605, 2009.
15. Chen H, Wang Y and Xue F: Expression and the clinical significance of Wnt10a and Wnt10b in endometrial cancer are associated with the Wnt/β-catenin pathway. Oncol Rep 29: 507-514, 2013.
16. Hsu RJ, Ho JY, Cha TL, Yu DS, Wu CL, Huang WP, Chu P, Chen YH, Chen JT and Yu CP: WNT10A plays an oncogenic role in renal cell carcinoma by activating WNT/β-catenin pathway. PLoS One 7: e47649, 2012.
17. Kirikoshi H, Inoue S, Sekihara H and Katoh M: Expression of WNT10A in human cancer. Int J Oncol 19: 997-1001, 2001.
18. Long A, Giroux V, Whelan KA, Hamilton KE, Tétreault MP, Tanaka K, Lee JS, Klein-Szanto AJ, Nakagawa H and Rustgi AK: WNT10A promotes an invasive and self-renewing phenotype in esophageal squamous cell carcinoma. Carcinogenesis 36: 598-606, 2015.
19. Feng C, Xu Z, Li Z, Zhang D, Liu Q and Lu L: Down-regulation of Wnt10a by RNA interference inhibits proliferation and promotes apoptosis in mouse embryonic palatal mesenchymal cells through Wnt/β-catenin signaling pathway. J Physiol Biochem 69: 855-863, 2013.
20. Saiselet M, Floor S, Tarabichi M, Dom G, Hébrant A, van Staveren WC and Maenhaut C: Thyroid cancer cell lines: An overview. Front Endocrinol (Lausanne) 3: 133, 2012.
21. van Engeland M, Nieland LJ, Ramaekers FC, Schutte B and Reutelingsperger CP: Annexin V-affinity assay: A review on an apoptosis detection system based on phosphatidylserine exposure. Cytometry 31: 1-9, 1998.
22. Vermes I, Haanen C, Steffens-Nakken H and Reutelingsperger C: A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods 184: 39-51, 1995.
23. Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ and Clevers H: Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 457: 608-611, 2009.
24. Holland JD, Klaus A, Garratt AN and Birchmeier W: Wnt signaling in stem and cancer stem cells. Curr Opin Cell Biol 25: 254-264, 2013.
25. Teissedre B, Pinderhughes A, Incassati A, Hatsell SJ, Hiremath M and Cowin P: MMTV-Wnt1 and -DeltaN89beta-catenin induce canonical signaling in distinct progenitors and differentially activate Hedgehog signaling within mammary tumors. PLoS One 4: e4537, 2009.
26. Vaillant F, Asselin-Labat ML, Shackleton M, Forrest NC, Lindeman GJ and Visvader JE: The mammary progenitor marker CD61/beta3 integrin identifies cancer stem cells in mouse models of mammary tumorigenesis. Cancer Res 68: 7711-7717, 2008.

27. Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ and Visvader JE: Generation of a functional mammary gland from a single stem cell. Nature 439: 84-88, 2006.
28. Malanchi I, Santamaria-Martínez A, Susanto E, Peng H, Lehr HA, Delaloye JF and Huelsken J: Interactions between cancer stem cells and their niche govern metastatic colonization. Nature 481: 85-89, 2011.
29. Morgan RG, Ridsdale J, Tonks A and Darley RL: Factors affecting the nuclear localization of β-catenin in normal and malignant tissue. J Cell Biochem 115: 1351-1361, 2014.
30. Zhao Z, Herman JG, Brock MV, Sheng J, Zhang M, Liu B and Guo M: Methylation of DACT2 promotes papillary thyroid cancer metastasis by activating Wnt signaling. PLoS One 9: e112336, 2014.
31. Vu-Phan D, Grachtchouk V, Yu J, Colby LA, Wicha MS and Koenig RJ: The thyroid cancer PAX8-PPARG fusion protein activates Wnt/TCF-responsive cells that have a transformed phenotype. Endocr Relat Cancer 20: 725-739, 2013.
32. Cho SW, Kim YA, Sun HJ, Ahn HY, Lee EK, Yi KH, Oh BC, Park DJ, Cho BY and Park YJ: Therapeutic potential of Dickkopf-1 in wild-type BRAF papillary thyroid cancer via regulation of β-catenin/E-cadherin signaling. J Clin Endocrinol Metab 99: E1641-E1649, 2014.
33. Zhao H, Li J, Li X, Han C, Zhang Y, Zheng L and Guo M: Silencing GPX3 expression promotes tumor metastasis in human thyroid cancer. Curr Protein Pept Sci 16: 316-321, 2015.
34. Deng X, Wu B, Xiao K, Kang J, Xie J, Zhang X and Fan Y: MiR-146b-5p promotes metastasis and induces epithelial-mesenchymal transition in thyroid cancer by targeting ZNRF3. Cell Physiol Biochem 35: 71-82, 2015.
35. Choi D, Ramu S, Park E, Jung E, Yang S, Jung W, Choi I, Lee S, Kim KE, Seong YJ, *et al*: Aberrant activation of Notch signaling inhibits PROX1 activity to enhance the malignant behavior of thyroid cancer cells. Cancer Res 76: 582-593, 2016.
36. Gilbert-Sirieix M, Makoukji J, Kimura S, Talbot M, Caillou B, Massaad C and Massaad-Massade L: Wnt/β-catenin signaling pathway is a direct enhancer of thyroid transcription factor-1 in human papillary thyroid carcinoma cells. PLoS One 6: e22280, 2011.
37. Oda K, Yatera K, Izumi H, Ishimoto H, Yamada S, Nakao H, Hanaka T, Ogoshi T, Noguchi S and Mukae H: Profibrotic role of WNT10A via TGF-β signaling in idiopathic pulmonary fibrosis. Respir Res 17: 39, 2016.
38. Kantaputra P, Kaewgahya M and Kantaputra W: WNT10A mutations also associated with agenesis of the maxillary permanent canines, a separate entity. Am J Med Genet A 164A: 360-363, 2014.
39. Mostowska A, Biedziak B, Zadurska M, Matuszewska-Trojan S and Jagodziński PP: WNT10A coding variants and maxillary lateral incisor agenesis with associated dental anomalies. Eur J Oral Sci 123: 1-8, 2015.
40. Zeng B, Xiao X, Li S, Lu H, Lu J, Zhu L, Yu D and Zhao W: Eight mutations of three genes (EDA, EDAR, and WNT10A) identified in seven hypohidrotic ectodermal dysplasia patients. Genes (Basel) 7: 7, 2016.
41. Cuellar-Partida G, Springelkamp H, Lucas SE, Yazar S, Hewitt AW, Iglesias AI, Montgomery GW, Martin NG, Pennell CE, van Leeuwen EM, *et al*: WNT10A exonic variant increases the risk of keratoconus by decreasing corneal thickness. Hum Mol Genet 24: 5060-5068, 2015.
42. Reddy S, Andl T, Bagasra A, Lu MM, Epstein DJ, Morrisey EE and Millar SE: Characterization of Wnt gene expression in developing and postnatal hair follicles and identification of Wnt5a as a target of Sonic hedgehog in hair follicle morphogenesis. Mech Dev 107: 69-82, 2001.
43. Ji H, Goode RJ, Vaillant F, Mathivanan S, Kapp EA, Mathias RA, Lindeman GJ, Visvader JE and Simpson RJ: Proteomic profiling of secretome and adherent plasma membranes from distinct mammary epithelial cell subpopulations. Proteomics 11: 4029-4039, 2011.
44. Kirikoshi H, Sekihara H and Katoh M: Up-regulation of WNT10A by tumor necrosis factor alpha and *Helicobacter pylori* in gastric cancer. Int J Oncol 19: 533-536, 2001.
45. Kirikoshi H, Sekihara H and Katoh M: WNT10A and WNT6, clustered in human chromosome 2q35 region with head-to-tail manner, are strongly coexpressed in SW480 cells. Biochem Biophys Res Commun 283: 798-805, 2001.
46. Clevers H and Nusse R: Wnt/β-catenin signaling and disease. Cell 149: 1192-1205, 2012.
47. Zhang J, Gill AJ, Issacs JD, Atmore B, Johns A, Delbridge LW, Lai R and McMullen TP: The Wnt/β-catenin pathway drives increased cyclin D1 levels in lymph node metastasis in papillary thyroid cancer. Hum Pathol 43: 1044-1050, 2012.
48. Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C and Niehrs C: Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. Nature 391: 357-362, 1998.