

# Tectonic-1 contributes to the growth and migration of prostate cancer cells *in vitro*

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Received September 22, 2014; Accepted June 5, 2015

DOI: 10.3892/ijmm.2015.2313

**Abstract.** Tectonic-1 (TCTN1) is an upstream gene involved in embryonic development. The aim of the present study was to investigate the effect of the TCTN1 gene on the viability and migration of prostate cancer cells. Lentivirus-mediated short hairpin RNA (shRNA) was constructed to silence the expression of TCTN1 in PC-3 and DU145 prostate cancer cells. Cell viability and proliferation were measured using MTT and colony formation assays, and the distribution of cells in phases of the cell cycle was determined using flow cytometry. Cell migration was detected using a Transwell assay. The results demonstrated that TCTN1 was widely expressed in several human prostate cancer cell lines. Knockdown of the TCTN1 gene by RNA interference markedly suppressed cell viability and colony formation in the PC-3 and DU145 cell lines. Cell cycle progression was also arrested by TCTN1 silencing. In addition, knockdown of the TCTN1 gene led to the inhibition of cell migration in the two cell lines. These findings confirmed the direct association between the TCTN1 gene and prostate cancer growth *in vitro*. With further understanding and clinical investigation, this indicates the potential for future development of a novel marker for early detection and gene therapy for prostate cancer.

## Introduction

Prostate cancer is a pathological change, associated with urinary dysfunction, in the prostate, which is one of the three most life threatening diseases in males worldwide (1). Although it has been observed that the incidence rate of pros-

tate cancer is decreasing, its incidence remains the highest in the world, particularly in the United States (2). The mortality rate of prostate cancer is lower than the sum of the mortality rates of lung and bronchus cancer (3). A number of males with prostate cancer never present with symptoms, therefore, early detection and prevention is challenging (4).

Conventional treatments for prostate cancer include surgery, chemotherapy, radiation therapy, radiofrequency ablation, high-intensity focused ultrasound, cryosurgery and hormonal therapy (5-7). The outcome of these therapeutic approaches is beneficial for benign prostate tumor, however, the survival rate of patients who present with cancer metastasis is low (8). It has been reported that the median overall survival rate of patients treated by chemotherapy with enzalutamide is 18.4 months (9). Therefore, a novel therapeutic strategy for prostate cancer is required. Gene therapy is one of the most promising therapeutic approaches, as it delivers therapeutic DNA into damaged cells, leading to fundamental healing in the patients (10). One of the most important issues is the identification of a specific gene responsible for the prostate cancer and universally impacting on different cell types in prostate cancer.

Garcia-Gonzalo *et al* reported that tectonic-1 (TCTN1) transports MKS1 and other MKS proteins to the transition zone, between the basal body and ciliary axoneme (12). MKS1, as a basal body protein, has a potential role in regulating Wnt signaling (11,12). The ciliary phenotype directly represents the de-regulation of Wnt signaling *in vitro* and *in vivo* (13,14). Wnt signaling regulates cell proliferation, contributing to high proliferation rates in the mutant kidney (15,16). Disturbance of Wnt signaling have been demonstrated to cause various diseases, including breast and prostate cancer (17,18).

It has also been reported that MKS1 acts upstream of Patched, and the loss of MKS1, which leads to Sonic hedgehog (Shh) signaling causes a reduction in high-level Shh signaling (19). Shh is the most extensively investigated ligand of the hedgehog signaling pathway among the mammalian signaling pathway families (20). It is critical in the differentiation and development of organs (21). MKS1 mutation in Hedgehog signaling leads to hypoplasia, in various types of tumor (22), and inhibition of the Shh signaling pathway has been identified as a possible treatment strategy for gastric cancer (23).

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**Key words:** tectonic-1, prostate cancer, short hairpin RNA, proliferation, migration

Therefore, changes in the gene expression levels of TCTN1 causes a chain reaction. Initially, it directly affects the expression level of MKS1, the further actions of which affect Wnt and Shh signaling. Variation in these two signaling pathways can lead to overproliferation of cells and the progression of cancer.

Despite substantial investigations in the gene therapy field (24-27), the mechanism of action of a specific gene target in prostate cancer remains unclear. Although the effect of the TCTN1 gene on prostate cancer has been revealed (28,29), a direct link between the TCTN1 gene and its effects on the viability of prostate cancer cells remains to be elucidated. To investigate the role of the TCTN1 gene in prostate cancer, the expression of TCTN1 gene was knocked down using RNA interference lentivirus system in four prostate cancer cell lines, PC-3, DU145, LNCaP and 22RV1. Biological function was further evaluated by analyzing the effects of TCTN1 on cell growth, cell cycle progression and cell migration. We aimed to reveal its contribution to the progression of prostate cancer

## Materials and methods

**Cell culture.** DU145, PC-3, LNCaP, 22RV1 and 293T cells were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). The DU145 cells were cultured in Ham's F-12 supplemented with 10% fetal bovine serum (FBS) and 1% NEAA. The PC-3 cells were cultured in Ham's F-12 supplemented with 10% FBS. LNCaP and 22RV1 cells were cultured in RPMI-1640 supplemented with 10% FBS. The 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. All cell lines were cultured at 37°C in humidified air with 5% CO<sub>2</sub>.

**Lentivirus vector design and production.** The two lentivirus vectors were designed to knock down the TCTN1 gene (NM\_001082537.2) and to avoid the non-specific knockdown effect, respectively. The short hairpin RNA (shRNA; Shanghai Hollybio, Shanghai, China), designed to silence TCTN1 had the following sequence: 5'-GCTCAGATGCATCAGTTCC TTCTCGAGAAGGAACTGATGCATCTGAGCTTTTTT-3'. The control shRNA had the following sequence: 5'-GCGGAGGGTTTGAAAGAATATCTCGAG ATATCTTTC AAACCCTCCGCTTTTTT-3'. The stem-loop-stem oligos (shRNAs) were synthesized, annealed and ligated into a *NheI*/*PacI*-linearized pFH-L vector (Shanghai Hollybio) containing the green fluorescent protein (GFP) gene as a reporter. Following DNA sequencing confirmation, using Lipofectamine<sup>®</sup> 2000 (Invitrogen Life Technologies, Carlsbad, CA, USA), the successfully constructed vectors, containing the pVSVG-I and pCMVΔR8.92 plasmids (Shanghai Hollybio), were transfected into the 80% confluent 293T cells for 48 h at 37°C, which were harvested 72 h following transfection and then purified by ultracentrifugation.

**Lentivirus transduction and gene knockdown.** The PC-3 and DU145 cells (5×10<sup>4</sup> cells/well) were seeded into 6-well plates and transduced with either the TCTN1 shRNA lentivirus (Lv-shTCTN1) or control shRNA lentivirus (Lv-shCon) at a multiplicity of infection of 40, respectively. Fluorescence microscopy was then used to observe the transduction efficiency 96 h post-infection.

**Quantitative polymerase chain reaction (qPCR).** qPCR was performed to determine the gene expression levels of TCTN1 in the PC-3, DU145, LNCaP and 22RV1 cell lines on a Bio-Rad Connet Real-Time PCR platform (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Total RNA was extracted using TRIzol<sup>®</sup> reagent (Invitrogen Life Technologies) and reverse-transcribed into cDNA using Moloney Murine Leukemia Virus Reverse Transcriptase (Promega Corp., Madison, WI, USA). The qPCR reaction system consisted of 2X SYBR Premix Ex Taq (10 μl), forward and reverse primers (2.5 μM; 0.8 μl), cDNA (5 μl) and ddH<sub>2</sub>O (4.2 μl). The β-actin gene was used as an internal control. For β-actin, the forward primer sequences were as follows: Forward 5'-GTGGACATCCGCAAAGAC-3' and reverse 5'-AAAGGGTGTAACGCAACTA-3'. For TCTN1, the primer sequences were as follows: Forward 5'-CCTTTGC GTGAATGTTGTTC-3' and reverse 5'-AGAGGGGACTG GCTGGGTATT-3'. The qPCR cycle was performed as follows: Initial denaturation at 95°C for 1 min, denaturation at 95°C for 5 sec and annealing extension at 60°C for 20 sec. A total of 40 cycles were performed. The cycle threshold (Ct) value, normalized with that of β-actin was used to determine the relative expression of TCTN1, using the 2<sup>-ΔΔCt</sup> formula (30).

**Western blotting.** The lentivirus-transduced cells (10,000 cells/well) were lysed in 2X SDS sample buffer, containing 100 mM Tris-HCl (pH 6.8), 10 mM EDTA, 4% SDS and 10% glycine. The proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In each lane of the gels, 30 μg protein was added and electrophoresis was performed under 50 V for 3 h. Subsequently a polyvinylidene fluoride (PVDF; Millipore, Bedford, MA, USA) transmembrane procedure was performed under 300 mA for 1.5 h. The membrane was then incubated with indicated primary antibodies [rabbit anti-TCTN1 (1:1,000; Cat. no. SAB3500518; Sigma-Aldrich, St. Louis, MO, USA) and rabbit anti-GAPDH (1:60,000; Cat. no. 10494-1-AP; Proteintech Group, Inc., Chicago, IL, USA)] at 4°C overnight, followed by incubation with horseradish peroxidase-conjugated secondary antibodies (1:5,000; Cat. no. sc-2054; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at room temperature for 2 h. Horseradish peroxidase glyceraldehyde 3-phosphate dehydrogenase protein was used as a loading control.

**MTT assay.** Following lentivirus transduction, the DU145 (2,500 cells/well) and PC-3 (2,000 cells/well) cells were seeded into 96-well plates, respectively. The numbers of cells were measured at time-points indicated in the figures. MTT solution (5 mg/ml; 20 μl) was added into each well. The MTT solution was aspirated off following incubation for 4 h at 37°C. Subsequently, 100 μl acidic isopropanol, containing 10% SDS, 5% isopropanol and 0.01 mol/L HCl, was added. The absorbance of each plate was measured at 595 nm using spectrophotometer (Epoch; BioTek, Winooski, VT, USA).

**Colony formation assay.** Following lentivirus transduction, the PC-3 (300 cells/well) and DU145 (500 cells/well) cells were seeded into 6-well plates and maintained at 37°C for 8 days, respectively. The culture media were replaced every 2-3 days. When the colonies were formed, the plate was washed and fixed with paraformaldehyde, stained with crystal purple for 20 min and washed three times using ddH<sub>2</sub>O, sequentially.

These cells were then photographed using a digital camera (D7000; Nikon Corp., Tokyo, Japan). The number of colonies containing >50 cells/colony was then counted.

**Flow cytometric analysis.** Following lentivirus transduction, the PC-3 and DU145 cells were seeded into 6-cm dishes at a density of  $1 \times 10^5$  and  $2 \times 10^5$  cells/dish, respectively. The cells were harvested following trypsinization, washed with phosphate-buffered saline (PBS) and fixed in 80% ethanol at  $-20^\circ\text{C}$  for 24 h. The cells were then collected and centrifuged at  $214.2 \times g$  for 10 min at  $4^\circ\text{C}$ , resuspended in the staining solution containing 100  $\mu\text{g/ml}$  RNase A and 50  $\mu\text{g/ml}$  propidium iodide in PBS, and incubated for 1 h at  $37^\circ\text{C}$ . The stained cells were subjected to flow cytometric analysis using a FACSCalibur II sorter and CellQuest FACS system (BD Biosciences, San Jose, CA, USA).

**Transwell migration assay.** The cell migration was determined by the number of cells that migrated through an 8- $\mu\text{m}$  pore Transwell polycarbonate membrane (Corning, Inc., Union City, CA, USA), separating the upper and lower chamber. An equal number of cells ( $5 \times 10^4$  PC-3 cells or  $1 \times 10^5$  DU145 cells) in the three groups were seeded into the upper chamber with 200  $\mu\text{l}$  of serum-free medium. Subsequently, 500  $\mu\text{l}$  medium, containing 10% FBS for the PC-3 cells or 20% FBS for the DU145 cells was added to the lower chamber. The fully prepared Transwell migration system was then placed in an incubator for 24 h at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$ . Finally, the cells on the surface of the basement membrane were removed, and the migrated cells were stained using crystal violet (0.05%) and counted under a microscope, in which five randomly-selected fields were observed for each sample.

**Statistical analysis.** Statistical analysis was performed using Prism 5 for Windows software (GraphPad Software, San Diego, CA, USA). Data are presented as the mean  $\pm$  standard deviation from at least three independent experiments, performed in triplicate. Statistical significance was determined using Student's t-test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Expression of TCTN1 is upregulated in prostate cancer cells.** The DU145, PC-3, LNCaP and 22RV1 human prostate cancer cell lines were cultured to measure the expression levels of TCTN1 using qPCR and western blot analysis. TCTN1 mRNA was detected in all the prostate cancer cell lines (Fig. 1A). The protein expression of TCTN1 was also confirmed in all the cell lines (Fig. 1B). These results indicated that TCTN1 was involved in prostate cancer. As the expression of TCTN1 was consistently high in these four cell lines, it may serve as a specific biomarker for the early detection of prostate cancer.

**Expression of TCTN1 is successfully downregulated by lentivirus-mediated RNAi in prostate cancer cells.** A specific Lv-shTCTN1 system was designed to silence the TCTN1 gene, and a control shRNA (Lv-shCon) was also constructed to eliminate the non-specific gene-silencing effect of the lentivirus alone. The lentiviral transduction efficiency was recorded using

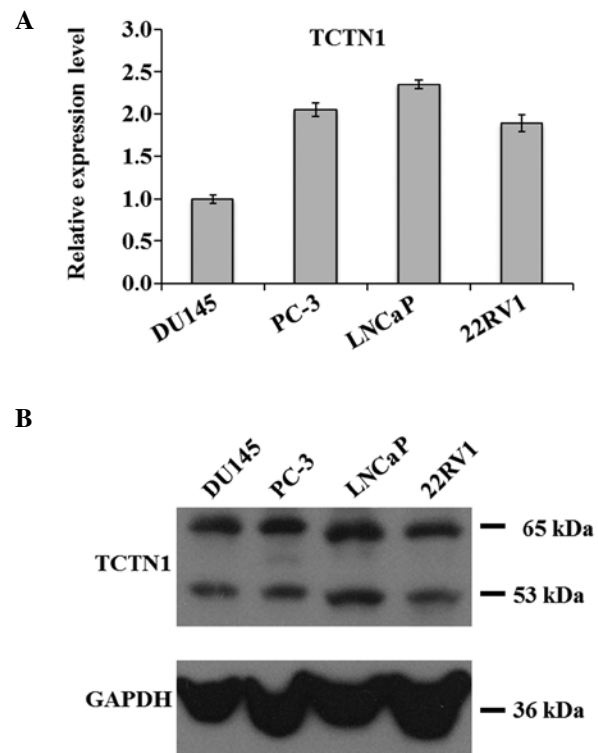


Figure 1. Expression analyses of levels of TCTN1 in four human prostate cancer cell lines using (A) quantitative polymerase chain reaction and (B) western blotting. Data are expressed as the mean  $\pm$  standard deviation. TCTN1, tectonic-1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

a fluorescence microscope (BX50; Olympus, Tokyo, Japan), in which >80% of the cells expressed fluorescence in the lentivirus transfection groups in the PC-3 and DU145 cell lines, whereas the non-infected control groups exhibited no fluorescence (Fig. 2A). This suggested the efficiency of the lentiviral transduction was stable and substantial. qPCR was then performed to evaluate the knockdown efficiency of TCTN1 following lentivirus transduction. The expression levels of TCTN1 in the control (Con) and Lv-shCon groups were similar, however, compared with the Lv-shCon group, the expression of TCTN1 was reduced by 90.3% in the PC-3 cells ( $P < 0.01$ ; Fig. 2B) and 94.0% in the DU145 cells ( $P < 0.001$ ; Fig. 2C). This indicated that the expression of TCTN1 was specifically knocked down by the lentivirus system in the two cell lines. Therefore, Lv-shTCTN1 was a stable lentivirus system which efficiently downregulated the expression of TCTN1 in the prostate cancer cells.

**Knockdown of the TCTN1 gene inhibits the viability and proliferation of prostate cancer cells.** In the PC-3 cells, the growth curves of the Con and Lv-shCon groups were overlapping, with almost no difference observed between them (Fig. 3A). By contrast, the growth curve of the Lv-shTCTN1 group was markedly lower, compared with that of the Lv-shCon group from day 2. On day 5, the optical density (OD) value at 595 nm of the PC-3 cells in the Lv-shTCTN1 group remained at  $0.1454 \pm 0.0042$ , which was markedly lower than that in the Con group ( $0.5334 \pm 0.0148$ ) and Lv-shCon group ( $0.5242 \pm 0.0199$ ). However, cell proliferation increased exponentially on day 5 in the Con and Lv-shCon groups. Similar growth curve trends

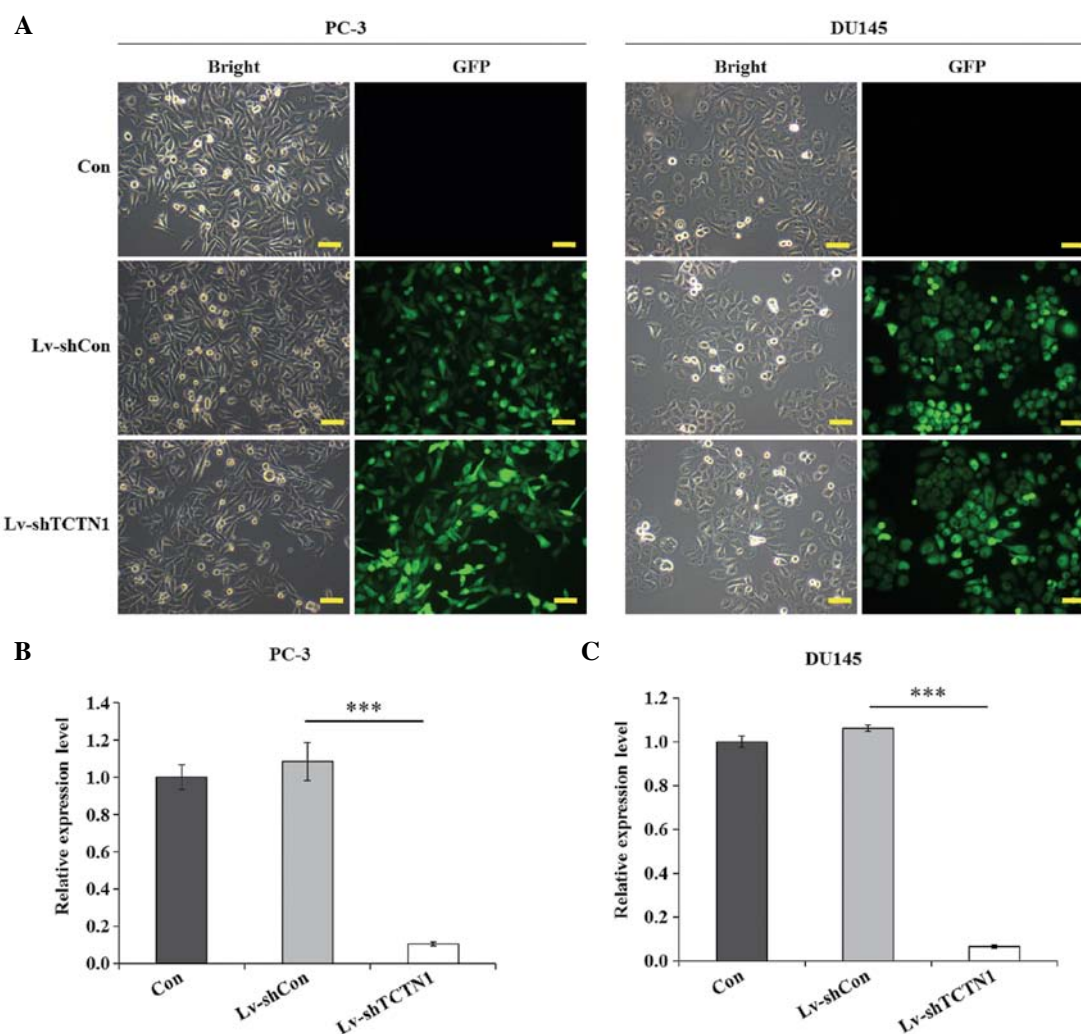


Figure 2. Lentivirus transduction efficiency recorded under a fluorescence microscope: (A, left) PC-3 and (A, right) DU145. Knockdown efficiency of the TCTN1 gene in the (B) PC-3 and (C) DU145 cells, determined using quantitative polymerase chain reaction analysis. Scale bar=100  $\mu$ m. Data are expressed as the mean  $\pm$  standard deviation. \*\*\*P<0.001, compared with Lv-shCon. TCTN1, tectonic-1; LV0shCon, control shRNA lentivirus; Con, control; GFP, green fluorescent protein.

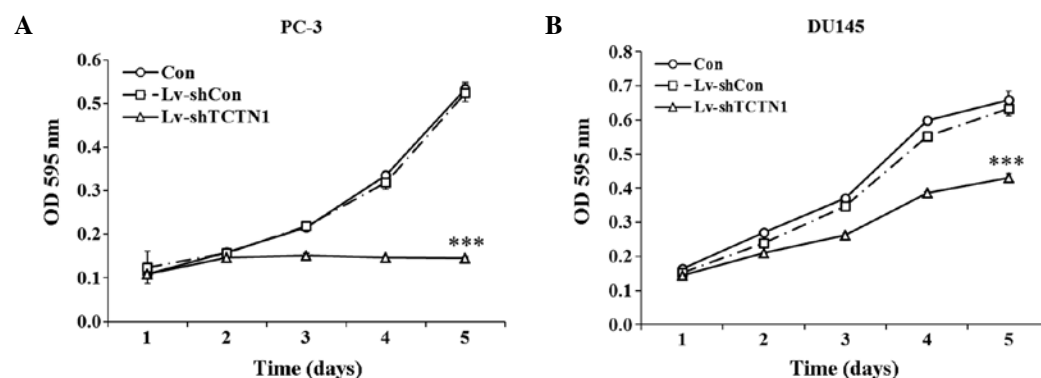


Figure 3. Knockdown of TCTN1 suppresses the viability of (A) PC-3 and (B) DU145 cells. \*\*\*P<0.001, compared with Lv-shCon. TCTN1, tectonic-1; LV0shCon, control shRNA lentivirus; Con, control; OD, optical density.

were observed in the DU145 cells, as shown in Fig. 3B. The number of viable cells in the Lv-shTCTN1 group was markedly lower than that in either the Con group or Lv-shCon group. On day 5, the OD value at 595 nm of the DU145 cells in the Lv-shTCTN1 group was  $0.4312 \pm 0.0115$ , which was lower than

that in the Con group ( $0.6594 \pm 0.0257$ ) and Lv-shCon group ( $0.6334 \pm 0.0211$ ).

The present study also performed a colony formation assay to determine the effect of TCTN1 on long-term cell proliferation in the PC-3 and DU145 cell lines. Compared



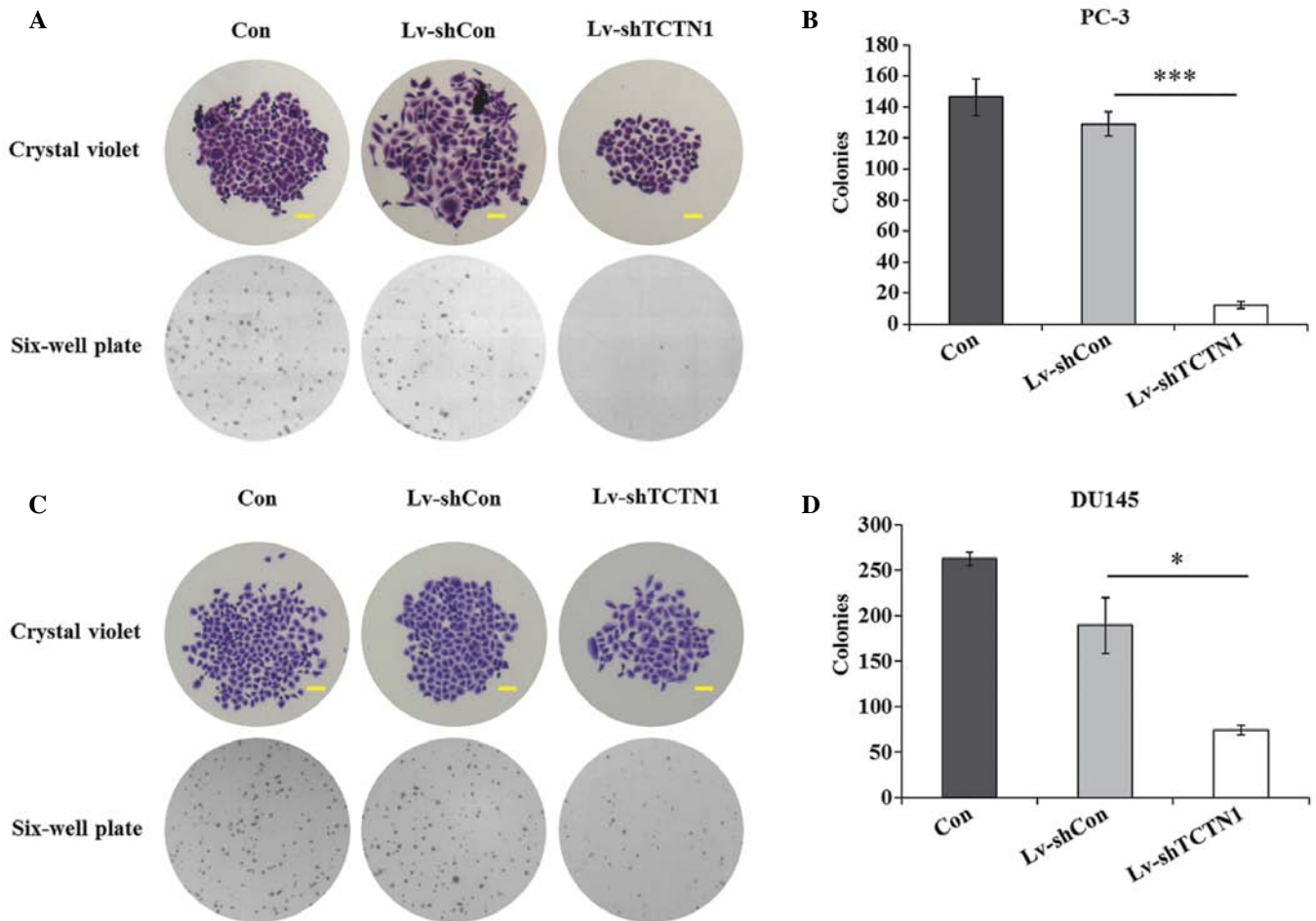


Figure 4. Knockdown of TCTN1 inhibits colony formation of the PC-3 and DU145 cells. Images recorded under micro and macro view, indicating the size and the number of colonies in each group of cells (A and C), and statistical analysis of the colony numbers (>50 cells/colony) in the (B) PC-3 and (D) DU145 cells. Scale bar=250  $\mu$ m. Data are expressed as the mean  $\pm$  standard deviation. \* $P$ <0.05 and \*\*\* $P$ <0.001, compared with Lv-shCon. Lv-shCon, TCTN1, tectonic-1; LV0shCon, control shRNA lentivirus; Con, control.

with the Con and Lv-shCon groups, the size of a single colony in the Lv-shTCTN1 group was substantially smaller in the images captured of the crystal violet staining and bright field (Fig. 4A and C). In addition, there were fewer colonies in the Lv-shTCTN1 group, compared with the Con and Lv-shCon groups. Compared with the Lv-shCon group, the number of colonies in the Lv-shTCTN1 group were reduced by 90.7% (Fig. 4B). Similar results were observed in the DU145 cells (Fig. 4D). Taken together, it is reasonable to conclude that lentivirus-mediated TCTN1 silencing had a suppressive effect on cell viability and proliferation of the prostate cancer cells. Therefore it was suggested that TCTN1 may act as a potential therapeutic target in prostate cancer.

**Knockdown of the TCTN1 gene inhibits the cell cycle progression of prostate cancer cells.** Flow cytometry, in conjunction with modeling algorithms, distinguished cells in the different stages of the cell cycle. TCTN1 silencing had a marked effect on cell cycle progression (Fig. 5A and C). In the PC-3 cells, the percentage of cells in the G0/G1 phase decreased from  $50.60 \pm 0.52\%$  in the Lv-shCon group to  $44.68 \pm 1.77\%$  in the Lv-shTCTN1 group. The percentage of cells in the G2/M phase increased from  $19.69 \pm 0.17\%$  in the Lv-shCon

group to  $27.34 \pm 0.77\%$  in the Lv-shTCTN1 group. However, no significant difference was observed between the Con and Lv-shCon groups (Fig. 5B). In the DU145 cells, the number of cells in the G2/M phase in the Lv-shTCTN1 group was also lower, compared with those in the Con and Lv-shCon groups. The percentages of cells in the S phase and G2/M phase were markedly elevated in the DU145 cells following TCTN1 silencing (Fig. 5D). There was a marked change in the number of cells in the S phase, which may have been due to the specific cell type. In conclusion, silencing of the TCTN1 gene arrested cells at the G2/M phase, which impaired cell proliferation.

**Knockdown of the TCTN1 gene inhibits the migration of prostate cancer cells.** Cell migration is a critical step during cancer progression. The present study subsequently aimed to determine the effect of TCTN1 knockdown in regulating prostate cancer cell migration using a Transwell assay (Fig. 6A). In the PC-3 cells, fewer cells in the Lv-shTCTN1 group ( $44.7 \pm 1.6$ ) migrated to the lower surface of the membrane, compared with the cells in the Con group ( $447.9 \pm 4.3$ ) or Lv-shCon group ( $440.1 \pm 2.3$ ; Fig. 6B). In addition, the crystal violet staining intensity was significantly lower in the Lv-shTCTN1 group than in the Con and Lv-shCon groups (Fig. 6C). Similar results

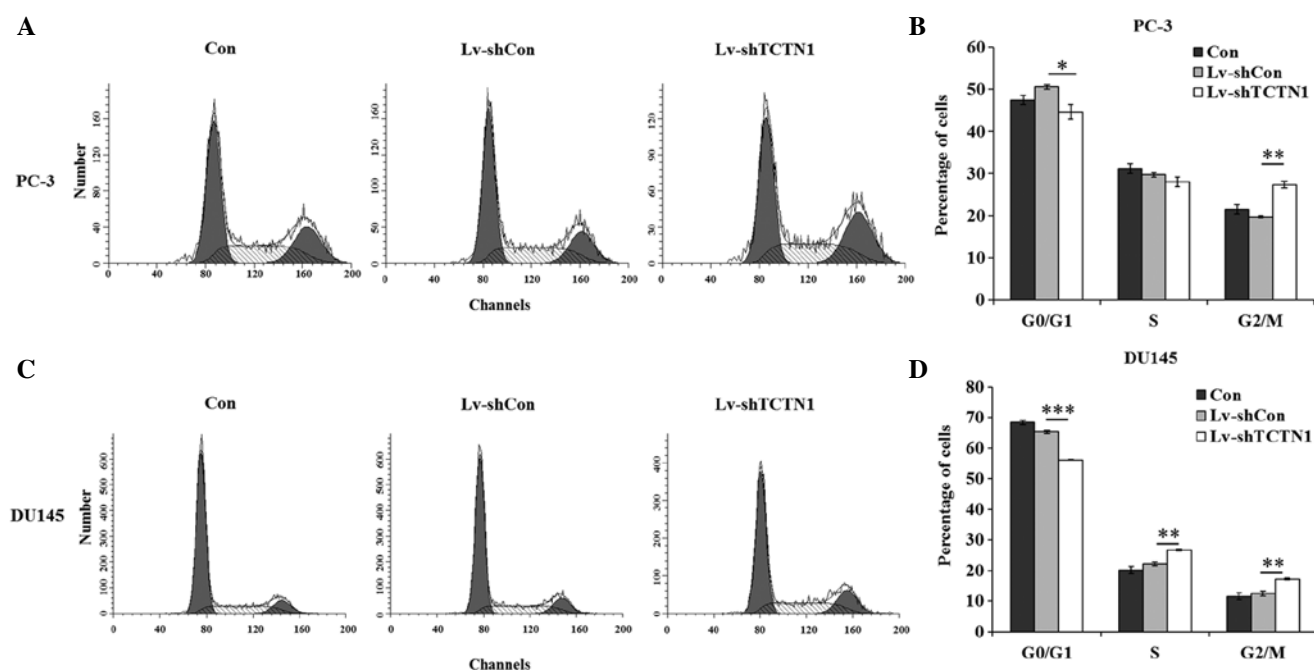


Figure 5. Knockdown of TCTN1 arrests cell cycle progression of the PC-3 and DU145 cells. Cell cycle distribution, determined using a flow cytometer, and statistical analysis of cell cycle distribution in the (A and B) PC-3 and (C and D) DU145 cells. Data are expressed as the mean  $\pm$  standard deviation. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ , compared with Lv-shCon. TCTN1, tectonic-1; LV0shCon, control shRNA lentivirus; Con, control.

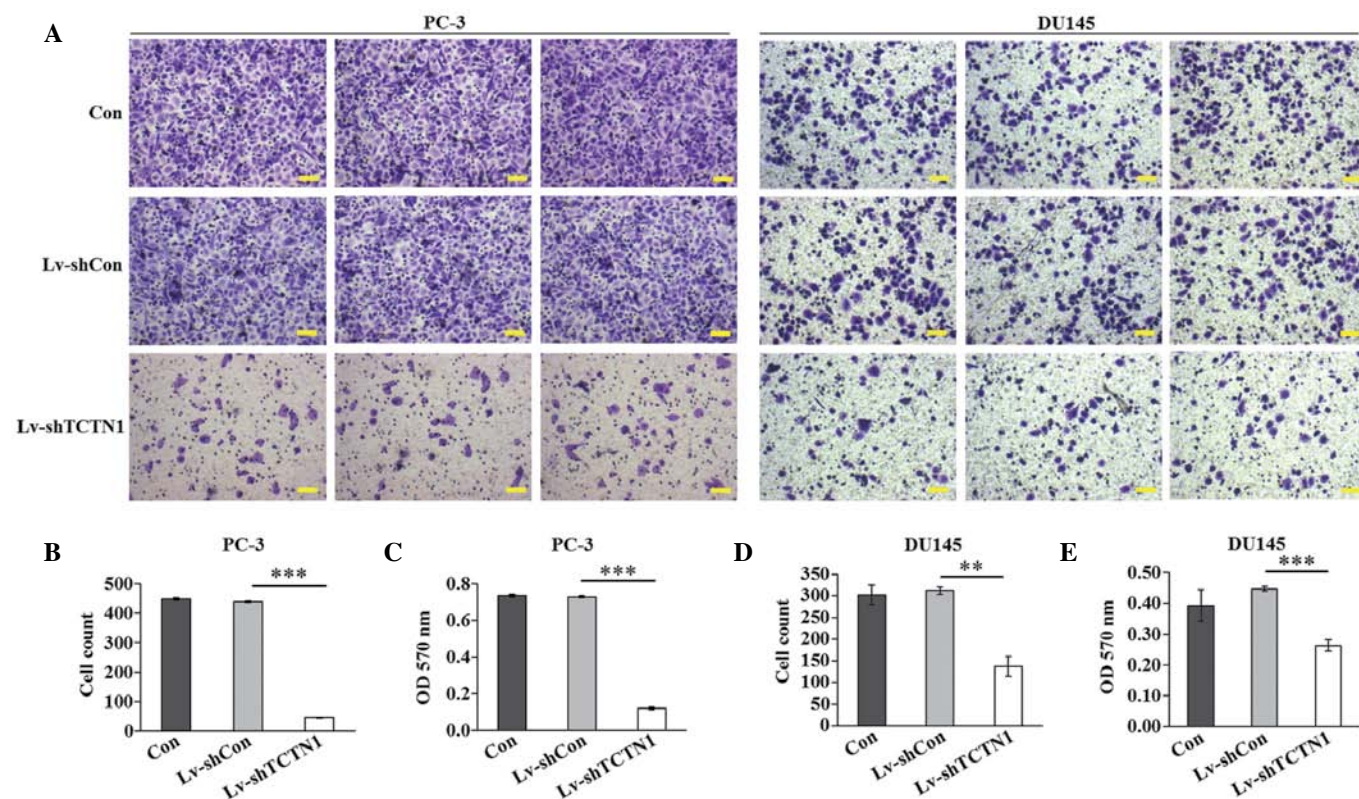


Figure 6. Knockdown of TCTN1 inhibits cell migration of PC-3 and DU145. (A) Images of PC-3 and DU145 cells, migrated through a Transwell membrane. Cell counts and quantitative analysis of the migrated (B and C) PC-3 and (D and E) DU145 cells. Quantitative analysis was performed by destaining and reading the OD at 570 nm. Scale bar=100  $\mu$ m. Data are expressed as the mean  $\pm$  standard deviation. \*\* $P < 0.01$  and \*\*\* $P < 0.001$ , compared with Lv-shCon. TCTN1, tectonic-1; LV0shCon, control shRNA lentivirus; Con, control; OD, optical density.

were observed in the DU145 cells (Fig. 6D and E), with knockdown of the TCTN1 gene also disrupting the migration of the

DU145 cells. These results suggested that TCTN1 may be key in prostate cancer metastasis.

## Discussion

Prostate cancer has the highest incidence rate among all types of cancers in males, and the mortality rate of prostate cancer remains high, as some prostate cancer cells migrate into lymph glands and through the lymphatic and circulatory systems (31,32). Traditional treatment approaches for prostate cancer are unable to cure it, with recurrence often occurring shortly following treatment (33,34). It is well accepted that cancer formation is a multi-step process, involving continuous gene mutation (35,36). Gene therapy is designed to deliver an effective gene into a target site to regulate the expression of a specific gene (37). Thus, it is important to identify the gene responsible for the viability and migration of prostate cancer cells to suppress cell growth *in situ*.

It has been reported that the TCTN1 gene regulates the expression of MKS1, which is associated with the expression of the Wnt signaling and Shh signaling pathways. These two signaling pathways share are involved in cell over-proliferation, which is one of six hallmarks of cancer cells (38). These findings prompted the present study to investigate the TCTN1 gene as a target site in prostate cancer therapy.

In the present study, the association between TCTN1 and characteristics of prostate cancer cells were initially examined. The expression of TCTN1 was detected in four prostate cancer cell lines, PC-3, DU145, LNCaP and 22RV1, which indicated that high expression levels of TCTN1 may be associated with prostate cancer. Subsequently, lentivirus-based shRNA expression systems were introduced to specifically knock down the expression of TCTN1 in the PC-3 and DU145 cells. In the absence of TCTN1, the viability and colony formation ability were impaired in the PC-3 and DU145 cell lines, which suggested that TCTN1 may be essential for the growth of prostate cancer cells *in vitro*. To determine the cause of this cell growth suppression, flow cytometry was performed to examine cell cycle progression. The results indicated that the number of cells in the G2/M phase were markedly increased in the Lv-shTCTN1 group, compared with the Con and Lv-shCon groups. It has been reported that TCTN1 is a complex that is localized at the transition zone of primary cilia (12), and that cilia are necessary for tissue development and homeostasis, which emerge during interphase prior to mitosis (39). Thus, the knockdown of TCTN1 gene may arrest cells at the M phase, which is consistent with the results of the present study. The arrest of the cell cycle was essential in reducing the rate of cell growth. TCTN1 regulates the primary cilia, which are critical in modeling cytoskeletal changes that impinge on cell migration (40,41). TCTN1 silencing also markedly inhibited the ability of cells to migrate in prostate cancer.

In conclusion, the present study demonstrated that TCTN1 was associated with the growth and migration of prostate cancer cells *in vitro*. Silencing of the TCTN1 gene markedly inhibited cell viability, proliferation and migration. Therefore, TCTN1 may offer potential as biomarker for the treatment of prostate cancer.

## Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (grant. no. 81170637), the

Shanghai Committee of Science and Technology General Program for Medicine (grant. no. 11JC1402302), the Key Project of Science and Innovation Foundation of Shanghai Ministry of Education (grant. no. 14zz084), and the Military Fund for Health Care (grant. no. 13BJZ29).

## References

1. Calle EE, Rodriguez C, Walker-Thurmond K and Thun MJ: Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. *New Engl J Med* 348: 1625-1638, 2003.
2. Siegel R, Naishadham D and Jemal A: Cancer Statistics, 2012. *Ca Cancer J Clin* 62: 10-29, 2012.
3. Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. *CA Cancer J Clin* 55: 74-108, 2005.
4. Smolensky M: Book review: Light pollution as a new risk factor for human breast and prostate cancers (Springer, pp168, 2013). *Chronobiol Int* 30: 1203-1204, 2013.
5. Peyromaure M, Valéri A, Rebillard X, *et al*: Characteristics of prostate cancer in men less than 50-year-old. *Prog Urol* 19: 803-809, 2009 (In French).
6. Crouzet S, Rouviere O, Martin X and Gelet A: High-intensity focused ultrasound as focal therapy of prostate cancer. *Curr Opin Urol* 24: 225-230, 2014.
7. Zhao X and Chua KJ: Regulating the cryo-freezing region of biological tissue with a controlled thermal device. *Med Eng Phys* 36: 325-334, 2014.
8. Baade PD, Youlten DR and Krnjacki LJ: International epidemiology of prostate cancer: geographical distribution and secular trends. *Mol Nutr Food Res* 53: 171-184, 2009.
9. Scher HI, Fizazi K, Saad F, *et al*: Increased survival with enzalutamide in prostate cancer after chemotherapy. *New Engl J Med* 367: 1187-1197, 2012.
10. Fitzgerald KA, Evans JC, McCarthy J, *et al*: The role of transcription factors in prostate cancer and potential for future RNA interference therapy. *Expert Opin Ther Targets* 18: 633-649, 2014.
11. Whewy G, Abdelhamed Z, Natarajan S, Toomes C, Inglehearn C and Johnson CA: Aberrant Wnt signalling and cellular over-proliferation in a novel mouse model of Meckel-Gruber syndrome. *Dev Biol* 377: 55-66, 2013.
12. Garcia-Gonzalo FR, Corbit KC, Sirerol-Piquer M, *et al*: A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. *Nat Genet* 43: 776-784, 2011.
13. Corbit KC, Shyer AE, Dowdle WE, Gaulden J, Singla V, Chen MH, Chuang PT and Reiter JF: Kif3a constrains beta-catenin-dependent Wnt signalling through dual ciliary and non-ciliary mechanisms. *Nat Cell Biol* 10: 70-76, 2008.
14. Gerdes JM, Liu Y, Zaghoul NA, *et al*: Disruption of the basal body comprises proteasomal function and perturbs intracellular Wnt response. *Nat Genet* 39: 1350-1360, 2007.
15. Bohnenpoll T, Trowe MO, Wojahn I, Taketo MM, Petry M and Kispert A: Canonical Wnt signaling regulates the proliferative expansion and differentiation of fibrocytes in the murine inner ear. *Dev Biol* 391: 54-65, 2014.
16. Liu YZ, Wu K, Huang J, *et al*: The PTEN/PI3K/Akt and Wnt/ $\beta$ -catenin signaling pathways are involved in the inhibitory effect of resveratrol on human colon cancer cell proliferation. *Int J Oncol* 45: 104-112, 2014.
17. Logan CY and Nusse R: The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 20: 781-810, 2004.
18. Komiyama Y and Habas R: Wnt signal transduction pathways. *Organogenesis* 4: 68-75, 2008.
19. Vierkotten J, Dildrop R, Peters T, Wang B and Ruether U: Ftm is a novel basal body protein of cilia involved in Shh signalling. *Development* 134: 2569-2577, 2007.
20. Ye W, Shimamura K, Rubenstein JL, Hynes MA and Rosenthal A: FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell* 93: 755-766, 1998.
21. Dahmane N and Ruiz i Altaba A: Sonic hedgehog regulates the growth and patterning of the cerebellum. *Development* 126: 3089-3100, 1999.
22. Hinterseher U, Wunderlich A, Roth S, *et al*: Expression of hedgehog signalling pathway in anaplastic thyroid cancer. *Endocrine* 45: 439-447, 2014.
23. Wan J, Zhou J, Zhao H, *et al*: Sonic hedgehog pathway contributes to gastric cancer cell growth and proliferation. *Biores Open Access* 3: 53-59, 2014.



24. Nishikawa R, Goto Y, Kojima S, *et al*: Tumor-suppressive microRNA-29s inhibit cancer cell migration and invasion via targeting LAMC1 in prostate cancer. *Int J Oncol* 45: 401-410, 2014.
25. Tetsu O and McCormick F: Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 398: 422-426, 1999.
26. Scholzen T and Gerdes J: The Ki-67 protein: From the known and the unknown. *J Cell Physiol* 182: 311-322, 2000.
27. Ambrosini G, Adida C and Altieri DC: A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med* 3: 917-921, 1997.
28. Shaw G, Price AM, Ktori E, Bisson I, Purkis PE, McFaul S, Oliver RT and Prowse DM: Hedgehog signalling in androgen independent prostate cancer. *Eur Urol* 54: 1333-1343, 2008.
29. Jamaluddin M, Liang Z, L J, Yao Q and Chen C: Tectonic1, a new hedgehog signaling molecule, upregulates endothelial nitric oxide synthase in human endothelial cells. *J Surg Res* 179: 331, 2013.
30. Gu J, Li K, Li M, Wu X, Zhang L, Ding Q, Wu W, Yang J, Mu J, Wen H, *et al*: A role for p21-activated kinase7 in the development of gastric cancer. *FEBS J* 280: 46-55, 2013.
31. Revelo MP, Cookson MS, Chang SS, Shook MF, Smith JA Jr and Shappell SB: Incidence and location of prostate and urothelial carcinoma in prostates from cystoprostatectomies: implications for possible apical sparing surgery. *J Urol* 171 (2 Pt 1): 646-651, 2004.
32. Revelo MP, Cookson MS, Chang SS, Shook MF, Smith JA Jr and Shappell SB: Incidence and location of prostate and urothelial carcinoma in prostates from cystoprostatectomies: Implications for possible apical sparing surgery. *J Urol* 179 (Suppl 5): S27-S32, 2008.
33. Bolla M, Gonzalez D, Warde P, *et al*: Improved survival in patients with locally advanced prostate cancer treated with radiotherapy and goserelin. *N Engl J Med* 337: 295-300, 1997.
34. D'Amico AV, Whittington R, Malkowicz SB, *et al*: Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA* 280: 969-974, 1998.
35. Hollstein M, Sidransky D, Vogelstein B and Harris CC: p53 mutations in human cancers. *Science* 253: 49-53, 1991.
36. Vogelstein B, Fearon ER, Hamilton SR, *et al*: Genetic alterations during colorectal-tumor development. *N Engl J Med* 319: 525-532, 1988.
37. Gleich LL: Gene therapy for head and neck cancer. *Laryngoscope* 110: 708-726, 2000.
38. Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. *Cell* 144: 646-674, 2011.
39. Parker JD, Hilton LK, Diener DR, *et al*: Centrioles are freed from cilia by severing prior to mitosis. *Cytoskeleton (Hoboken)* 67: 425-430, 2010.
40. Christensen ST, Pedersen SF, Satir P, Veland IR and Schneider L: Chapter 10 The primary cilium coordinates signaling pathways in cell cycle control and migration during development and tissue repair. In: *Current Topics in Developmental Biology*. Bradley KY (ed). Vol 85, Academic Press, pp261-301, 2008.
41. Schneider L, Cammer M, Lehman J, *et al*: Directional cell migration and chemotaxis in wound healing response to PDGF-AA are coordinated by the primary cilium in fibroblasts. *Cell Physiol Biochem* 25: 279-292, 2010.