Int J Clin Exp Pathol 2017;10(1):377-384
www.ijcep.com /ISSN:1936-2625/IJCEP0026280

Original Article

Gelsolin-like actin-capping protein is highly expressed in prostate cancer and affects cell apoptosis, proliferation and migration

Haiqi Mu[1,2], Bo Qiu[3], Qipeng Xie[4], Yijun Wang[2], Cunjin Nan[2], Wei Han[5], Tong Zhao[3], Shan-Chao Zhao[1]

[1]Department of Urology, Nanfang Hospital, Southern Medical University, Guangzhou, China; [2]Department of Urology, The Second Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China; [3]Department of Pathology, Nanfang Hospital, Southern Medical University, Guangzhou, China; [4]Department of Laboratory Medicine, The Second Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China; [5]Cancer Research Institute, Southern Medical University, Guangzhou, China

Received February 19, 2016; Accepted July 12, 2016; Epub January 1, 2017; Published January 15, 2017

Abstract: Objective: To investigate the expression of Gelsolin-like actin-capping protein (CapG) and its biological functions in prostate cancer. Methods: CapG expression was examined in 90 cases of prostate cancer (PCa) and 30 cases of adjacent normal prostate (NP) by immunohistochemistry (IHC). Then, the correlation between CapG expression and clinicalpathological characteristics was assessed in PCa. In addition, CapG expression was examined in PC3, DU145 and Lncap cells by qRT-PCR and Western blot. When CapG expression was knocked down by specific siRNA, cell apoptosis, proliferation and migration were evaluated, respectively. Results: The positive rate of CapG expression in prostate cancer and adjacent normal prostate tissues were 54.4% and 16.7%, and the difference was statistically significant (P<0.001). Meanwhile, CapG expression was significantly correlated with PSA level, Gleason score, T stage and lymph node metastasis in prostate cancer (P<0.001). Furthermore, higher expression of CapG was observed in PC3 cells compared with that in DU145 and Lncap cells by qRT-PCR and Western blot. CapG down-regulation significantly inhibited the proliferation and migration, but promoted the apoptosis in PC3 and Lncap cells. Conclusions: CapG is highly expressed in prostate cancer and affects cell apoptosis, proliferation and migration.

Keywords: Prostate cancer, CapG, apoptosis, proliferation, migration

Introduction

Prostate cancer (PCa) is one of most common malignancy, which has increasing incidence and mortality rates in men all over the word [1, 2]. Meanwhile, PCa is a major cause of cancer related deaths in China [3, 4]. Although the outcome of PCa patients with localized or regional disease has been markedly improved, the 5-year survival rate remains unsatisfied [5, 6]. Furthermore, the mechanism of carcinogenesis and progression of PCa have not yet been fully understood. Thus, molecular markers associated with PCa should be identified, which may be helpful for the diagnosis and therapy of PCa.

Gelsolin-like actin-capping protein (CapG), a member of the gelsolin protein family, is an actin binding protein, which is crucial for modulating the motility of cells [7, 8]. Studies demonstrated that CapG was involved in various types of carcinomas [9-11]. Higher expression of CapG was observed in breast cancer [12], non-small cell lung cancer [11] and ovarian carcinoma [13]. However, loss of CapG protein expression was found in stomach cancer cell lines, lung cancer cell lines and melanoma cell lines [14]. In addition, CapG plays key roles in regulating cell division [15], motility [16], migration and invasiveness [13]. However, it is not fully identified about the relationship between CapG expression and prostate cancer.

In this study, we aim to investigate the expression of CapG and its biological functions in prostate cancer.

Materials and methods

Patient and tissue samples

A total of 90 cases of PCa tissues and 30 cases of adjacent normal prostate tissues were collected from Nanfang hospital and the Second Affiliated Hospital of Wenzhou Medical University between January 2013 and December 2015. All samples were obtained by surgery resection and none of patients received chemotherapy, radiotherapy or immunotherapy before surgery. The clinicopathological characters of patients, including age, smoke history, PSA level, Gleason score, T stage and lymph node metastasis were obtained by hospital records. This study was supported by the Medical Ethics Committee of Nanfang hospital of southern medical university and the Second Affiliated Hospital of Wenzhou Medical University, also supported with patients' approval.

Immunohistochemistry (IHC)

All samples were formalin-fixed, paraffin-embedded and cut with two-micrometer sections. Sections were dipped into xylene and rehydrated with graded alcohol under standard procedures. Subsequently, antigen retrieval was heated with 0.01 mol/L citrate buffer (pH 6.0) for five minutes. After incubation with 3% hydrogen peroxide and 5% goat serum individually, sections were incubated with polyclonal antibody CapG antibody (dilution, 1:150) (Proteintech Co, USA) for 2 h at room temperature. After washing with PBS (Phosphate Buffered Saline), sections were incubated with biotinylated secondary antibody for 30 minutes. Then, sections were developed by 3,3'-diaminobenzidine (DAB) and stained with hematoxylin. Goat serum substituted primary antibody as negative control.

According to the staining intensity and the percentage of positive-staining cells, CapG expression was classified into negative and positive expression. Staining intensity was recorded as absent, weak, moderate and strong. The percentage of positive-staining cells divided into less than and more than 25%. Only these sections with moderate or strong staining and more than 25% of positive-staining cells were regarded as CapG positive expression. Others were definited as CapG negative expression.

Cell culture and transfection

Human prostate cancer cell lines PC3, DU145 and Lncap were purchased from American Type Culture Collection and cultured with RPMI-1640 (Gibco, USA) supplemented with 12% fetal bovine serum (HyClone, USA) at 37°C in a humid atmosphere with 5% CO_2. Using Lipofectamine2000 Transfection Reagent (Invitrogen, USA), cells were transfected with CaPG-specific siRNA and those in normal controls (NC) were transfected with scramble sequence.

RNA preparation and quantitative reverse transcription-polymerase chain

After transfection for 48 h, cells were collected to extract total RNA using the Isogen (Nippon Gene, Tokyo, Japan). Then the concentration of total RNA was quantitatively measured and reversely transcribed into cDNA using the reverse transcription kit (Takara Biotechnology, Japanese). The gene expression was quantified by using SYBR Green PCR kit (Toyobo, Osaka, Japan). The PCR reaction was conducted as follow: 95°C for 30 s, 95°C for 5 s and 60°C for 30 s. Each sample was repeatedly measured three times and the mRNA levels of genes were calculated using the $2^{-\Delta\Delta Ct}$. The primer sequences of GAPDH were 5'-GCATCCTGCACCACCAACTG-3' and 5'-GCCTGCTTCACCACCTTCTT-3'. The primer sequence of CapG were 5'-CGAACACTCAGGTGGAGATT3' (F) and 5'-CGAACACTCAGGTGGAGATT-3' (R).

Western blot

After transfection for 48 h, cells were collected, homogenized with RIPA lysis buffer (Thermo Scientific, USA) and measured by BCA Protein Assay Kit (Beyotime). 50 μg of protein was separated by 10% SDS-polyacrylamide gels (SDS-PAGE) and transferred onto PVDF membranes. After blocking with 5% nonfat milk, membranes were incubated with polyclonal antibody CapG antibody (dilution, 1:500) (Proteintech Co, USA) and monoclonal antibody GAPDH antibody (dilution, 1:2000) (Abcam, USA) overnight at 4°C. Then, membranes were washed with PBS and Tween-20 solution, and incubated with HRP-conjugated secondary antibodies (zhongshanJingqiao, China) for 1 hour at room temperature. Protein bands were visualized by ECL chromogenic substrate (Beyotime, China).

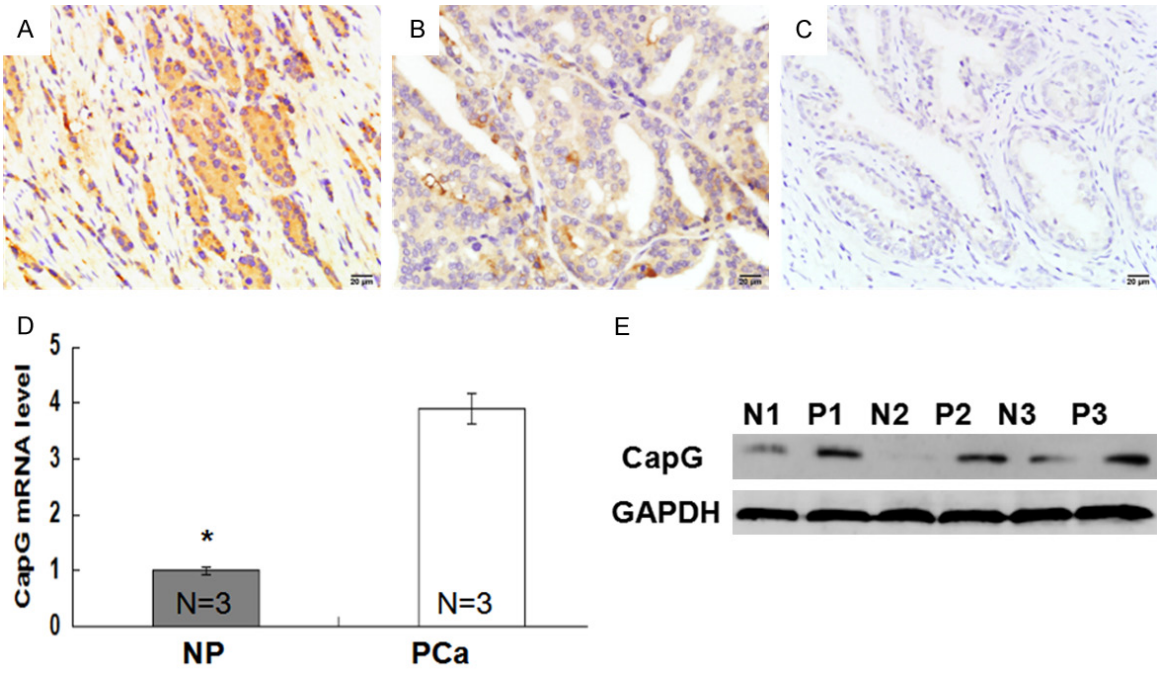

Figure 1. CapG expression was evaluated in prostate cancer. A: CapG expression was detected in high grade of prostate cancer by IHC. B: CapG expression was detected in low grade of prostate cancer by IHC. C: CapG expression was detected in normal prostate by IHC. D: CapG mRNA level was examined in normal prostate (NP) and prostate cancer (PCa) tissues by qRT-PCR. E: CapG protein expression was detected in normal prostate (N1, N2 and N3) and prostate cancer (P1, P2 and P3) tissues by Western blot. *: $P<0.001$.

Flow cytometry analysis of apoptosis

After transfection for 48 h, cells were harvested for the apoptosis analysis by flow cytometry. Simply, 1×10^5 cells was collected and washed with PBS. Then, cells were resuspended in 400 μl binding buffer and stained with 10 μl propidium iodide and 5 μl Annexin V-FITC (BestBio, Shanghai, China) for 15 min at room temperature. Stained cells were measured by flow cytometry. Each test was repeated for three times.

CCK-8 cell proliferation assay

Cell proliferation was determined by CCK-8 assay as previously described [17]. In briefly, 2×10^3 cells were cultured in a 96-multiwell plates, and transfected with CaPG-specific siRNA and scramble sequence, respectively. After 24 h transfection, cells in each well were incubated with 10 μl CCK-8 (5 mg/ml) solution for 4 h at 37°C. Then, the optical density (OD) in each well was determined by using a Thermomax microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm. Each test was repeated for three times.

Cell migration

3×10^4 cells were cultured with RPMI-1640 (Gibco, USA) supplemented with 5% fetal bovine serum (HyClone, USA) in 24-well inserts and transfected with control RNA or CapG siRNA. The inserts filled in RPMI-1640 medium with 25% fetal bovine serum was considered as an attractant. After 48 h, cells were fixed in 95% ethanol solution and stained with Giemsa's staining solution. Then, migrated cells were analyzed under optical microscopy. Each experiment was repeated for three times.

Statistical analysis

All data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Pearson's chi-square test (χ^2) was used to evaluate the CapG expression in prostate cancer and normal prostate and to assess the correlation between CapG expression and clinicalpathological characters. Independent-sample t-test was used to compare the difference in two groups. The statistically significant difference was $P<0.05$.

Table 1. Expression of CapG in prostate cancer and normal prostate tissues

Tissue types	N	CapG (%)		*P* value
		Negative	Positive	
Prostate cancer	90	41 (45.6)	49 (54.4)	<0.001
Normal prostate	30	25 (83.3)	5 (16.7)	

Table 2. Correlation between CapG expression and clinical pathological characters in prostate cancer

Clinicalpathological characteristics	N	CapG		*P* value
		Negative	Positive	
Age (years)				
≤58	59	29	30	0.380
>58	31	12	19	
Smoke history				1.000
Negative	33	15	18	
Positive	57	26	31	
PSA (ng/ml)				<0.001
>10	42	29	13	
≤10	48	12	36	
Gleason score				
≤7	45	31	14	<0.001
>7	45	10	35	
T stage				
T1-T2	37	30	7	<0.001
T3-T4	53	11	42	
Lymph node metastasis				
Negative	62	37	25	<0.001
Positive	28	4	24	

Results

CapG is highly expressed in prostate cancer and correlated with clinicalpathological characters

IHC results shown that positive CapG expression in prostate cancer tissues was located in the cytoplasm of cells and stained as yellow brown (**Figure 1A** and **1B**). While positive CapG expression was hard to be detected in adjacent normal prostate tissues (**Figure 1C**). The positive rates of CapG expression in prostate cancer and adjacent normal prostate were 54.4% and 16.7%, and the difference was statistically significant (**Table 1**, *P*<0.001). These results indicated that CapG was highly expressed in prostate cancer compared with those in adjacent normal prostate. To further validate the observation, we detected the expression of CapG in 3 cases of fresh prostate cancer tissues and matched normal prostate tissues by qRT-PCR and Western blot. As expected, qRT-PCR and Western blot results also indicated that higher expression of CapG was found in prostate cancer in comparison with those in normal prostate (**Figure 1D** and **1E**), which was consistent with IHC results.

Then, we evaluated the correlation between CapG expression and clinicalpathological characteristics in prostate cancer. As shown in **Table 2**, significant correlation between CapG expression with PSA level, Gleason score, T stage and lymph node metastasis was recorded in prostate cancer (*P*<0.05). However, CapG expression wasn't significantly associated with age and smoke history (*P*>0.05).

CapG down-regulation affects cell apoptosis, proliferation and migration

To investigate the biological functions of CapG, we knocked down the expression of CapG in human prostate cancer cell lines by siRNA. Firstly, we detected the expression of CapG in three human prostate cancer cell lines by qRT-PCR and Western blot and found that CapG highly expressed in PC3 cells compared with that in DU145 and Lncap cells (**Figure 2A**). Then, CapG expression in PC3 and Lncap cells was knocked down by siRNA, respectively. Both qRT-PCR and Western blot results indicated CapG expression was successfully down-regulated in PC3 and Lncap cells (**Figure 2B**). After CapG down-regulation, the apoptotic rate was significantly increased in PC3 and Lncap cells (**Figure 3**, *P*<0.05). Meanwhile, CapG depletion significantly inhibited cell proliferation compared with those in normal controls (**Figure 4A**, *P*<0.001). Furthermore, the migrated number of cells was markedly reduced when CapG expression was down-regulated in PC3 and Lncap cells (**Figure 4B**, *P*<0.001).

Discussion

In this study, we are the first to evaluate the expression of CapG in PCa and normal prostate tissues. IHC results indicated that CapG was highly expressed in PCa compared with normal prostate, indicating that high expression of

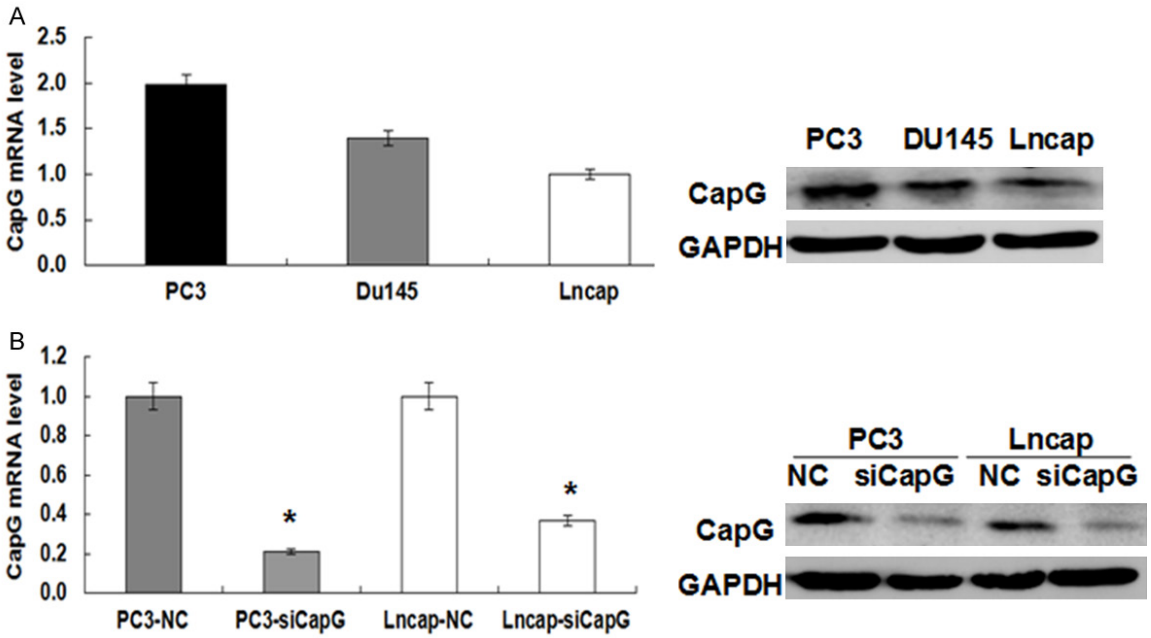

Figure 2. CapG expression was measured in human prostate cancer cell lines. A: CapG expression was measured in PC3, Du145 and Lncap cell lines by qRT-PCR and Western blot. B: CapG down-regulation in PC3 and Lncap cells was confirmed by qRT-PCR and Western blot. *: $P<0.001$.

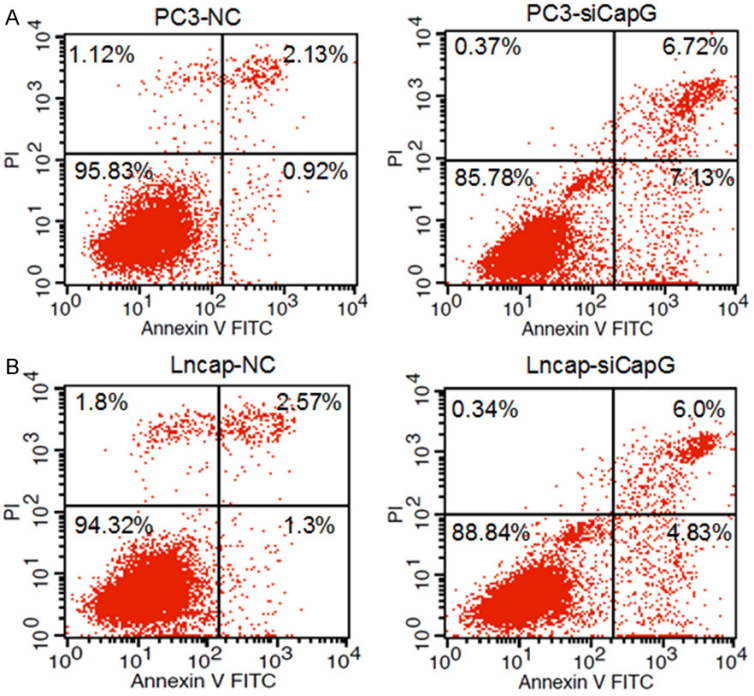

Figure 3. Effect of CapG down-regulation on cell apoptosis was evaluated by flow cytometry. A: The apoptotic rates in PC3-NC and PC3-siCapG cells were 3.14%±0.27 and 14.78%±1.16, respectively, and the difference was statistically significant ($P<0.001$). B: The apoptotic rates in Lncap-NC and Lncap-siCapG cells were 3.90%±0.33 and 11.10%±1.72, respectively, and the difference was statistically significant ($P<0.001$).

CapG might be correlated with the formation of PCa. Meanwhile, the same evidence that CapG highly expressed in PCa was confirmed by qRT-PCR and Western blot. These observations indicated that abnormal high-expression of CapG was contributed to the formation of PCa, which might be helpful for the diagnosis of PCa. Our data was also consistent with the observations in non-small cell lung cancer [11], breast cancer [12] and ovarian carcinoma [13], indicating that CapG was a carcinogenic gene. However, Watari et al. [14] reported that CapG was lost in the tumorigenic cell line, such as lung cancer, stomach cancer and melanoma, and might be a candidate tumor suppressor.

Then, we evaluated the correlation between CapG and clin-

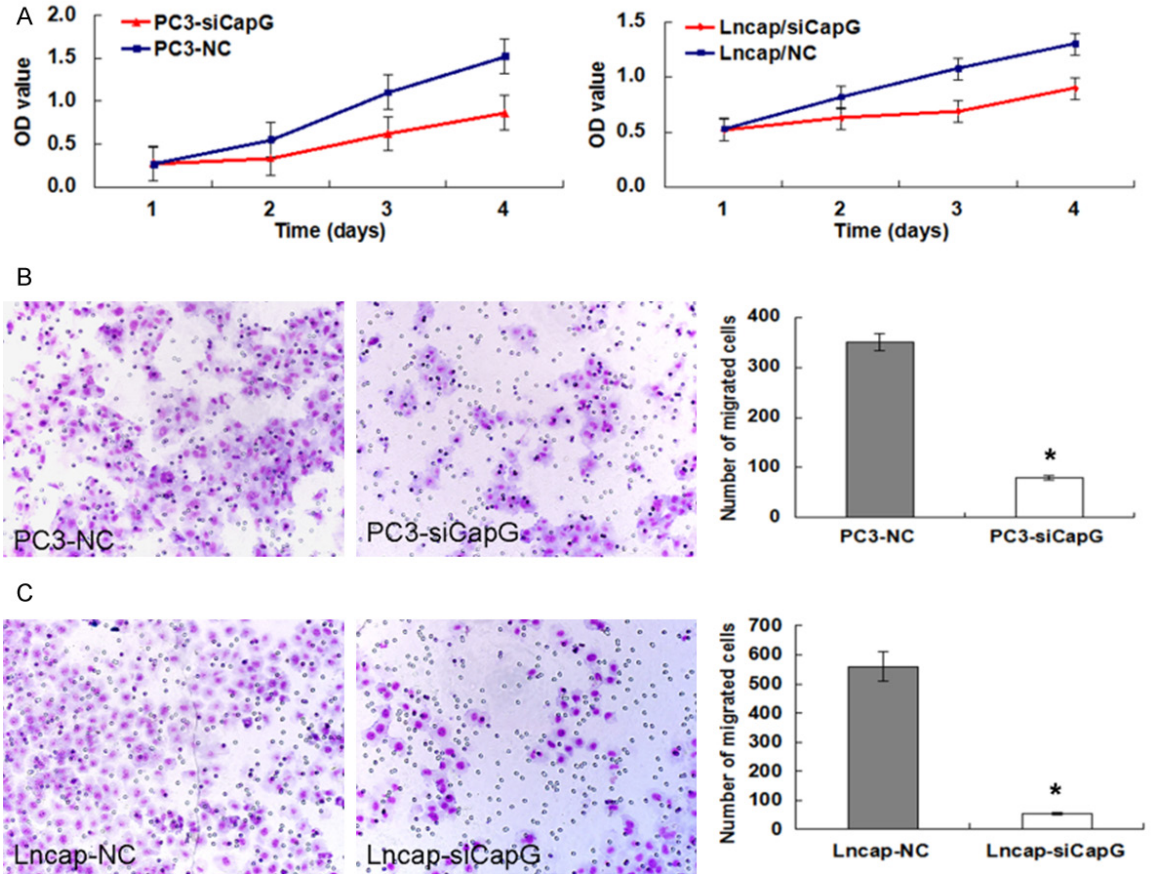

Figure 4. CapG depletion significantly inhibited cell proliferation and migration in PC3 and Lncap cells. A: CapG depletion significantly inhibited cell proliferation by CCK-8 assay (P<0.001). B: CapG depletion significantly inhibited the migration of PC3 cells (P<0.001). C: CapG depletion significantly inhibited the migration of Lncap cells (P<0.001). *: P<0.001.

icalpathological characteristics in PCa. Results exhibited that CapG expression was significantly correlated with PSA level, Gleason score, T stage and lymph node metastasis. Higher expression of CapG was found in patients with higher level of PSA (>10 ng/ml). Meanwhile, CapG expression was markedly increased in patients with higher Gleason score (>7) and T stage (T3-T4), and was also significantly elevated in patents with lymph node metastasis. It is well known that PSA is beneficial for the diagnosis and prognostic prediction in patients with PCa [18, 19]. Gleason score, T stage and lymph node metastasis were implicated with the progression of PCa and might be served as prognostic factors in patients with PCa [20-22]. Thus, these data further validated that CapG was a carcinogenic gene in PCa and higher expression of CapG was relative to the progression of PCa.

At present, CapG has been reported to be connected with cell invasion, division and motility [7, 9, 12, 13, 15, 16]. However, the effect of CapG expression on cell apoptosis, proliferation and migration was little to know. In present study, we observed that CapG expression was higher in PC3 cell lines compared with that in Du145 and Lncap cell lines, suggesting that CapG was highly expressed in human PCa cell lines. Subsequently, we found that CapG depletion significantly promoted the apoptosis in PC3 and Lncap cells, while markedly inhibited the proliferation and migration compared with those in normal controls. Meanwhile, Li et al. [23]. also reported that CapG was expressed in PC3, Du145 and Lncap cell lines, and CapG downregulation significantly inhibited the proliferation, invasion and metastasis of DU145 cells. Thus, these data confirmed that high CapG expression was contributed to cell prolif-

eration and migration, which might promote the progression of PCa. Meanwhile, these data further validated the observations in PCa tissues, and supported that CapG might be served as a therapeutic target in Pca. In addition, CapG downregulation significantly suppressed the migration and invasion in ovarian carcinoma and breast cancer [13, 24].

In summary, our results indicate that CapG is highly expressed in PCa and affects cell apoptosis, proliferation and migration. However, the mechanism of CapG in PCa remains unknown. Further investigations are needed.

Acknowledgements

This work was supported by a National Natural Science Foundation of China (No.81328017), a Natural Science Foundation of Guangdong Province (2016A030313583), two Science and Technology Planning Projects of Guangdong Province (No.2013B051000050, No.2014A-020212538), and two grants from President Foundation of Nanfang Hospital, Southern Medical University (No.G2011014, No.2013Z011).

Disclosure of conflict of interest

None.

Address correspondence to: Shan-Chao Zhao, Department of Urology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China. E-mail: zhaoshanchaosmu@sina.cn

References

[1] Petrakis D, Pentheroudakis G, Kamina S, Pappa L, Papadiotis E, Malamou-Mitsi V and Pavlidis N. An unusual presentation of a patient with advanced prostate cancer, massive ascites and peritoneal metastasis: Case report and literature review. J Adv Res 2015; 6: 517-521.

[2] Zhao L, Yu N, Guo T, Hou Y, Zeng Z, Yang X, Hu P, Tang X, Wang J and Liu M. Tissue biomarkers for prognosis of prostate cancer: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev 2014; 23: 1047-1054.

[3] Lei F, Zhang L, Li X, Lin X, Wu S, Li F and Liu J. Overexpression of prostate tumor overexpressed 1 correlates with tumor progression and predicts poor prognosis in breast cancer. BMC Cancer 2014; 14: 457.

[4] Jiang X, Li X, Huang H, Jiang F, Lin Z, He H, Chen Y, Yue F, Zou J, He Y, You P, Wang W, Yang W, Zhao H, Lai Y, Wang F, Zhong W and Liu L. Elevated levels of mitochondrion-associated autophagy inhibitor LRPPRC are associated with poor prognosis in patients with prostate cancer. Cancer 2014; 120: 1228-1236.

[5] Dimakakos A, Armakolas A and Koutsilieris M. Novel tools for prostate cancer prognosis, diagnosis, and follow-up. Biomed Res Int 2014; 2014: 890697.

[6] Rachner TD, Thiele S, Gobel A, Browne A, Fuessel S, Erdmann K, Wirth MP, Frohner M, Todenhofer T, Muders MH, Kieslinger M, Rauner M and Hofbauer LC. High serum levels of Dickkopf-1 are associated with a poor prognosis in prostate cancer patients. BMC Cancer 2014; 14: 649.

[7] De Corte V, Van Impe K, Bruyneel E, Boucherie C, Mareel M, Vandekerckhove J and Gettemans J. Increased importin-beta-dependent nuclear import of the actin modulating protein CapG promotes cell invasion. J Cell Sci 2004; 117: 5283-5292.

[8] Van Impe K, De Corte V, Eichinger L, Bruyneel E, Mareel M, Vandekerckhove J and Gettemans J. The Nucleo-cytoplasmic actin-binding protein CapG lacks a nuclear export sequence present in structurally related proteins. J Biol Chem 2003; 278: 17945-17952.

[9] Renz M and Langowski J. Dynamics of the CapG actin-binding protein in the cell nucleus studied by FRAP and FCS. Chromosome Res 2008; 16: 427-437.

[10] Tonack S, Patel S, Jalali M, Nedjadi T, Jenkins RE, Goldring C, Neoptolemos J and Costello E. Tetracycline-inducible protein expression in pancreatic cancer cells: effects of CapG overexpression. World J Gastroenterol 2011; 17: 1947-1960.

[11] Zhu WY, Hunag YY, Liu XG, He JY, Chen DD, Zeng F, Zhou JH and Zhang YK. Prognostic evaluation of CapG, gelsolin, P-gp, GSTP1, and Topo-II proteins in non-small cell lung cancer. Anat Rec (Hoboken) 2012; 295: 208-214.

[12] Renz M, Betz B, Niederacher D, Bender HG and Langowski J. Invasive breast cancer cells exhibit increased mobility of the actin-binding protein CapG. Int J Cancer 2008; 122: 1476-1482.

[13] Glaser J, Neumann MH, Mei Q, Betz B, Seier N, Beyer I, Fehm T, Neubauer H, Niederacher D and Fleisch MC. Macrophage capping protein CapG is a putative oncogene involved in migration and invasiveness in ovarian carcinoma. Biomed Res Int 2014; 2014: 379847.

[14] Watari A, Takaki K, Higashiyama S, Li Y, Satomi Y, Takao T, Tanemura A, Yamaguchi Y, Katayama I, Shimakage M, Miyashiro I, Takami K, Kodama K and Yutsudo M. Suppression of tumorigenicity, but not anchorage independence, of hu-

man cancer cells by new candidate tumor suppressor gene CapG. Oncogene 200; 625: 7373-7380.

[15] Hubert T, Van Impe K, VandekerckhoveJ and Gettemans J. The actin-capping protein CapG localizes to microtubule-dependent organelles during the cell cycle. Biochem Biophys Res Commun 2009; 380: 166-170.

[16] Tonack S, Patel S, Jalali M, Nedjadi T, Jenkins RE, Goldring C, Neoptolemos J and Costello E. Tetracycline-inducible protein expression in pancreatic cancer cells: effects of CapG overexpression. World J Gastroenterol 2011; 17: 1947-1960.

[17] Bao JM, He MY, Liu YW, Lu YJ, Hong YQ, Luo HH, Ren ZL, Zhao SC and Jiang Y. AGE/RAGE/Akt pathway contributes to prostate cancer cell proliferation by promoting Rb phosphorylation and degradation. Am J Cancer Res 2015; 5: 1741-1750.

[18] Giannarini G, Scott CA, Moro U, Pertoldi B, Beltrami CA and Selli C. Are PSA density and PSA density of the transition zone more accurate than PSA in predicting the pathological stage of clinically localized prostate cancer? Urol Oncol 2008; 26: 353-360.

[19] Eminaga O, Hinkelammert R, Titze U, Abbas M, Eltze E, Bettendorf O and Semjonow A. The presence of positive surgical margins in patients with organ-confined prostate cancer results in biochemical recurrence at a similar rate to that in patients with extracapsular extension and PSA </= 10 ng/ml. Urol Oncol 2014; 32: 32 e17-25.

[20] Amin A, Partin A and Epstein JI. Gleason score 7 prostate cancer on needle biopsy: relation of primary pattern 3 or 4 to pathological stage and progression after radical prostatectomy. J Urol 2011; 186: 1286-1290.

[21] Deng FM, Mendrinos SE, Das K and Melamed J. Periprostatic lymph node metastasis in prostate cancer and its clinical significance. Histopathology 2012; 60: 1004-1008.

[22] Carlsson SV, Tafe LJ, Chade DC, Sjoberg DD, Passoni N, Shariat SF, Eastham J, Scardino PT, Fine SW and Touijer KA. Pathological features of lymph node metastasis for predicting biochemical recurrence after radical prostatectomy for prostate cancer. J Urol 2013; 189: 1314-1318.

[23] Li B, Guo KK, Li CY, Li HL, Zhao PP, Chen K and Liu CX. Influence of suppression of CapG gene expression by siRNA on the growth and metastasis of human prostate cancer cells. Genet Mol Res 2015; 14: 15769-15778.

[24] Van Impe K, Bethuyne J, Cool S, Impens F, Ruano-Gallego D, De Wever O, Vanloo B, Van Troys M, Lambein K, Boucherie C, Martens E, Zwaenepoel O, Hassanzadeh-Ghassabeh G, Vandekerckhove J, Gevaert K, Fernandez LA, Sanders NN and Gettemans J. A nanobody targeting the F-actin capping protein CapG restrains breast cancer metastasis. Breast Cancer Res 2013; 15: R116.