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

Haiqi Mu¹,², Bo Qiu³, Qipeng Xie¹, Yijun Wang², Cunjin Nan², Wei Han⁵, Tong Zhao³, Shan-Chao Zhao¹

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

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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 clinical pathological 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 world [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 unsatisfactory [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 characteristics 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 defined 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% CO₂. 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^ΔΔCt. The primer sequences of GAPDH were 5’-GCATCCTGCACCACCAACTG-3’ and 5’-GCCTGCTTCACACCTTTT-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 (zhongshanJinqiao, China) for 1 hour at room temperature. Protein bands were visualized by ECL chromogenic substrate (Beyotime, China).
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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, 2×10³ 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 (χ²) was used to evaluate the CapG expression in prostate cancer and normal prostate and to assess the correlation between CapG expression and clinical pathological characters. Independent-sample t-test was used to compare the difference in two groups. The statistically significant difference was P<0.05.
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**Results**

CapG is highly expressed in prostate cancer and correlated with clinical pathological 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 clinical pathological 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

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

<table>
<thead>
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<th>Tissue types</th>
<th>N</th>
<th>CapG (%)</th>
<th>P value</th>
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<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
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<tr>
<td>Prostate cancer</td>
<td>90</td>
<td>41 (45.6)</td>
<td>49 (54.4)</td>
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<td>Normal prostate</td>
<td>30</td>
<td>25 (83.3)</td>
<td>5 (16.7)</td>
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### Table 2. Correlation between CapG expression and clinical pathological characters in prostate cancer

<table>
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<td>Negative</td>
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</tr>
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<td>12</td>
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<td>Gleason score</td>
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</table>
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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-
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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 patients 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-

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.
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Apoptosis 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.

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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

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


