Capping Actin Protein Overexpression in Human Colorectal Carcinoma and Its Contributed Tumor Migration

Tsung-Jung Tsai, Yun-Ping Lim, Wen-Ying Chao, Chien-Chin Chen, Yi-Ju Chen, Ching-Yen Lin, and Ying-Ray Lee

1Department of Gastroenterology, Ditmanson Medical Foundation Chiayi Christian Hospital, Chiayi, Taiwan
2Department of Pharmacy, College of Pharmacy, China Medical University, Taichung 404, Taiwan
3Department of Nursing, Min-Hwei College of Health Care Management, Tainan 736, Taiwan
4Department of Pathology, Ditmanson Medical Foundation Chiayi Christian Hospital, Chiayi, Taiwan
5Department of Cosmetic Science, Chia Nan University of Pharmacy and Science, Tainan, Taiwan
6Department of Medical Research, Ditmanson Medical Foundation Chiayi Christian Hospital, Chiayi, Taiwan

Correspondence should be addressed to Ching-Yen Lin; jouyuan22@gmail.com and Ying-Ray Lee; yingray.lee@gmail.com

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Objective. Human colorectal cancer (CRC) is the third most common cancer; patients with metastatic colorectal cancer (mCRC) show poor prognosis than those with CRC cases. There are no reliable molecular biomarkers for the diagnosis of CRC prognosis except with pathological features. Therefore, it is urgent to develop a biomarker for diagnosis and/or prediction of human CRC. In addition, capping actin protein (CapG) belongs to the gelsolin family and has been reported to contribute on tumor invasion/metastasis in multiple human cancers. Here, we are the first to evaluate the expression of CapG in human CRCs.

Study Design. To investigate the expression levels of CapG in human tissue array by immunohistochemistry (IHC) staining. Moreover, the mRNA and protein levels were also confirmed in four CRC cell lines and determined using real-time RT-PCR and Western blotting. Finally, a Matrigel transwell invasion assay was used to evaluate the invasion ability in CapG high or low expression cells.

Results. We demonstrated that CapG could be determined in the normal colon tissue and human CRC specimens. However, CapG was significantly overexpressed in the mCRC specimens compared with that in CRC specimens and normal cases. It was also detectable in the four CRC cell lines including mRNA and protein levels. We also found that knockdown of the expression of CapG reduced tumor migration. Conclusions. In this study, we suggested that CapG could be used as a biomarker for metastatic CRC in the clinical specimens. Moreover, our in vitro study demonstrated that CapG might contribute on tumor metastasis in human CRCs.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer-related death in men and women worldwide. The risk factors for CRC are age, lifestyle, familial history of CRC, and patients with inflammatory bowel disease (IBD) and Crohn’s disease [1]. Current clinical treatment strategy for CRC includes surgical resection, chemotherapy, and target therapies. However, the prognosis of patients with CRC is determined by pathological features and the diagnostic stage of the tumor. In patients with in situ carcinoma of CRC, the 5-year survival rate is 90%. However, patients with metastatic colorectal cancer (mCRC) have poor prognosis, and the five-year survival rate is 10–20% [2]. The target therapies including monoclonal antibodies against receptor tyrosine kinases (RTK) such as epidermal growth factor receptor (EGFR) may contribute to the improvement in survival of mCRC patients. Therefore, early diagnosis for CRC patients or identification of predictive markers for treatment of mCRC patients is urgent.

The main cause of death from CRC is usually related to metastasis and resistance to chemotherapeutics after surgery. If prognosis could be determined more precisely before treatment, patients would be more effectively treated with
individualized therapy. The spread of solid tumor from the
primary site and subsequent dissemination is facilitated by
detachment of malignant cells. This metastatic cascade is
critically contributed by the cytoskeleton and extracellular
matrix (ECM) remodeling through migratory and invasive
signal regulation [3, 4].

CapG, a membrane of the gelsolin family, has the
common property of binding to the barbed end of actin
filaments with high affinity and has been identified as playing
important roles in tumor invasion and metastasis including
breast, lung, and prostate carcinoma [5–7]. CapG is contrib-
uting on the modulation of cellular motility through interacting
differentially with the actin cytoskeleton [8]. It also has
been reported to act on the tumor invasion and migration
[5, 6, 8]. Although gelsolin has been reported to overexpress
and induce tumor invasion in CRC cases [6], the role and the
association of CapG with CRC are still unclear.

In this study, we identified the expression of CapG from
immunohistochemical staining on tissue microarray (TMA)
of CRC patients and carried out clinical validation with
pathology. Moreover, the mRNA and protein levels of CapG
are also evaluated in the various CRC cell lines. Finally, cellu-
lar invasion is also confirmed in the CapG-overexpressed
cell lines.

2. Materials and Methods

2.1. Patient Samples. A human colorectal cancer-metastasis-
normal tissue microarray slide (CDA3) was purchased from
SuperBioChips Laboratories (Seoul, Korea). A total of 50
patients (including 10 patients with metastatic carcinoma)
who were pathologically diagnosed with CRC and 9 nor-
mal cases were selected and included in this TMA (the data
category3=14). However, in this slide, there were two
cases exceptionally having no tumor cells. The diagnosis
with tumor stage and pTNM in these cases could be
found in the TMA data sheet. Moreover, the prognosis
of these cases also could be found in the supplementary
data of this TMA. This study was approved by the Institu-
tional Review Board (IRB) of Ditmanson Medical Founda-
tion Chiayi Christian Hospital (IRB106018).

2.2. Cell Lines and Cell Culture. Human colorectal cancer cell
lines, including HCT116, HT29, SW1116, and DLD-1, were
obtained from ATCC. HT29 and DLD-1 cells were cultured
in Dulbecco’s Modified Eagle’s Medium (DMEM); HCT116,
cells were cultured in RPMI 1640 medium, and SW1116
were cultured in L-15 medium (all media were
supplemented with 10% fetal bovine serum (FBS)).

2.3. Immunohistochemistry Staining. Tissue specimens in the
TMA were deparaffinized, boiled in citric acid, and treated
with hydrogen peroxide before incubation with anti-CapG
primary antibody (GeneTex Inc., Hsinchu, Taiwan) over-
night at 4°C. Then, the TMA was incubated with polymer-
horseradish peroxidase-conjugated secondary antibody at
room temperature. Specimens were developed with diami-
benzidine (DAB) and counterstained with hematoxylin
(Dako, Glostrup, Denmark). The images were determined
with an Olympus microscope. The intensity of staining was
graded from 0 (undetectable), 1 (weak staining), 3 (medium
staining) to 5 (intense staining), while the proportion of
positive staining tumor cells within a tissue was scored from
0% to 100% of tumor cells identified. The staining score
was expressed as the product of intensity of staining and propor-
ton of tumor positivity.

2.4. Real-Time Quantitative RT-PCR. Real-time PCR was
used to evaluate the expression of CapG in the four CRC cell
lines. The total RNAs from HCT116, HT29, SW1116, and
DLD-1 cells were extracted by RNA isolation kit (GE Health-
care, Munich, Germany), and the cDNAs were prepared by
using Transcriptor First Strand cDNA Synthesis Kit (Roche,
Mannheim, Germany), carried out on the CFX96 Real-Time
System (Bio-Rad). The conditions of real-time PCR were
as follows: one cycle at 50°C for 2 min and 95°C for
10 min and followed by 40 cycles of denaturation at 95°C
for 15 seconds and annealing extension at 55°C for 1 min.
The primers used included the following:

(1) CapG-F: CGAACACTCAGGGAGATT
(2) CapG-R: TCCAGTTTTGAAAAATTTGC
(3) GAPDH-F: TGCACCAACTGCTTAGC
(4) GAPDH-R: GGCATGGACTGTGGTCATGAG

2.5. Western Blotting. Total cell lysates of human colorectal
cancer cell lines, HCT116, HT29, SW1116, and DLD-1, were
extracted with RIPA buffer (containing PMSF, EGTA, apro-
tinin, leupeptin, Na3VO4 (Sigma, MO, USA), and EDTA
(Merck, Darmstadt, Germany)). Proteins from each sample
were analyzed with 10% sodium dodecyl sulfate polyacryl-
amide gel electrophoresis (SDS-PAGE), transferred to
PVDF membrane (Bio-Rad Laboratories Inc., CA, USA),
and blocked and incubated with primary antibody (CapG
antibody; GeneTex Inc.) overnight at 4°C followed by sec-
dary antibody staining for 1 h at room temperature.
Finally, the data were examined using the BioSpectrum Imaging
system (UVP, CA, USA). The expression levels of the
proteins in the cells were quantified using Image-J software.

2.6. Migration Assay. A control shRNA (CCGGACACTCG
AGCATTTTGG) and CapG shRNA (CCGGCGCAACA
CTCAGGTGAGATTCTCGAGAATCTCCACCTGAGTG
TTCCGTATTTT) were transfected into HT29 cells, and the
migration activity of the cells was determined under
transwell migration assay. 1 × 105 cells/well were seeded and
cultured in the transwell upper chamber for 24 h, and 10% FBS
was then used as chemoattractant in the bottom cham-
ber for 24 h. Cells migrated in the bottom chamber were
stained with 2% crystal violet solution (Sigma, MO, USA).
2.7. Statistical Analysis. All data were analyzed using Graph-Pad Prism for Windows, version 6 (GraphPad Software Inc., San Diego, CA, USA). The data presented as the mean ± SD, and *p* values was calculated by Microsoft Excel and SPSS, version 21.0 (IBM SPSS Statistics, USA) through the non-parametric tests. *p* values less than 0.05 were considered as "statistically significant".

3. Results

3.1. CapG Expression Is Illustrated in the Human Metastatic Colorectal Carcinoma. The demographic data and the clinical characteristics of the patients were showed in Table 1. The expression index of CapG in these specimens was definite into high (expression index \( \geq 1.5 \)) and low (expression index \(< 1.5\)). There were no significant difference regarding CapG expression between groups including age, sex, tumor differentiation, tumor size and invasive, lymph node status, and tumor stage (Table 1). The expression of CapG in the CRC clinical specimens including 9 normal cases, 39 CRC cases, and 9 metastatic CRC cases was validated by IHC staining and determined under microscopy (Supplementary data 1). CapG expression in tumor tissues as well as in normal tissues was determined by Dr. Chen Chien-Chin (a clinical doctor of pathology) and scored for the intensity of staining (nonexpression: 0, weak expression: 1, medium expression: 3, and intense expression: 5) and proportion of tumor positivity (0–100%). CapG overexpression was significantly determined in the metastatic CRC specimens compared with that in CRC specimens and normal cases (Figure 1). This finding was consistent with the reports in various human cancers [5, 6]. However, our data also showed that no statistically significant expression of CapG between CRC cases and normal specimens (Figure 1). Herein, our data suggested that CapG might be a prognostic marker for metastasis but could not be used as a diagnostic tumor marker for CRC patients.

2. Analytical Cellular Pathology

<table>
<thead>
<tr>
<th>Clinical classification</th>
<th>Total number</th>
<th>CapG immunostaining intensity</th>
<th></th>
<th>p value</th>
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<tr>
<td></td>
<td></td>
<td>Low (number)</td>
<td>High (number)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td>p = 0.988 (male versus female)</td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>20</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td>p = 0.789 (≥55 versus &lt;55)</td>
</tr>
<tr>
<td>≥55</td>
<td>30</td>
<td>14</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>&lt;55</td>
<td>26</td>
<td>15</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>T-primary tumor</td>
<td></td>
<td></td>
<td></td>
<td>p = 0.742 (T1 + T2 versus T3 + T4)</td>
</tr>
<tr>
<td>T1 + T2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>T3 + T4</td>
<td>36</td>
<td>22</td>
<td>14</td>
<td></td>
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<tr>
<td>Lymph node status</td>
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<td></td>
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<td>p = 0.902 (negative versus positive)</td>
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<tr>
<td>Negative</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td>p = 0.399 (I + II versus III + IV)</td>
</tr>
<tr>
<td>I + II</td>
<td>13</td>
<td>7</td>
<td>6</td>
<td></td>
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<td>III + IV</td>
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<td>10</td>
<td></td>
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<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
<td>p = 0.827 (pairwise comparison randomly)</td>
</tr>
<tr>
<td>Well differentiation</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Moderate differentiation</td>
<td>21</td>
<td>13</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Poor differentiation</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
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</tbody>
</table>

3.2. mRNA and Protein Expression Levels of CapG in the Human Colorectal Carcinoma Cell Lines. In order to evaluate the expressions and the further bioactivity of CapG in the
human CRC cells, four CRC cell lines were examined. Human CRC cell lines including SW1116, HT29, HCT116, and DLD-1 were cultured, and the mRNA and total protein of CapG were determined by real-time RT-PCR and Western blotting. Figure 2(a) showed that the expression level of CapG mRNA in these cells was SW1116 > HT29 > HCT116 > DLD-1. Consistently, the protein expression level of CapG in these cells was SW1116 > HT29 > HCT116 > DLD-1 (Figure 2(b)). Here, we firstly illuminated the differential expressions of mRNA and protein of CapG in SW1116, HT29, HCT116, and DLD-1 cells.

3.3. Knockdown of the Expression of CapG in HT29 Cells Reduces the Cellular Migration Ability. Our data (Figure 1) illustrates that CapG was overexpressed in the metastatic CRC cases, suggesting that CapG may contribute on tumor metastasis in human CRC. To address this hypothesis, a shRNA of CapG was transfected into HT29 cells and the cellular migration was further determined under a transwell migration assay. Figure 3(a) demonstrated that CapG shRNA could significantly suppress the expression of CapG in HT29 cells. Moreover, the migration ability was reduced in HT29 cells with CapG knockdown (Figures 3(b) and 3(c)). These findings suggested that CapG overexpression in the human colorectal cancer cells might contribute on the tumor cell migration.

4. Discussion

In this study, we are the first to address the expression of CapG in human colorectal cancers. IHC staining showed that CapG was highly expressed in metastatic CRC specimens compared with that in CRC specimens and normal cases (Figure 1). It indicated that high expression of CapG might be correlated with the migration of CRC, which might be a useful prognostic marker for the early diagnosis of mCRC.
Importantly, our finding was consistent with previous findings in human glioma [9], breast cancer [10], gastric cancer [11], and ovarian cancer [12], suggesting that CapG could be an oncoprotein. In addition, the previous report showed that patients with lymph node metastases were associated with overexpression of CapG in 75 pulmonary adenocarcinomas [13]. Among them, overexpression of CapG in the advanced stage of pulmonary adenocarcinoma (stages III and IV) was also higher than that in the earlier stages of pulmonary adenocarcinomas [13]. Most of the literatures have demonstrated that CapG is associated with invasion and migration of cancer cells. The previous study demonstrated CapG lost exhibiting in the small-cell lung cancer (H69, Lu22, Lu139, Lu134, and H209), lung adenocarcinoma (PC7, RERF-LCMS), gastric cancer (AZ521), and melanoma (A2058) [14]. To test ectopic CapG from the tumorigenic stages of human diploid fibroblast strain (RBT) and gastric cancer cell line AZ521, the results showed that CapG overexpression could suppress tumorigenicity but showed no influence in the anchorage-independent growth of RBT and AZ521 cells and might be a candidate tumor suppressor [14]. CapG protein is known to possess actin-modulating activity [15], and it is possible that an alteration of microfilaments contributes to the acquisition or loss of tumorigenicity.

Furthermore, CapG is belonging in the gelsolin/villin family of actin-regulatory proteins. Gelsolin is associated with the invasion and metastasis of cancer cells, such as prostate cancer [7] and lung adenocarcinoma [13]. Gelsolin promotes invasion and metastasis of HCT116 and DLD-1 cells via modulation of the invasion-associated urokinasetype plasminogen activator (uPA) [6]. Moreover, the uPA system plays the role in causing aggressive tumor behavior, which promotes invasion and metastasis in several tumors [6]. Westbrook et al. demonstrated that CAPG and PDZ domain-containing protein GIPC1 (GIPC1) were independently associated with bone metastasis for breast cancer [10]. CAPG showed a weak association, and GIPC1 expressed a stronger relation with bone metastasis [10]. Moreover, CRCs are classified as microsatellite instability (MSI) or microsatellite stable (MSS). To further confirm the role of CapG in human CRC, shRNA of CapG was transfected into HT29 cells and the inhibition of cellular migration was also demonstrated (Figure 3). Therefore, we suggest that CapG may play the role of tumor metastasis in human colorectal cancers.

5. Conclusion

CapG has been showed to overexpress and contribute on malignancy in multiple human cancers. However, the expression and the role of CapG in the human colorectal cancers are still unknown. In the present study, we demonstrated that CapG could be determined in the normal colon tissue and human CRC specimens. Most importantly, CapG was significantly overexpressed in the metastatic CRC specimens compared with that in CRC specimens and normal cases. Further study demonstrates that knockdown of the expression of CapG in human CRC cells decreases the tumor migration ability. Our in vitro study suggests that CapG could be used as a biomarker for metastatic CRC in the clinical specimens and might play a role in tumor metastasis.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no financial relationship with any company involved in this study. There is no conflict of interest involved in this study.

Supplementary Materials

Supplementary data 1: CapG expresses in the human colorectal carcinoma and normal specimens in tissue microarray. The tissue microarray was used to examine the expression of CapG by immunohistochemistry, and the photographs were determined by microscopy. There were no tumor specimens in the samples number 35 and number 45. A blank was showed in the number 60. (Supplementary Materials)

References


