Academia Journal of Scientific Research 7(8): 000-000, February 2018
DOI: 10.15413/ajsr.2019.0206
ISSN 2315-7712


Academia Publishing

*Research Paper*

# Small interfering RNA target for long non-coding RNA PCGEM1 increases sensitivity of LNCaP cells to baicalein

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Accepted

**ABSTRACT**

The aim of this research is to investigate the inhibitory effect and mechanism of long non-coding RNA PCGEM1 siRNA in combination with baicalein on prostate cancer LNCaP cells. The effect of baicalein or lentiviral vector (LV3-shRNA-PCGEM1) alone and the combination of the two on the proliferation of LNCap cells were detected with WST-8. The capability of cell colony formation was detected by a colony formation experiment, while the effect on cell cycle was detected by flow cytometry. The relative expression of PCGEM1 was detected by RT-PCR. The formation of autophagosomes was observed by immunocytochemistry and the levels of protein were detected by western blotting. LNCaP cells transfected with small hair RNA lentiviral vector targeting PCGEM1 were constructed and their expression in LNCaP cells silenced. The stable cell line of LNCaP cells infected with LV3-shRNA-PCGEM1 was successfully constructed. LV3-shRNA-PCGEM1 was also able to increase the baicalein-induced inhibitory effects on LNCaP cells. The increased susceptibility multiples was 2.3. LV3-shRNA-PCGEM1 combined with baicalein inhibited the formation, increased G2 and S phase cells, inhibited the expression of PCGEM1, and induced autophagy of LNCaP cells. LV3-shRNA-PCGEM1 may improve the sensitivity of LNCaP cells to baicalein, and the molecular mechanism may be associated with the decrease of PCGEM1 expression and the induction of autophagy, providing an experimental basis for the combined treatment of Chinese traditional and Western medicine on prostate cancer in a clinical setting.

**Key words:** Long non-coding RNA, prostate cancer gene expression marker 1 (PCGEM1), baicalein, prostate cancer cells, small interfering RNA sequences, autophagy.

**Abbreviations: (lncRNA):** Long non-coding RNA; (**siRNA):** Small interfering RNA; **(shRNA):** Small hair RNA; **(CCK-8):** Cell counting Kit-8; **(PCGEM1):** Prostate cancer gene expression marker 1; **(FBS):** Fetal bovine serum; microtubule-associated protein 1 light chain 3 (**LC3): (RT-PCR):** Reverse transcription polymerase chain reaction; **(HRP):** horseradish peroxidase; (**PCa):** prostate cancer; **(MOI):** multiplicity of infection; **(shRNA):** Short hairpin RNA.

## INTRODUCTION

Prostate cancer has become the most frequent tumor among American males and ranks third in terms of fatalities caused by tumors in American males after the lung and colorectal cancer (Siegel et al., 2018). With the aging population in China, the incidence of prostate cancer increases yearly and the prognosis is poor (Song et al., 2018). Thus, it is imperative to elaborate on the pathogenesis of prostate cancer and find new ways to enhance curative effects.

Recently, several studies have shown that multiple abnormally that expressed long non-coding RNAs (lncRNAs) exist in prostate cancer (Aird et al., 2018). LncRNAs are RNA modules with a transcript length of 200 bp. They do not encode proteins but widely participate in almost all physiological and pathological processes and are closely associated with several diseases (Tang et al., 2017).

In tumor cells, the expression level of some specific lncRNAs changes significantly, and this change may be closely associated with several biological processes (Sun et al., 2018). Extremely high expression of an lncRNA called prostate cancer gene expression marker1 (PCGEM1) is closely associated with the occurrence of prostate cancer. It promotes the proliferation and clone formation of cancer cells and is expected to be the potential marker of prostate cancer (Ho et al., 2016).

For tumor treatment, traditional Chinese medicine is superior to radiotherapy and chemotherapy in terms of comprehensiveness, low toxicity, and immunity promotion (Xue, 2016). As studies on the mechanisms of eliminating pathogens to support healthy mechanisms of promoting immunity of traditional Chinese medicine progress, the application of traditional Chinese medicine in the prevention of cancers and the treatment of early-stage cancers and even medium or advanced cancers has distinct advantages (Yan et al., 2017).

*Scutellaria baicalensis* Georgi, an East Asian skullcap plant that contains abundant flavonoids, is widely used in traditional Chinese medicine (TCM) as a remedy for the clinical treatment of inflammation, allergy, and fever as well as, a treatment for several cancers (Michiko et al., 2017; Sowndhararajan et al., 2018).

Baicalein is one of the monomer extracted from *S. baicalensis* Georgi. Studies have shown that baicalein inhibit the proliferation as well as, invasion and metastasis of tumor cells in human breast cancer, liver cancer, pancreatic cancer, and other cell lines (Bie et al., 2017). In one preliminary study, the expression level of PCGEM1 decreased with small interference RNA (siRNA), which effectively inhibited the proliferation of LNCaP cells of prostate cancer (He et al., 2014).

In the present study, baicalein in combination with siRNA-PCGEM1 was used to act on LNCaP cells of prostate cancer. Its effect on cell proliferation was observed and the potential action mechanism was further evaluated in order to provide an experimental basis for the combined treatment of Chinese traditional and Western medicine on prostate cancer in a clinical setting.

## MATERIALS AND METHODS

### Cell culture

LNCaP cells were purchased from the Shanghai Institute of Cell Biology (Shanghai, China). Cell lines were cultured in Dulbecco's modified Eagle medium (Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS, HyClone; Invitrogen, Camarillo, CA, USA), 100 U/ml of penicillin (Invitrogen), and 100 μg/ml of streptomycin (Invitrogen). Cells were maintained in a humidified incubator at 37°C in the presence of 5% $CO_2$. All cell lines were passaged for less than 6 months.

### Construction of lentiviral expression vector of recombinant PCGM1-siRNA and stable LNCaP cell line

For target sites of the PCGEM1 gene, coding genes of siRNA were designed and constructed by Shanghai GenePharma Co., Ltd. (Shanghai, China). The nucleotide sequences were as follows: siRNA sequence of PCGM1 (sense: 5'-GCCCTACCTATGATTTCATAT-3', antisense: 5'-CAUUAGUGAAAGCACCUGAGC-3'); negative control (NC) sequence (sense: 5'-TTCTCCGAACGTGTCACGTTTC-3', antisense: 5'-GAAACGUGACACGUUCGGAGAA-3'). The nucleotide sequences of coding hairpin shRNA were as follows: (sense: 5'-GATCCGCCCTACCTATGATTTCATATTTCAAGAGAATATGAAATCATAGGTAGGGCTTTTTTG-3', antisense: 5'-AATTCAAAAAAGCCCTACCTATGATTTCATATTCTCTTGAAATATGAAATCATAGGTAGGGCG-3').

The insertion segment of the earlier mentioned shRNA contained a siRNA segment with 21 bases. TTCAAGAGA was selected for the loop structure in the shRNA template in the case of the formation of a termination signal. GATCC was added to the 5' end of the sense strand of the template, complementary to the cohesive end after BamHI digestion. AATTC was added to the 5' end of the antisense strand template complementary to the cohesive end after EcoRI digestion. The constructed lentiviral expression vector of recombinant PCGEM1-siRNA was designated LV3-shRNAPCGEM1. Construction of stable cell line was infected with recombinant siRNA-PCGM1 lentiviral according to the manufacturer's instructions (He et al., 2014).

### Cell proliferation assays

Proliferation of LNCaP was evaluated by the CCK-8 assay (Dojindo, Kumamoto, Japan), according to the manufacturer's instructions. Cells ($5\times10^4$/ml) were divided into control group, NC group (LV3 +scramble sequence [20 nM]), transfection group (LV3-shRNAPCGEM1 [20 nM]), baicalein group (5, 10, 15, 20, 25 μM), and LV3-shRNAPCGEM1 combined with the baicalein group. Cells were cultured for 1, 2, or 3 days before the addition of 10 μl of CCK-8 (5 mg/ml) to the culture medium in each well. After 1 h incubation at 37°C, the absorbance at 450 nm of each well was measured using a ThermoMax microplate reader (thermo Labsystems). Each experiment was repeated thrice, and the data used to represent the mean of

all measurements.

**Quantitative real-time RT-PCR**

Cells were seeded at a density of $1.0\times10^6$ cells/ml (500 µl/well) in 6-well plates (corning, sigma) and divided into a control group, transfection group (LV3-shRNAPCGEM1 [20 nM]), baicalein group (15 µM), and LV3-shRNAPCGEM1 combined with baicalein group. Total RNA samples were extracted using Trizol (Invitrogen), according to the manufacturer's instructions. Real-time quantitative PCR analysis was performed using an Applied Biosystems 7500 Real-Time PCR System (Foster City, CA, USA), and the U6 level was an internal control for miRNAs. Primers used in quantitative real-time PCR analysis were: U6 (forward: 5'-CTCGCTTCGGCAGCACA -3', reverse: 5'-AACGCTTCACGAATTTGCGT-3'); and PCGEM1 (forward: 5'-CACGTGGAGGACTAAGGGTA-3', reverse: 5'-TTGCAACAAGGGCATTTCAG-3'); The expression level was calculated using CT and $2^{-\Delta\Delta Ct}$.

**Colony formation assay**

Cells were seeded at a density of (100 cells/well) in 6-well plates (Costar), while cell groups were divided as previously mentioned and incubated at 37°C for 12 to 14 days. Cells were fixed for 15 min in 3:1 (v/v) methanol: acetic acid and stained for 15 min with 0.5% (w/v) crystal violet (Sigma, St. Louis, MO, USA) in methanol. After staining, colonies were counted using a cut-off of 50 viable cells/colony. Experiments were performed in triplicate and mean, SD, and P-values calculated.

**Flow cytometric analysis of the cell cycle**

Cells were seeded at a density of $1.0\times10^6$ cells/ml (500 µl/well) in 6-well plates (Costar), and cell groups divided as previously mentioned. Cells were continuously incubated for 48 h, harvested, washed twice with PBS, and fixed with 70% ethanol. Cells were thereafter treated with RNase A (1 mg/ml) following the elimination of ethanol. Finally, the cells were stained with PI solution (50 µg/ml). Cell cycles were analyzed by flow cytometry according to the content of DNA (Beckman Coulter Elite, Fullerton, CA, USA) (He et al., 2016).

**Western blot assays**

LNCaP cells seeded at a density of $1.0\times10^6$ cells/ml (500 µl/well) in 6-well plates, and cell groups same as earlier mentioned. Whole cell lysates were prepared by adding 5×SDS sample buffer. Equal amounts of protein were electrophoresed on 10% SDS-PAGE gels and transferred to PVDF membranes. The membranes were then blocked for 60 min at room temperature with 5% non-fat dry milk/TBST (TBS-Tween 20) and reacted with appropriate antibodies for LC3, p62 and GAPDH (1:1000 dilution in blocking buffer) at 4°C overnight. Following incubation with the primary antibody, membranes were washed in TBST and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Proteins were visualized by incubation with SuperSignal west pico reagents (NCI5079, Thermo), followed by exposure to radiograph film.

**Immunocytochemistry**

Cells were seeded at a density of $1.0\times10^6$ cells/ml (500 µl/well) in 6-well plates (Costar), and cell groups as earlier mentioned. Cells were plated on cover slips and treated as indicated for 48 h. Cells were then fixed at 4°C for 10 min in pre-cooled 100% methanol (-20°C), rinsed with phosphate-buffered saline (PBS) thrice, permeabilized at room temperature in 0.2% Triton X-100 for 5 min, and blocked at room temperature for 30 min in 5% BSA and 0.1% Triton X-100. Samples were incubated with primary antibodies at 4°C overnight, followed by a secondary antibody conjugated to rabbit (1:500 Alexa Fluor 488; Molecular Probes, Eugene, OR, USA) for 1 h. After rinsing thrice with PBS, the cells were incubated with 0.5 ng/ml of DAPI for 15 min at room temperature before mounting. All cover slips were examined under a spectral laser scanning confocal microscope (Nikon C1-Si, Mississauga, Canada). Images were analyzed using EZ-C1 3.20 FreeViewer software (He et al., 2013).

**Statistical analysis**

All results and data were confirmed in at least three separate experiments. Data were expressed as mean ± standard deviation. SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA) was used to conduct statistical analyses. Statistical comparisons were made using one-way ANOVA. $P<0.05$ was considered statistically significant.

**RESULTS**

**Successful construction of LV3-shRNAPCGEM1, siRNA PCGEM1, and stable cell line infected with LV3-shRNAPCGEM1**

The lentiviral vector is a gene therapy vector modified based on HIV-1, which belongs to retrovirus and is widely applied in studies of *in vitro* living cell transfection and gene therapy (Hu et al., 2018; Benskey and Manfredsson, 2016). HIV-1

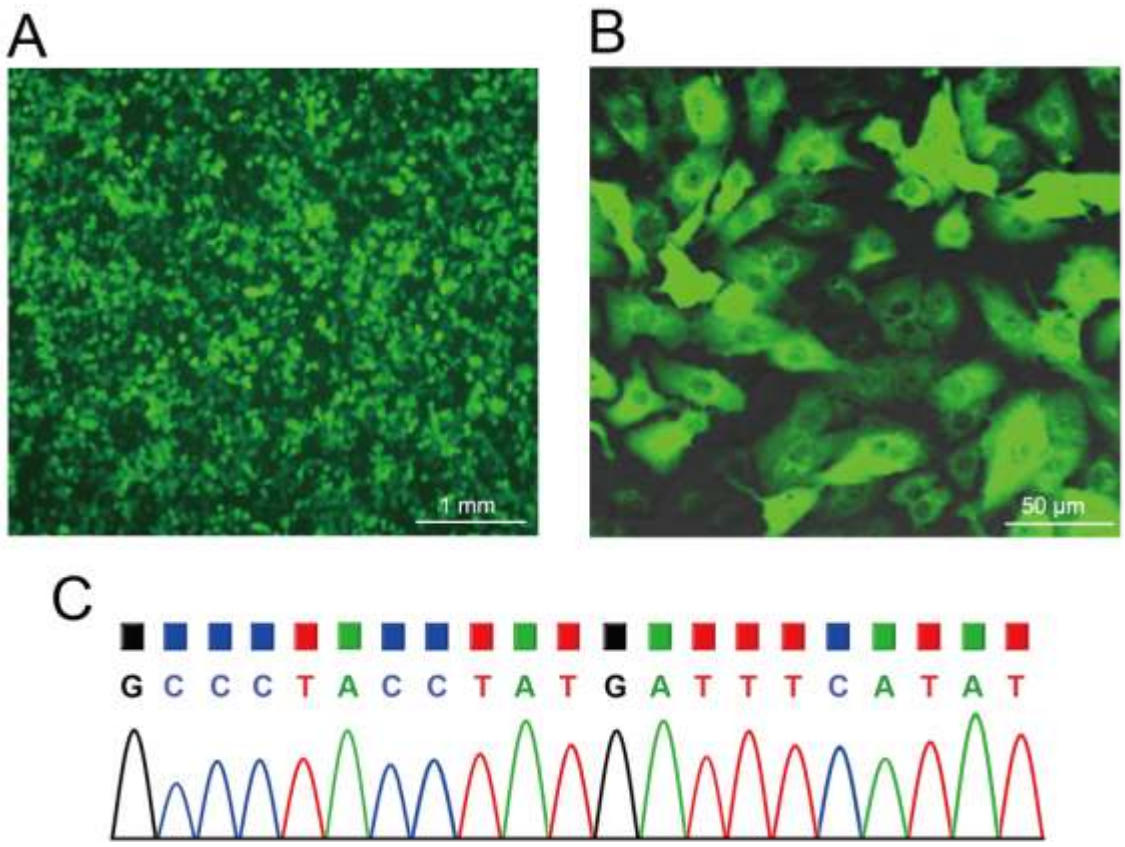


**Figure 1:** Construction of lentiviral expression vector of the coding siRNAPCGEM1 gene. (A) Lentiviral titers under a fluorescence microscope; (B) Transfection efficiency of LLNCaP cells under fluorescence microscope; (C) Correct coding sequence of siRNAPCGEM1 by sequencing.

lentiviral vector particles carrying target genes not only retain efficient infection and integration, but also avoid damage to cells of virus replication. Moreover, lentiviral vector carries green fluorescence, which is convenient for observing whether the target genes are transfected into the cell line (Del et al., 2019). In order to obtain the cell line stably expressing lentivirus new viral particles generated by host cells are not used. siRNA sequences may be cloned to the vector as "short hairpin," such as adenovirus vector. The hairpin sequence is expressed in cells, forming a "double-chain RNA" (short hairpin RNA; shRNA), and processed by RNA interfering (RNAi) channel. Target genes were silenced by shRNA, automatically processed to siRNA in cells (Li and Chen, 2013).

A shRNA was directionally connected to the pGLV3/H1/GFP+Puro vector plasmid. In addition, recombinant, packaging, and envelope plasmids (Shanghai GenePharma Co.) were co-transfected into 293T cells for 72 h. Viruses were then collected and the titer determined to be $2 \times 10^9$ TU/ml (Figure 1A). Figure 1C shows that the recombinant plasmid was sequenced and the correct siRNA PCGEM1 sequence was obtained indicating that the lentiviral expression vector (LV3-shRNAPCGEM1) of the coding siRNA PCGEM1 gene was successfully constructed, hence, creating the basis for subsequent study involving the function of the coding siRNA PCGEM1 gene. The constructed lentiviral vector (LV3-shRNAPCGEM1) infected the LNCaP cells temporarily. Figure 1B shows that when MOI=50, the infection efficiency was higher than 95% after 4 days. These results indicated that the stable cell line of LNCaP cells infected with lentiviral vector (LV3-shRNAPCGEM1) was successfully constructed.

## LV3-shRNAPCGEM1 combined with baicalein inhibited LNCaP cell proliferation

This study proposed to determine the influence of LV3-shRNAPCGEM1 on cell proliferation (alone or in combination with baicalein) and established negative control. As shown in Figure 2A, LV3-shRNAPCGEM1 or baicalein alone significantly inhibited cell proliferation ($P<0.05$ as compared with NC controls) and LV3-shRNAPCGEM1 combined with baicalein effectively inhibited LNCaP cell proliferation ($P<0.05$ as compared with NC controls). Proliferation of cell LNCaP was negligible in the NC group.

Furthermore, our findings indicated that LV3-

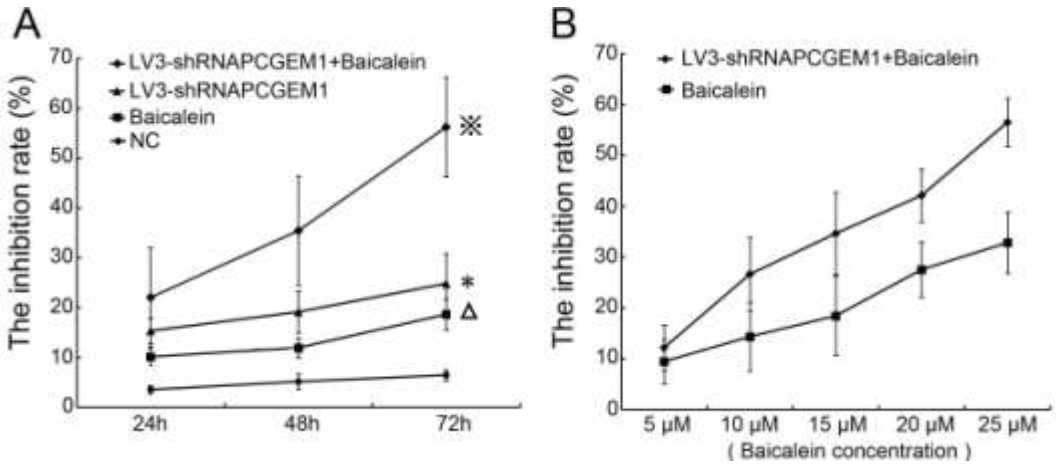


**Figure 2:** LV3-shRNAPCGEM1 promotes sensitivity of LNCaP cells to baicalein. (A) Effects on proliferation of LNCaP cells with NC, LV3-shRNAPCGEM1 and baicalein alone at certain concentrations, as well as, LV3-shRNAPCGEM1 combined with baicalein acting on LNCaP cells after 24, 48, and 72 h ($^{*\Delta※}P<0.05$ as compared with NC group); (B) Effects on proliferation of LNCaP cells with baicalein at different concentrations combined with LV3-shRNAPCGEM1 at certain concentration after 48 h.

shRNAPCGEM1 increased baicalein-induced inhibitory effects on LNCaP cells. As shown in Figure 2B, LV3-shRNAPCGEM1 significantly decreased the $IC_{50}$ values of baicalein. When used alone, the $IC_{50}$ of baicalein was 44.04 μM; in combination with LV3-shRNAPCGEM1, the $IC_{50}$ of baicalein was 19.4 μM. The increased susceptibility multiples was 2.3.

### LV3-shRNAPCGEM1 combined with baicalein inhibits colony formation of LNCaP cells

In order to explore whether LV3-shRNAPCGEM1 combined with baicalein inhibits the colony formation of LNCaP cells, the effects of LV3-shRNAPCGEM1 (20 nM), baicalein (15 μM) alone, or LV3-shRNAPCGEM1 in combination with baicalein were investigated in LNCaP cells. After treatment for 10 days, cells were fixed and stained as shown in Figure 3. Baicalein, LV3-shRNAPCGEM1 alone, and LV3-shRNAPCGEM1 in combination with baicalein were shown to significantly inhibit the colony formation ability of LNCaP cells (N=198±19, 135±16, and 68±8, respectively; $P<0.05$ compared with control). The results indicate that LV3-shRNAPCGEM1 combined with baicalein inhibits the colony formation of LNCaP cells more strongly (Figure 3A and B).

### Effects of LV3-shRNAPCGEM1 combined with baicalein on cell cycle of LNCaP cells

To explore the effects of LV3-shRNAPCGEM1 or LV3-shRNAPCGEM1 combined with baicalein on the cell cycle, LV3-shRNAPCGEM1 (20 nM) treatment alone or in combination with baicalein (15 μM) was investigated in LNCaP cells. Cells were stained with PI solution. Cell cycles were analyzed by flow cytometry according to DNA content. As shown in Figure 4, LV3-shRNAPCGEM1 and baicalein were able to independently induce G1 phase arrest ($P<0.05$). Furthermore, decreased G2 ($P<0.05$) and S ($P<0.05$) phase cells indicated that both could induce apoptosis in LNCaP cells.

### LV3-shRNAPCGEM1 combined with baicalein down-regulates expression of PCGEM1

The potential mechanism of LV3-shRNAPCGEM1 combined with baicalein in the inhibition of LNCaP cell proliferation was subsequently explored. LNCaP cells were processed and analyzed for mRNAs. PCGEM1 expression was determined by quantitative real-time PCR. U6 were used as the internal controls. The fold-change for PCGEM1 expression level was calculated using $2^{-\Delta\Delta CT}$, as earlier described. Figure 5 shows that LV3-shRNAPCGEM1 (20 nM) or baicalein (15 μM) alone down-regulated the relative expression levels significantly ($P<0.05$ compared with controls). These results showed that LV3-shRNAPCGEM1 combined with baicalein inhibits the expression of PCGEM1.

### LV3-shRNAPCGEM1 combined with baicalein induces autophagy of LNCaP cells

Autophagy is an evolutionarily conserved process of protein degradation associated with tumor promotion and tumor suppression in different situations. The microtubule-associated protein 1 light chain 3 (LC3), a homolog of yeast Atg8, is present on isolated autophagosomal membranes. The amount of LC3-II correlates well with the number of

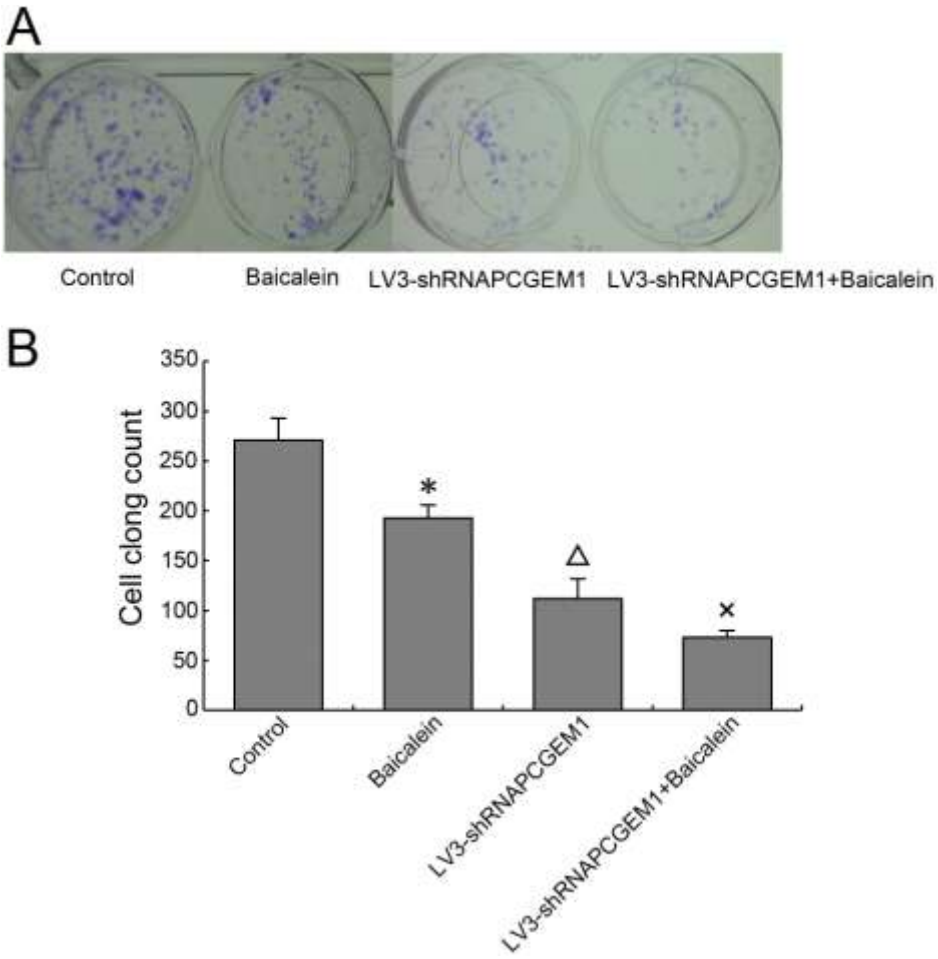


**Figure 3:** Effect of LV3-shRNAPCGEM1 combined with baicalein on the formation of LNCaP cells. (A) Clone formed by each group of cells in the well plate; (B) Colony count of each group of cells.

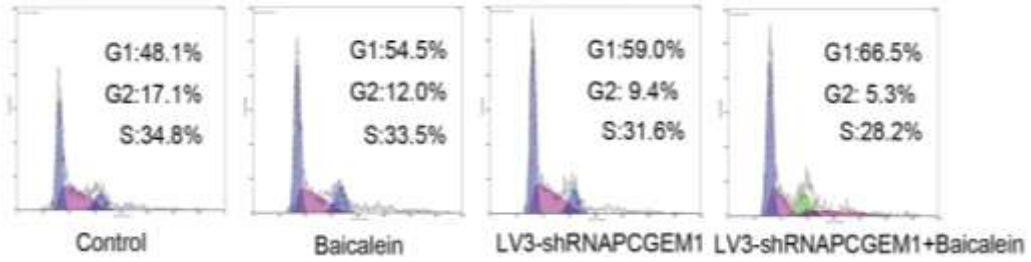


**Figure 4:** LV3-shRNAPCGEM1 combined with baicalein affects cell cycles in LNCaP cells. Cell cycles were analyzed by flow cytometry 48 h post-transfection. The results indicated that both LV3-shRNAPCGEM1 and baicalein decreased G2 and S phase cells.

autophagosomes. Enhancement of conversion of LC3-I to LC3-II, and up-regulation of LC3 expression occurs when autophagy is induced (Thorburn, 2018; Deretic and Levine, 2018).

Protein p62, also known as SQSTM1, has a short region that interacts with LC3. p62 participates in autophagy and is degraded in autolysosomes (Lamark et al., 2017). Conversion of LC3-I to LC3-II and p62 degradation yields two reliable markers of autophagy. To determine whether LV3-shRNAPCGEM1 induces autophagy of LNCaP cells, LNCaP cells were treated with 20 nM LV3-shRNAPCGEM1 or 15 μM baicalein for 24 h, and thereafter, stained with anti-LC3 antibody. Morphologic changes such as enhanced fluorescence aggregation in cells by fluorescence confocal microscopy were monitored, suggesting the occurrence of autophagy (Figure 6A). After LNCaP cells were treated with

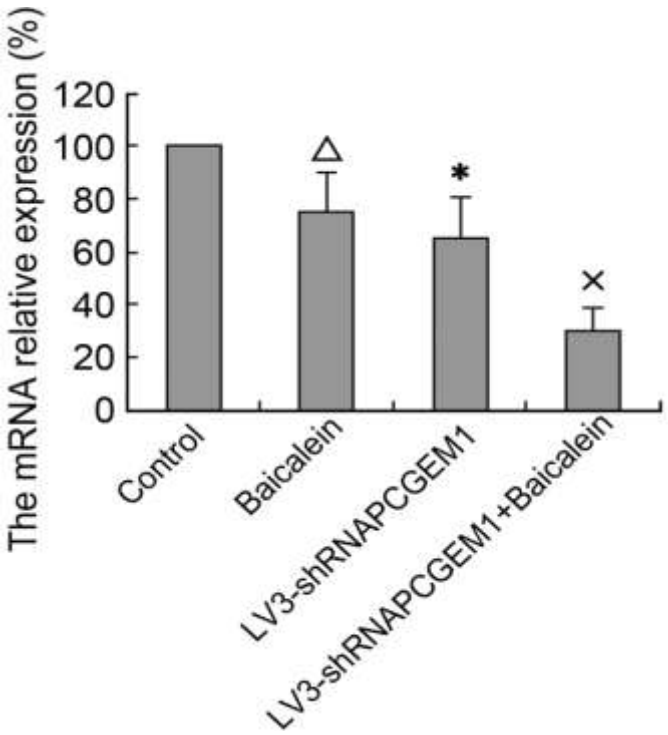


**Figure 5:** Effects of LV3-shRNAPCGEM1 combined with baicalein on the relative expression levels of PCGEM1. Total RNA from treated LNCaP cells was extracted in Trizol and quantified by ultraviolet spectrophotometry at 48 h after transfection. The PCGEM1 mRNA relative expression level was assessed by SYBR-Green real-time PCR. The data showed that alone or combined use down-regulates PCGEM1 mRNA expression in LNCaP cells $^{\Delta *\times}P<0.01$ as compared with the control groups.

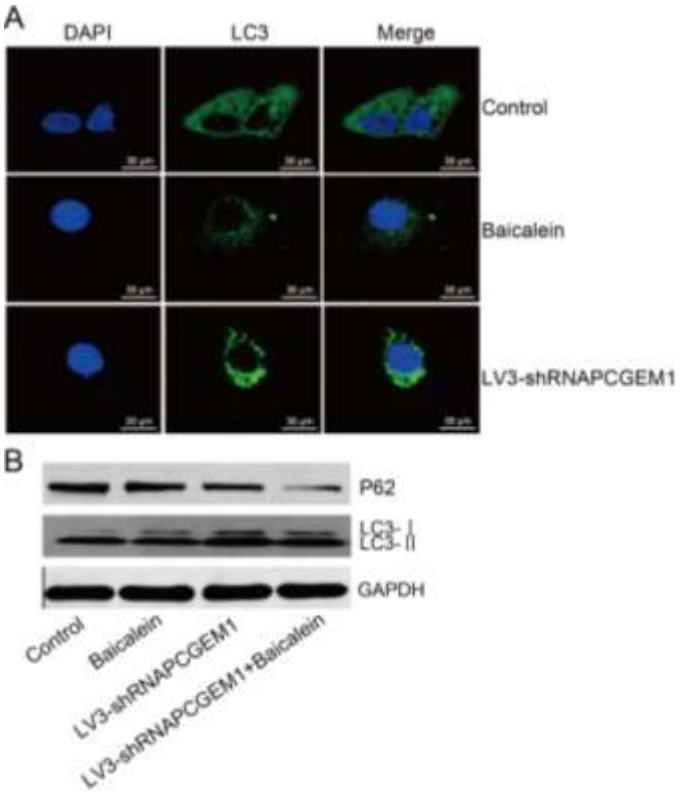


**Figure 6:** LV3-shRNAPCGEM1 combined with baicalein induced cell autophagy in LNCaP cells. LNCaP cells were treated with 20 nM or baicalein 15 μM for 24 h, and then stained with anti-LC3 antibody. Cells were examined by fluorescence confocal microscopy. Green: FITC-labeled LC3; Blue: DAPI-labeled nucleus. Magnification: × 400. (A) LNCaP cell morphologic changes by fluorescence confocal microscopy; (B) the expression of LC3 and p62 was performed by Western blotting and LNCaP cells were treated with LV3-shRNAPCGEM1 (20 nM), baicalein 15 μM or LV3-shRNAPCGEM1 combined with baicalein for 48 h. GAPDH was used as a loading control.

certain concentrations of LV3-shRNAPCGEM1, baicalein, or LV3-shRNAPCGEM1 combined with baicalein, the levels of LC3 and p62 expression were measured by immunoblotting. Figure 6B shows that expression of LC3-II increased, while p62 protein decreased in LNCaP cell lines. These results show that LV3-shRNAPCGEM1 combined with baicalein induces autophagy of LNCaP cells.

## DISCUSSION

Long non-coding RNAs (lncRNAs) are non-coding RNA molecules that do not encode proteins and have transcript length >200 nucleotides. LncRNAs account for 98% of total RNAs and can regulate gene expression by epigenetic regulation, transcriptional regulation, and post-transcriptional regulation. Moreover, they are involved in genome modification, transcriptional activation, transcriptional interference, chromosome silencing and medication, and other processes etc (Saha et al., 2017). At present, it is thought that their mechanism of action includes interfering with the expression of downstream gene, influencing the transgenesis of encoding proteins, and regulating protein functions (Wang et al., 2017).

Clinical observations and experimental studies have shown that lncRNAs play an important role in the occurrence and development of tumors of urinary system, normal tissues and tumor tissues which differ significantly in their expression of lncRNAs (Terracciano et al., 2017). LncRNA expression also varies at different stages of the same tumor (Smolle et al., 2017). PCGEM1 is the recognized lncRNA; it does not express protein products and is found in LNCaP, DU145, and PC3 prostate cancer cell lines (Hirsch et al., 2015). PCGEM1 with the length of 16 kb is at chromosome 2q32, which is the specific gene of prostate tissue and is closely associated with the occurrence of prostate cancer. Thus, PCGEM1 is also known as a prostate cancer-related gene (Ho et al., 2016). In NIH3T3 cells, the expression of PCGEM1 leads to cell proliferation. The increase of PCGEM1 expression in prostate cancer cells may promote cell proliferation (Srivastava et al., 2004), while the decrease may affect the proliferation of prostate cancer cells. The expression vectors encoding shRNAs are used to

generate shRNAs, realizing the purpose of RNAi (RNA interfering), which is a common method used in studies.

In this study, small hair RNA (shRNA) for PCGEM1 was directionally connected to the pGLV3/H1/GFP+Puro vector plasmid. In addition, recombinant, packaging, and envelope plasmids were co-transfected into 293T cells for 72 h. Viruses were then collected and the titer was determined to be $2 \times 10^9$ TU/ml (Figure 1A and B). The constructed lentiviral vector (LV3-shRNAPCGEM1) infected LNCaP cells. Figure 1C shows that when MOI=50, the infection efficiency was higher than 95% after 4 days. These results indicate that the stable cell line of LNCaP cells infected with lentiviral vector (LV3-shRNAPCGEM1) was successfully constructed.

Compared with the chemotherapeutics of Western medicine, antineoplastic traditional Chinese medicine is characterized by low cost, low toxicity, and few side effects. Therefore, traditional Chinese medicine is promising in the treatment of tumors. The most studied antitumor treatment in traditional Chinese medicine is baicalein. Baicalein is a monomer extracted from radix *Scutellariae* with the molecular formula of $C_{15}H_{1005}$ and the molecular weight of 270.24 (Chiu et al., 2011). Baicalein may significantly inhibit the proliferation as well as, invasion and metastasis of human breast cancer MDA-MB-231 cells (Wang et al., 2015).

In the present study, baicalein in combination with LV3-shRNAPCGEM1 at certain concentrations was used on LNCaP cells of prostate cancer, and was shown to effectively inhibit the proliferation of LNCaP cells and colony formation as well as, inducing apoptosis. Moreover, in combination with LV3-shRNAPCGEM1 at certain concentrations, LV3-shRNAPCGEM1 may improve the sensitivity of LNCaP cells to baicalein. The increased susceptibility factor was 2.3 (Figure 2B).

In the present study, the molecular mechanism by which LV3-shRNAPCGEM1 improves the sensitivity of LNCaP cells to baicalein was studied. LNCaP cells were processed and analyzed for mRNAs. Expression was determined by quantitative real-time PCR. The results showed that LV3-shRNAPCGEM1 combined with baicalein inhibits the expression of PCGEM1 (Figure 5). At the same time, we monitored morphologic changes such as enhanced fluorescence aggregation in cells by fluorescence confocal microscopy, which suggested the occurrence of autophagy (Figure 6A). It was observed that expression of LC3-II increased, whereas that of p62 protein decreased in LNCaP cell lines (Figure 6B). LNCaP cells were treated with certain concentrations of LV3-shRNAPCGEM1, baicalein, or LV3-shRNAPCGEM1 combined with baicalein. Our results show that LV3-shRNAPCGEM1 combined with baicalein induces autophagy of LNCaP cells. The existing molecular mechanism may be associated with the decrease of PCGEM1 expression and the induction of autophagy. Furthermore, PCGEM1 may inhibit the apoptosis of LNCaP cell line of prostate cancer induced by adriamycin, thus, delaying the expression of p53 and p21 cancer suppressor genes (Fu et al., 2006).

Other studies showed that curcumin may inhibit the invasion and metastasis of HOTAIR-mediated renal carcinoma and the mechanism may be curcumin-induced decreased HOTAIR expression in renal carcinoma cells (Pei et al., 2014). Traditional Chinese medicine in combination with lncRNAs may become a new antineoplastic treatment trend.

In conclusion, the analysis carried out indicated that LV3-shRNAPCGEM1 at a certain concentration may improve the sensitivity of LNCaP cells to baicalein in this study. The existing molecular mechanism may be associated with decreased PCGEM1 expression and induction of autophagy. There is need to further evaluate their combination on the action and possible mechanisms in animal solid tumors. Results obtained provided an experimental basis for combined treatment with Chinese traditional and Western medicine on prostate cancer in a clinical setting, a modality that may help in alleviating the pain and the economic burden faced by patients. PCGEM1 may be a target of baicalein action.

## ACKNOWLEDGEMENTS

The present study was supported by grants from Administration of Traditional Chinese Medicine of Guangdong Province (No. 20192073 and No. 20151057), the Guangzhou medicine and health care technology project (No. 20191A011119), the Guangzhou Health and Family Planning Commission Program (No. 20181A011118), the National Natural Science Foundation of Guangdon Province (No. 2018A0303130191), the Medical and Health Science and Technology Project of Panyu District, Guangzhou (No. 2017-Z04-18, and No. 2018-Z04-59), and the Science and Technology Planning Project of Guangdong Province (No. 2017ZC0372).

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