MEF2-activated long non-coding RNA PCGEM1 promotes cell proliferation in hormone-refractory prostate cancer through downregulation of miR-148a

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Abstract. Prostate cancer gene expression marker 1 (PCGEM1) is a prostate-specific gene overexpressed in prostate cancer cells that promotes cell proliferation. To study the molecular mechanism of PCGEM1 function in hormone-refractory prostate cancer, the interaction between myocyte enhancer factor 2 (MEF2) and PCGEM1 was assessed by a luciferase reporter assay and chromatin immunoprecipitation (ChIP) assay. In addition, the underlying mechanism of PCGEM1 regulating expression of microRNA (miR)-148a in PC3 prostate cancer cells was evaluated. Relative expression levels were measured by reverse transcription-quantitative polymerase chain reaction, and early apoptosis was measured by flow cytometry. PCGEM1 was demonstrated to be overexpressed in prostate cancer tissues compared with noncancerous tissues. Expression levels of PCGEM1 in PC3 cancer cells were demonstrated to be regulated by MEF2, as PCGME1 mRNA was increased by MEF2 overexpression but decreased by MEF2 silencing. MEF2 was also demonstrated to enhance the activity of PCGEM1 promoter and thus promote PCGEM1 transcription. In addition, downregulation of PCGEM1 expression in PC3 cells increased expression of miR-148a. By contrast, overexpression of PCGEM1 decreased miR-148a expression. Finally, PCGME1 silencing by small interfering RNA significantly induced early cell apoptosis but this effect was reduced by a miR-148a inhibitor. In conclusion, the present study demonstrated a positive regulatory association between MEF2 and PCGEM1, and a reciprocal negative regulatory association between PCGEM1 and miR-148a that controls cell apoptosis. The present study, therefore, provides new insights into the mechanism of PCGEM1 function in prostate cancer development.

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Introduction

Long non-coding RNAs (lncRNAs) are untranslated transcripts longer than 200 nucleotides that structurally resemble mRNAs but do not encode proteins (1). They are composed of several typical mRNA structural characteristics, including a polyA tail, 5'-capping, and a promoter structure (2). Previous studies have revealed that lncRNAs have important effects on the regulation of gene expression at the epigenetic, transcriptional, and post-transcriptional level (3). In addition, it has been revealed that lncRNAs display important roles in a number of physiological and pathological processes, including the pathogenesis of human cancers (1,2). Aberrant expression of lncRNAs is correlated with tumorigenesis through several distinct modes of action (4). It is hypothesized that the regulation of lncRNAs is a crucial part of tumorigenesis, however, the details of lncRNAs regulation and their mechanisms of action in specific cancers remain unclear (4).

Recent studies have demonstrated that microRNAs, small noncoding RNAs comprising of 20-22 nucleotides, function as oncogenes or as tumor suppressors; they inhibit cell proliferation by binding to mRNA sequences and preventing their translation (5-9). Given the structural similarities with mRNAs, miRNAs could also potentially target lncRNAs, suggesting a novel mode of regulatory interactions between noncoding RNA families (10). In addition, the reciprocal regulation of lncRNAs and miRNAs has been correlated with tumor invasion and metastasis (2). For example, both miR-31 and its host gene lncRNA LOC554202 were downregulated in triple-negative breast cancer lines (11). The loss of miR-31 expression was demonstrated to be mediated by the hypermethylation of its promoter-associated CpG islands (11). Mitochondrial dynamic related lncRNAs (MDRL) inhibit mitochondrial fission and apoptosis by directly binding to miR-361 and downregulating its expression, which in turn relieves miR-361-mediated inhibition of miR-484 processing (12). The lncRNA urothelial carcinoma-associated 1 (UCA1) functions by directly binding to miR-216b and downregulating miR-216b expression. In addition, UCA1 downregulates fibroblast growth factor receptor 1 (FGFR1) expression to reverse the inhibitory effect of miR-216b on the growth and metastasis of human hepatocellular carcinoma cells (13). Several studies have demonstrated

that miRNA-148a inhibits cell proliferation and promotes cell apoptosis in pancreatic (14), colorectal (15), bladder (16), ovarian (17), gastric (18), and hepatocellular carcinoma (19).

Prostate cancer is the most common malignancy afflicting men in the United States and the second leading cause of cancer mortality (20). Prostate cancer gene expression marker 1 (PCGEM1), a prostate-specific gene, is a novel class of androgen-regulated lncRNAs (2). Previous studies have revealed that elevated expression of PCGEM1 is associated with high-risk prostate cancer (21,22). PCGEM1 was expressed exclusively or in higher levels in primary prostate tumor specimens than in matched normal tissues. In addition, PCGEM1 expression was detected exclusively in the androgen receptor-positive cell line LNCaP among various prostate cancer cell lines analyzed (20).

The myocyte enhancer factor 2 (MEF2) profoundly influences cell differentiation, proliferation and metastasis (23,24). MEF2 directly binds to muscle A-kinase anchoring protein (mAKAP) which leads to inhibition of MEF2 activation during the early stages of muscle cell differentiation (23). In addition, class I myosin-epididymal binding protein 1 (E12) heterodimers interact with MEF2, resulting in the activation of myogenesis. However, homodimers of E12 do not interact with MEF2 due to lack of the conserved alanine and threonine residues in the basic domain. The interaction between the myogenic basic helix-loop-helix and MEF2 is uncoupled from transcriptional activation (25). A gene expression analysis study of the prostate cancer cell line LNCaP demonstrated that MEF2 is differentially expressed following exposure to androgen (26). MEF2 transcription factors binding site is present in the promoter of co-expressed genes (26).

To date, the effect of MEF2 on PCGEM1 regulation remains unclear. Furthermore, functional analysis of lncRNAs PCGEM1 potential interactions with miRNAs is warranted by previous studies. Therefore, in the present study, the regulatory interaction of MEF2 with PCGEM1, and of PCGEM1 with miR-148a were explored. The results indicated novel insights in the function of PCGEM1 on prostate cancer cells.

Materials and methods

Experimental sample collection. 60 random cases of prostate cancer and adjacent tumor-free prostate cancer tissue specimens were collected between April 2016 and November 2016 at Ji'nan Central Hospital Affiliated to Shandong University (Ji'nan, China). All cases were confirmed by pathological diagnosis, and the surgery during which specimens were obtained was the first surgical treatment in each case. No chemotherapy, radiotherapy or other treatments for prostate cancer were performed prior to surgery. All tissues were placed immediately in liquid nitrogen and stored in the central laboratory of Ji'nan Central Hospital. All experiments were approved by the Medical Ethics Committee of Ji'nan Central Hospital and written informed consent documents were signed by all patients. The large samples of prostate cancer tissue and tumor surrounding tissue were processed, and RNAs were extracted for sequencing analysis. Using an Illumina HiSeq sequencing platform (Illumina, Inc., San Diego, CA, USA), the sequencing data were obtained, the quality of the original sequencing data was evaluated, and processed to obtain the clean reads. The fragments per kilobase of transcript per million mapped reads (FPKM) method was used for quantitative analysis, and the prostate cancer tissue samples were used for analysis of differential gene expression (27).

Cell culture and treatments. LNCaP, DU145, and PC-3 prostate cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). PrEC normal human prostate epithelial cells were obtained from Clonetics (Lonza, Basel, Switzerland) and cultured as recommended by the supplier. All cell lines were cultured in RPMI 1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% (v/v) fetal bovine serum (FBS; HyClone; GE Healthcare Life Sciences, Logan, UT, USA), and were maintained at 37˚C in a humidified atmosphere of 5% CO_2. PC-3 cells ($1.5x10^4$ cells/well) were seeded into 96-well plates. The slow-growing LNCaP cells were seeded at a density of $2.0x10^4$ cells/well into 96-well plates. Cells were cultured to attach to the wells for 24 h.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The tissue samples were ground to powder by a frozen tissue pulverizer. The cells were harvested following wash with PBS twice and 12,000 x g centrifugation for 15 min at 4˚C. The harvested cells were resuspended in a solution containing 4 M guanidine isothiocyanate, 100 mM β-mercaptoethanol, 25 mM sodium citrate pH 7.0 and 0.5% sarcosyl. Total RNA extraction was performed as previously described (26). The quality of total RNA was analyzed by 1% agarose gel electrophoresis and ethidium bromide staining. qPCR was performed according to the manufacturer's protocol of the PCR kit (cat. no. 0960211; Beijing Kuangbo Biotechnology Co., Ltd., Beijing, China). qPCR reactions were performed using a preheated 7500 RT-PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) with SYBR-Green detection. Reaction conditions were as follows: Stage 1, 95˚C for 30 sec; stage 2, 40 cycles of 95˚C for 5 sec and 60˚C for 34 sec; and stage 3 (dissolution curve), 95˚C for 15 sec, 60˚C for 1 min and 95˚C for 15 sec. The primers for miR-148a were designed by Applied Biosystems (Thermo Fisher Scientific, Inc.). Other primers were as follows: PCGEM1, 5'-tccacccaatacacaggat-3' (forward), 5'-aattgggagctgatgaggac-3' (reverse); U6, 5'-agcttcggcagcacatatactaaaattggaat-3' (forward), 5'-tcttcacgaatttgcgtgtcatccttga-3' (reverse); β-actin, 5'-aaactggaacggtgaaggtg-3' (forward) and 5'-agagaagtggggtggctttt-3' (reverse). The relative level of miR-148a was normalized to U6, and the relative amount of PCGEM1 was normalized to β-actin. The data were analyzed using the $2^{-\Delta\Delta Cq}$ method (28).

Western blot analysis. Cells were lysed by radio immunoprecipitation assay buffer containing protease inhibitor cocktail (Beyotime Institute of Biotechnology, Haimen, China). The total protein concentration was determined using a bicinchoninic acid protein assay kit (Shanghai Haoyang Biological Technology Co., Ltd., Shanghai, China), and were subjected to 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes with Western Blocking Buffer (100 μg; Shanghai Yansheng Industrial Co., Ltd, Shanghai, China). Proteins were electrophoretically resolved on 8-10% Tris-Glycine gels and transferred onto a nitrocellulose membrane. After blocking the non-specific binding sites with 5% skimmed milk for 1 h at 20˚C, the membrane was incubated with the primary antibody

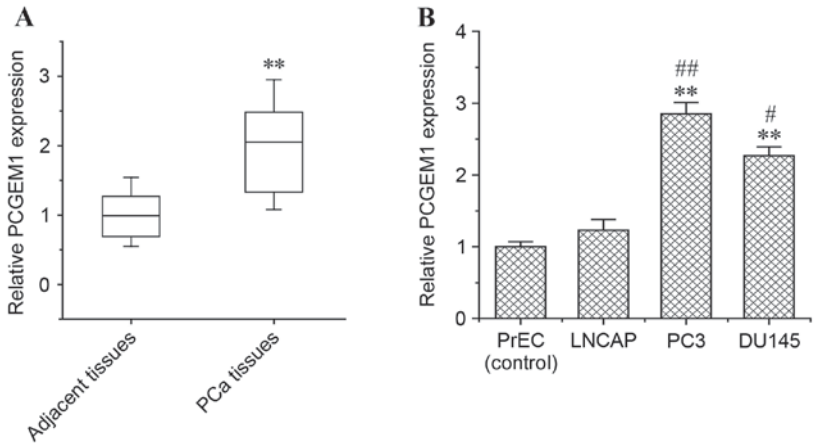

Figure 1. PCGEM1 mRNA expression in prostate tumor tissues and cell lines. (A) Relative PCGEM1 mRNA expression levels in prostate cancer tissues and in adjacent non-cancerous tissues (control). (B) Relative expression of PCGEM1 in the normal prostate epithelial cell line PrEC (control) and three prostate cancer cell lines LNCaP, PC3 and DU145. **P<0.01 vs. control. $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. LNCAP. PCGEM1, prostate cancer gene expression marker 1; PCa, prostate carcinoma.

(1:800 dilution; cat. no. ADI-950-100-0001; Enzo Life Sciences, Inc., Farmingdale, NY, USA) at 4˚C overnight. The membranes were then incubated with a horseradish peroxidase-conjugated secondary antibody (1:800 dilution; rabbit secondary antibodies, 6 vials; cat. no. NB910-95603; Novus Biologicals, LLC, Littleton, CO, USA) for 1 h at 20˚C. Immunoreactive proteins were detected with the ECL Plus western blotting Detection System (GE Healthcare Life Sciences, Little Chalfont, UK) and exposed to X-ray film. The samples were analyzed using enhanced chemiluminescence (cat. no. 320002; Best Biotechnology Co., Ltd., Shanghai, China) and quantified using an image analyzer (LabWorks LLC, Lehi, UT, USA). The density of the bands on the membrane was quantified using Quantity One software (version 4.62; Bio-Rad Laboratories, Inc., Hercules, CA, USA). β-actin (1:2,000 dilution; cat. no. A01010; Abbkine Scientific Co., Ltd., Wuhan, China) was used as a control.

Cell transfection and luciferase reporter assay. Human MEF2-directed and PCGEM1-directed small interfering RNA (siRNA) (cat. no. sc-29528) and a non-specific control siRNA (cat. no. sc-29533) were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). PC-3 cells were seeded one day prior to transfection. siRNA (100 nm) was transfected into the cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The medium was replaced by complete RPMI 1640 medium 3 h post-transfection and cultured for another 48 h. Following transfection, cells were harvested after 12,000 x g centrifugation for 15 min at 4˚C and analyzed for mRNA and protein expression, using the aforementioned RT-qPCR and western blotting protocols. For transfection of the pcDNA3.1 expression vector, PC3 cells were cultured in a mixture of Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12, 1:1) (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), supplemented with 10% FBS (HyClone; GE Healthcare Life Sciences) and 25 μg/ml gentamicin (Gibco; Thermo Fisher Scientific, Inc.). PC3 cells were transfected with pcDNA3.1-MEF2 plasmid or pcDNA3.1-PCGEM1 plasmid (Shanghai Kyrgyzstan Biological Technology Co., Ltd., Shanghai, China) and control cells were transfected with pcDNA3.1 empty vector. Stable transfectants were selected by adding 400 μg/ml of G418 (Geneticin) in the medium. Individual colonies were picked and maintained in RPMI-1640 media enriched with 5% FBS, penicillin-streptomycin and 200 μg/ml of G418 (29). Luciferase activity was measured using the dual luciferase reporter assay system kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's instructions, on a Tecan M200 luminescence reader (Tecan Group, Ltd. Zurich, Switzerland).

Chromatin immunoprecipitation (ChIP) assay. ChIP assay was carried out as previously described (30). Native protein-DNA complexes were cross-linked by 1% formaldehyde treatment for 10 min. Equal aliquots of isolated chromatin were used for immunoprecipitation with specific antibodies (cat. no. FHP004-100, FHP003-100 and FHP003-050; Beijing Zheng Bo Biological Technology Co., Ltd., Beijing, China). DNA associated with specific immunoprecipitates or with mouse immunoglobulin G as a negative control was isolated and used as a template for PCR amplifying the PCGEM1 promoter sequence containing the MEF2 binding site (30).

Apoptosis assay. PC3 cells were seeded for 24 h in 24-well plates and transfected with non-specific control siRNA or PCGEM1 siRNA, in the presence (5 nmol/l) or absence of miRNA-148a inhibitor (cat. no. M101; Shanghai Tuoran Biotechnology Co., Ltd., Shanghai, China), in serum free RPMI-1640 for 5 h. Following transfection, each well was supplemented with 500 μl of appropriate growth medium containing 20% FBS. PC3 cells ($5x10^5$) were harvested after incubating for another 48 h, via 12,000 x g centrifugation for 10 min and washed with PBS at room temperature. Cells were then stained with Annexin V-fluorescein isothiocyanate (10 μM; SouthernBiotech, Birmingham, AL, USA) and 50 μg/ml propidium iodide (PI) for 1 h at 25˚C and analyzed by flow cytometry. All the experiments were performed at least three times.

Statistical analysis. Values are presented as the means + standard deviation. Statistical significance analysis between two groups was carried out by the student's t-test using

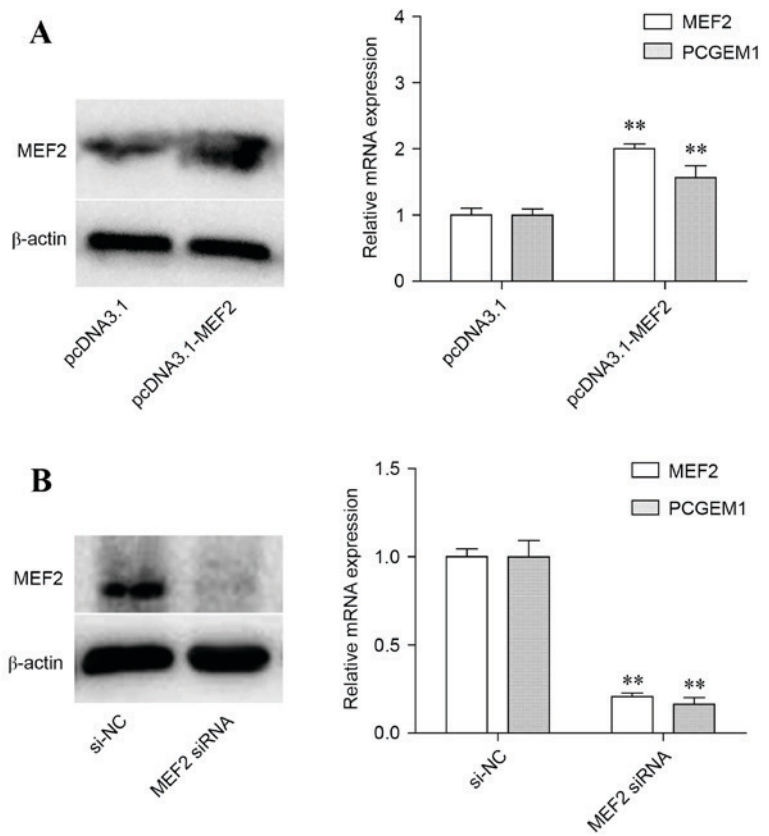

Figure 2. Effect of MEF2 on PCGEM1 expression. MEF2 protein and mRNA expression and PCGEM1 mRNA expression were analyzed in (A) PC3 cells that were stably transfected with either empty vector control pcDNA3.1 or pcDNA3.1-MEF2 and (B) PC3 cells transfected for 48 h with either non-targeting control siRNA (si-NC) or MEF2 siRNA. β-actin was used in western blots as an internal control. **P<0.01 vs. control. MEF2, myocyte enhancer factor 2; PCGEM1, prostate cancer gene expression marker 1; siRNA, small interfering RNA.

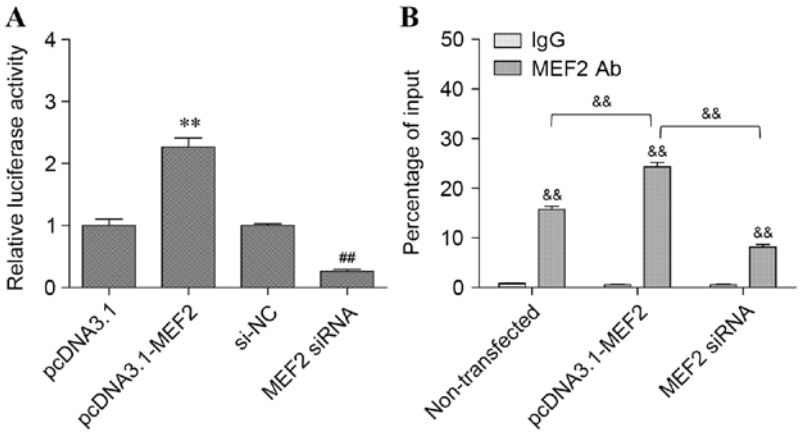

Figure 3. MEF2 binds and activates the PCGEM1 promoter. (A) Relative luciferase activity driven by the PCGEM1 promoter was measured in PC3 cells that were stably transfected with either empty vector control pcDNA3.1 or pcDNA3.1-MEF2, and in PC3 cells that were transfected for 48 h with either non-targeting control siRNA (si-NC) or MEF2 siRNA. (B) PC3 cells were transfected with either pcDNA3.1-MEF2 vector or MEF2 siRNA and analyzed by ChIP assay to measure enrichment of PCGEM1 promoter sequences. IgG was used as a negative control for the MEF2 antibody. **P<0.01 vs. pcDNA3.1. $^{\#\#}$P<0.01 vs. si-NC. $^{\&\&}$P<0.01 vs. IgG control, or as indicated by brackets. MEF2, myocyte enhancer factor 2; PCGEM1, prostate cancer gene expression marker 1; siRNA, small interfering RNA; ChIP, chromatin immunoprecipitation; IgG, immunoglobulin; Ab, antibody.

GraphPad Prism software version 4.0 (GraphPad Software, Inc., La Jolla, CA, USA). Significance among multiple groups was analyzed by one-way analysis of variance followed by Tukey's test, using DPS software (31). Significance analysis for the clinical samples was performed by Wilcoxon rank sum test. P<0.05 was considered to indicate a statistically significant difference.

Results

PCGEM1 is overexpressed in prostate tumor tissues and cell lines. To determine the difference in PCGEM1 expression in prostate cancer vs normal prostate, semi-quantitative RT-PCR analysis was performed in prostate cancer tissue samples and adjacent non-cancerous tissue samples (Fig. 1A). PCGEM1

mRNA expression was significantly higher in prostate carcinoma tissues than in the adjacent non-cancerous tissues (P<0.01; Fig. 1A), which is consistent with a previous report demonstrating that PCGEM1, a prostate specific gene, is overexpressed in prostate cancer (20). In addition, PCGEM1 mRNA expression was investigated in a normal prostate epithelial cell line (PrEC), in the LNCaP hormone-sensitive prostate cancer cell line, and in the PC3 and DU145 hormone-refractory prostate cancer cell lines. PCGEM1 mRNA expression was significantly higher in PC3 and DU145 cells compared with LNCaP cells and with the normal PrEC cells (Fig. 1B). In addition, PC3 cells expressed the highest levels of PCGEM1 mRNA among the cell lines tested (Fig. 1B). Thus, the PC3 cell line was chosen for further examinations of the function of PCGEM1 in prostate cancer.

MEF2 effect on PCGEM1 expression. MEF2 expression was evaluated at the mRNA and protein level in PC3 cells following transfection with pcDNA3.1-MEF2 overexpression vector or with MEF2 siRNA, in order to confirm successful overexpression or silencing respectively. PC3 cells transfected with empty pcDNA3.1 vector or non-targeting siRNA were used as the respective controls. As expected, MEF2 mRNA and protein levels were significantly increased by pcDNA3.1-MEF2 transfection compared with control (Fig. 2A). Similarly, MEF2 mRNA and protein expression was markedly decreased following MEF2 siRNA transfection, compared with control (Fig. 2B). The mRNA expression levels of PCGEM1 were then analyzed. The results demonstrated that PCGEM1 mRNA expression was significantly increased by MEF2 overexpression (Fig. 2A), but significantly decreased by MEF2 silencing (Fig. 2B), suggesting that MEF2 regulated expression of PCGEM1.

MEF2 effect on PCGEM1 promoter activity. Previous studies have indicated that MEF2 is differentially expressed by androgen exposure and that MEF2 binding sites are present in the promoters of co-expressed genes (26). Therefore, it was hypothesized that MEF2 could regulate PCGEM1 expression by directly interacting with its promoter and driving gene transcription. To test this hypothesis, pcDNA3.1 and pcDNA3.1-MEF2 vectors were transfected into PC3 cells to induce MEF2 overexpression, and transcription efficiency by the PCGEM1 promoter was determined by luciferase assay (Fig. 3A). The results demonstrated that MEF2 overexpression increased PCGEM1 promoter activity ~2.0-2.5-fold compared with control (P<0.01; Fig. 3A). By contrast, promoter activity was significantly reduced in PC3 cells transfected with MEF2 siRNA compared with control (P<0.01; Fig. 3A).

To validate the existence of MEF2 binding sites on the PCGEM1 promoter, ChIP analysis was used to evaluate its enrichment. As expected, PCGEM1 promoter sequences were highly enriched in PC3 cells immunoprecipitated with the MEF2 specific antibody compared with IgG control (P<0.01; Fig. 3B). To determine whether PCGEM1 enrichment was dependent on MEF2, ChIP analysis was repeated following MEF2 overexpression or silencing (Fig. 3B). The results indicated that enrichment of PCGEM1 promoter in pcDNA3.1-MEF2 transfected cells was increased by ~2-fold compared with control cells (P<0.01; Fig. 3B). In addition, PCGEM1 enrichment was significantly decreased in MEF2 siRNA transfected cells compared with control (P<0.01; Fig. 3B). In conclusion, the present results

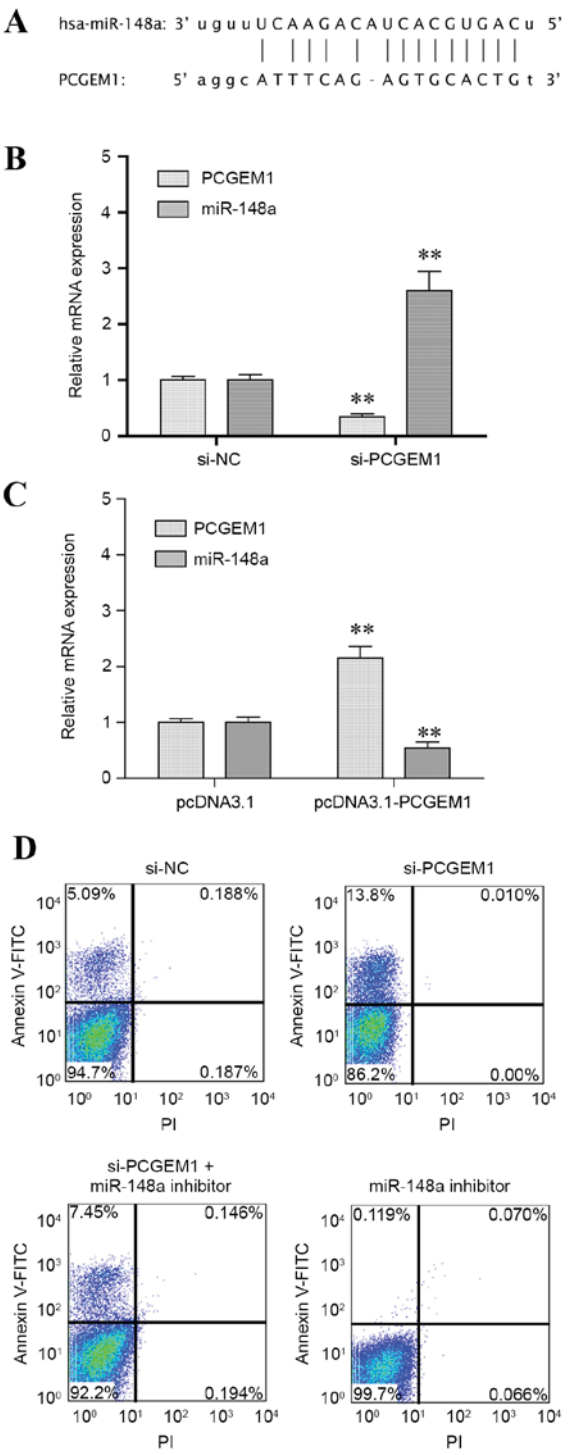

Figure 4. Identification of miR-148a as a target of PCGEM1. (A) Alignment of potential PCGEM1 and miR-148a complementary sequences, as identified by RegRNA analysis. (B) PC3 cells were transfected for 48 h with either non-targeting control siRNA (si-NC) or PCGEM1 siRNA (si-PCGEM1) and mRNA expression of PCGEM1 and miR-148a was analyzed. (C) PC3 cells were transfected with either empty vector control pcDNA3.1 or pcDNA3.1-PCGEM1 and mRNA expression of PCGEM1 and miR-148a was analyzed. (D) PC3 cells were transfected with either non-targeting control siRNA (si-NC) or PCGEM1 siRNA (si-PCGEM1), in the absence or presence of miR-148a inhibitor (1 μg/ml). The percentage of apoptotic cells was 13.8% in the si-PCGEM1 group, 0.119% in the mRNA-148a inhibitor group and 7.45% in the si-PCGEM1 + mRNA-148a inhibitor group, which were compared with the si-NC group (5.09%). Flow-cytometric analysis of apoptosis was performed following staining for PI (x axis) and Annexin V-FITC (y axis). *P<0.05 and **P<0.01 vs. control. miR, microRNA; PCGEM1, prostate cancer gene expression marker 1; siRNA, small interfering RNA; PI, propidium iodide; FITC, fluorescein isothiocyanate.

indicated that MEF2 activated the lncRNA PCGEM1 expression via targeting its promoter.

Identification of miR-148a as a target of PCGEM1. To elucidate the molecular mechanism underlying the role of PCGEM1 in prostate cancer cells, the effect of PCGEM1 in regulation of miR-148a expression was analyzed. Based on the web-based RegRNA analysis for prediction of functional RNA motifs (32), a putative PCGEM1 binding site was identified in the 5' untranslated region (UTR) of miR-148a (Fig. 4A). To confirm the regulation of miR-148a by PCGEM1, PC3 cells were transfected with PCGEM1 siRNA and miR-148a expression was analyzed. Expression of miR-148a was significantly increased following PCGEM1 silencing compared with control ($P<0.05$; Fig. 4B). By contrast, when PCGEM1 was overexpressed, miR-148a expression was significantly downregulated compared with control ($P<0.05$; Fig. 4C). The results indicated a negative regulation between PCGEM1 and miR-148a. Finally, the effect of PCGEM1 and miR-148a on PC3 cell apoptosis was evaluated by flow cytometry (Fig. 4D). The results demonstrated that the number of early-stage apoptotic cells was increased in the PCGEM1 siRNA-transfected cells compared with control (13.8% vs. 5.09%, respectively; Fig. 4D). However, when cells were additionally treated with a miR-148a inhibitor, the cell apoptosis rate was reduced compared with PCGEM1 siRNA transfection alone treatment (7.45% vs. 13.8%, respectively; Fig. 4D).

Discussion

The present study demonstrated that MEF2-induced activation of PCGEM1 altered the apoptosis rate in PC3 cancer cells by downregulating miR-148a. PCGEM1 has been demonstrated to serve a role in castration-resistant proliferation of cancer cells (33). It has been reported that expression of the lncRNA PCGEM1 is significantly higher in prostate cancer tissues of African-American patients (33). In the present study, it was demonstrated that this prostate cancer-associated noncoding RNA gene was also highly expressed in prostate cancer tissues of Asian patients. PCGEM1 expression was significantly elevated in tumor tissues compared with non-cancerous tissues, which was in accordance with a previous report (20).

Although the number of reports related to lncRNAs has sharply risen in recent years, their role in enhancing cell growth and promoting cell proliferation still remains to be elucidated (34-36). The putative PCGEM1 promoter contains a 5' flanking region. The interaction between ligand-induced enhancer and promoter is impaired by depletion of PCGEM1 (37).

Based on previous bioinformatics analyses, MEF2 transcription factor binding sites are present in the promoters of co-expressed genes (38). In addition, various members of the MEF2 family of transcription factors have been detected in diverse cell types and display an important regulatory role in cell development and differentiation (39). Proteins of the MEF2 family are calcium-dependent regulators of cell division, differentiation and death (40). The regulatory function of MEF2 in accelerating myeloid leukemia has also been confirmed. However, its role in multiple human cancers remains largely unknown (41). In the present study, the interaction between MEF2 and PCGEM1 was assessed and MEF2 was demonstrated to positively regulate PCGEM1 expression by targeting the PCGEM1 promoter. MEF2 directly bound to the promoter of PCGEM1 and enhanced its activity. Taken together, MEF2 regulated the expression of PCGEM1 at the mRNA level by activating transcription. Further studies will be required to fully explore their reciprocal regulation and the underlying mechanisms.

Of note, miR-148a has been identified as a tumor suppressor in human cancer cell lines (40). Its expression was significantly reduced in the PC3 and DU145 hormone-refractory prostate cancer cell lines compared with the PrEC normal prostate epithelial cell line and the LNCaP hormone-sensitive prostate cancer cell line (42). Using the prediction analysis RegRNA software (43) and an online prostate cancer genomic database (cbio.mskcc.org/cancergenomics/prostate/data) (44,45), a complementary sequence of miR-148a was identified against the 5'-UTR of PCGEM1. In order to understand the regulation of miR-148a expression by PCGEM1, their interaction was further examined following PCGEM1 overexpression or silencing. RT-qPCR results revealed that PCGEM1 silencing in PC3 cells significantly elevated miR-148a expression. By contrast, PCGEM1 overexpression in PC3 cells resulted in miR-148a downregulation, indicating that miR-148a expression was regulated by a PCGEM1-dependent mechanism. Apoptosis of PC3 cancer cells was also evaluated by flow cytometry. PCGEM1 silencing increased the number of PC3 apoptotic cells, while simultaneous treatment with a miR-148a inhibitor reduced cell apoptosis. Thus, downregulation of miR-148a mediated by the lncRNA PCGEM1 may be a potential strategy promoting cell proliferation in prostate cancer cells.

In conclusion, the present study demonstrated a reciprocal regulation between MEF2 and PCGEM1. MEF2 enhanced the activity of the PCGEM1 promoter and upregulated PCGEM1 expression. Furthermore, the tumor-promoting lncRNA PCGEM1 promoted downregulation of the tumor suppressor miR-148a, resulting in reduced cell apoptosis in PC3 prostate cancer cells. In conclusion, it was demonstrated that lncRNA PCGEM1 and miR-148a may be novel biomarkers and targets for the early prevention and treatment of prostate cancer.

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