**INTRODUCTION**

Prostate cancer (PCa) is a big health threat for men worldwide with increasing incidence (Castillejos-Molina & Gabilondo-Navarro, 2016). What is worse, the pathogenesis of PCa remains largely unknown, which results in the lack of treatment options, leading to worse overall survival of PCa patients (Murillo-Garzón & Kypta, 2017). Hence, further investigations are needed to understand the mechanisms behind PCa progression.

Noncoding RNAs (ncRNAs) including microRNA (miRNA), long noncoding RNA (lncRNA), and circular RNA (circRNA) have been revealed to function as crucial roles in PCa progression (Greene et al., 2019; Kanwal, Plaga, Liu, Shukla, & Gupta, 2017; Wu, Xiao, Zhou, Zhou, & Yan, 2019). Increasing evidence suggested that ncRNAs can be developed as biomarkers for prognosis prediction and treatment (Greene et al., 2019; Kanwal et al., 2017; Wu et al., 2019). miRNAs are short RNAs (18–24 nucleotides in length) without protein-coding capability (Ha & Kim, 2014). Dual functions of miRNAs in cancers have been reported, as some miRNA can promote cancer development, while some of them can inhibit tumorigenesis (Suzuki, Maruyama, Yamamoto, & Kai, 2013). By 3′-untranslated region (3′-UTR) binding, miRNAs can regulate multiple gene expressions, associated

**MIR-138-5P inhibits the progression of prostate cancer by targeting FOXC1**

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Abstract

**Background:** Studies have suggested that micro-RNAs (miRNAs) can function as an oncogene or a tumor suppressor in cancers. However, the role of **MIR-138-5P** (613394) in prostate cancer (PCa) remains unclear.

**Methods:** Expression level of **MIR-138-5P** in PCa cell lines and normal cell line was analyzed with the quantitative real-time PCR method. Cell counting kit-8 assay, colony formation assay, wound-healing assay, and transwell invasion assay were performed to analyze the biological functions of **MIR-138-5P**.

**Results:** We showed **MIR-138-5P** expression level was significantly decreased in PCa cell lines compared with the normal cell line. Overexpression of **MIR-138-5P** inhibits PCa cell proliferation, colony formation, cell migration, and cell invasion in vitro. Mechanistically, we showed Forkhead box C1 (**FOXC1**, 601090) was a direct target for **MIR-138-5P** in PCa. We confirmed that overexpression of **FOXC1** partially reversed the effects of **MIR-138-5P** on PCa cell behaviors.

**Conclusions:** Collectively, we showed that **MIR-138-5P** functions as a tumor suppressor gene in PCa via targeting **FOXC1**.

**KEYWORDS**

**FOXC1**, **MIR-138-5P**, prostate cancer, tumor suppressor gene
signaling pathways, and eventually affect the hallmarks of cancer (Acuño, Romano, Wernicke, & Croce, 2015).

**MIR-138-5P** (613394, NC_000003.12) is an miRNA that reported to function as a tumor suppressive role in several human cancers. For instance, **MIR-138-5P** was revealed to be a decreased expression in colorectal cancer, and its low expression was significantly correlated with advanced tumor stage and poor overall survival (Zhao et al., 2016). Additionally, programmed cell death ligand 1 was identified to be a decreased expression in colorectal cancer, and its low expression was significantly correlated with advanced tumor stage and poor overall survival (Zhao et al., 2016).

**2.2 | Cell treatment**

**MIR-138-5P** mimic (5'-AGCUGGUGUGUGAAUCAGGC CG-3') and the corresponding negative control (NC-miR, 5'-GGGUUGUGUGUCAUGUAUA-3') were synthesized by RiboBio Inc. **MIR-138-5P** overexpression was accompanied by transfecting **MIR-138-5P** mimic into PCA cell lines using Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific, Inc.). pcDNA3.1 contains the coding sequence of FOXC1 (pcFOXC1) and an empty vector were purchased from GenScript. Transfection was also conducted using Lipofectamine 2000 using the manufacturer's instruction.

**2.3 | Quantitative real-time PCR (RT-qPCR)**

RNA from the cells was isolated with TRIZOL reagent (Beyotime). After concentration determination, RNA was reverse transcribed into complementary DNA with PrimeScript RT Reagent (Takara). RT-qPCR was conducted at ABI 7500 system (Applied Biosystems) using SYBR Green Mix (Takara). The method of 2−ΔΔCt was used to measure the relative expression level of **MIR-138-5P** or FOXC1 using U6 small nuclear RNA (U6 snRNA, 180962) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 138400) as endogenous control. Primers used were as follows: **MIR-138-5P**: Forward: 5'-GCGAGCTGGTGTTGTGAATC-3', Reverse: 5'-AGTGCA TTCGGCAGCACA-3'; **FOXC1**: Forward: 5'-CGGTATCCAGCCAGTC GGTTGTTGTGAATC-3', Reverse: 5'-AGTGCA TTCGGCAGCACA-3'; **U6 snRNA**: Forward: 5'-CTCGC TTCGGCAGCACA-3', Reverse: 5'-AACGCTTCACGAATT TCCGAT-3'; **GAPDH**: Forward: 5'-CGGTATCCAGCCAGTC TTCGGCAGCACA-3', Reverse: 5'-GCTTGTTGTTGAGGGTG TC-3'; **MIR-138-5P** mimic: 5'-AGCUGGUGUGUGAAUCAGGC CG-3', Reverse: 5'-GGGUUGUGUGUCAUGUAUA-3'. Experiments were repeated in triplicate.

**2.4 | Cell counting kit-8 (CCK-8) assay**

Cells in the density of 1 x 10⁴ cells/well were seeded into 96-well plates. After 0, 24, 48, or 72 hr incubation, CCK-8 reagent purchased from Beyotime was filled into the plate and further incubated for 4 hr. Optical density in each well was analyzed at a wavelength of 450 nm. Experiments were repeated in triplicate.
Cells were incubated into 6-well plate at the density of 800 cells/well. Colonies generated from the cultured cells were fixed with methanol, stained with crystal violet, and then counted under the microscope. Experiments were repeated in triplicate.

2.6 | Cell migration assay

Cells were seeded in 6-well plates and incubated until about 80% confluence. The pipette tip was used to create a wound at the cell surface. Thereafter, the cells were washed with PBS three times to remove cell debris. After incubation for 0 or 48 hr, cell images were captured under the microscope to evaluate the effects of MIR-138-5P or FOXC1 expression on cell migration. Experiments were repeated in triplicate.

2.7 | Cell invasion assay

In this experiment, $1 \times 10^5$ cells in serum-free medium were filled into the upper chamber of the Matrigel-coated insert.
The lower chamber was filled with medium contains FBS. After incubation for 48 hr, noninvasive cells were removed with cotton. Then, invasive cells were fixed with methanol, stained using crystal violet, and counted under microscope. Experiments were repeated in triplicate.

2.8 | Bioinformatic analysis

TargetScan was used to detect the putative targets for MIR-138-5P. Among all these targets, FOXC1 was selected for the following analyses.

2.9 | Luciferase activity reporter assay

To validate the direct connection of MIR-138-5P and FOXC1, luciferase activity reporter assay was performed. The wild-type (wt) or mutant (mt) 3’-UTR sequence of FOXC1 was inserted into psiCHECK-2 to obtain wt-FOXC1 or mt-FOXC1 luciferase vectors. For the luciferase activity reporter assay, cells were co-transfected with luciferase vectors and miRNAs using Lipofectamine 2000. After transfection for 48 hr, relative luciferase activity was measured with a dual-luciferase reporter kit (Promega) with Renilla luciferase activity as the internal control. Experiments were repeated in triplicate.

2.10 | Statistical analyses

Data were obtained from three independent experiments and then expressed as mean ± SD after analyses using GraphPad Prism 6.0 (GraphPad Software). Differences in groups were assessed with Student’s t test or one-way ANOVA and Tukey post hoc test. p value less than .05 was considered as a statistically significant difference.

3 | RESULTS

3.1 | Decreased expression of MIR-138-5P in PCa cells

To explore the function of MIR-138-5P, we first analyzed its expression level in PCa cell lines and in the normal cell line. We found the MIR-138-5P expression level was significantly decreased in PCa cells compared with the normal cell line (Figure 1).

3.2 | MIR-138-5P overexpression inhibits PCa cell growth, migration, and invasion

We then detected the roles of MIR-138-5P overexpression on PCa cells. Introduction of MIR-138-5P mimic significantly
increased MIR-138-5P levels in PCa cells (Figure 2a). CCK-8 assay and wound-healing assay revealed that the overexpression of MIR-138-5P inhibits PCa cell proliferation and colony formation (Figure 2b,c). Moreover, we found in PCa cells transfected with MIR-138-5P mimic cell migration and invasion abilities were significantly inhibited (Figure 2d,e).

### 3.3 MIR-138-5P can interact with FOXC1

Subsequently, we are interested to explore the targets of MIR-138-5P using TargetScan. We found FOXC1 was a putative target for MIR-138-5P (Figure 3a). Luciferase activity reporter assay validated the direct connection of MIR-138-5P and the 3’-UTR of FOXC1 (Figure 3b). The RT-qPCR analysis results indicated that overexpression of MIR-138-5P could inhibit the expression level of FOXC1 in PCa cells (Figure 3c).

### 3.4 MIR-138-5P regulates PCa cell malignancy behaviors via targeting FOXC1

To clarify the relationship between MIR-138-5P and FOXC1, rescue experiments were conducted. We showed pcFOXC1 transfection increased the levels of FOXC1 in PCa cell (Figure 4a). In addition, we found the effects of MIR-138-5P mimic on FOXC1 expression can be reversed by pcFOXC1 (Figure 4a). In vitro functional experiments showed that overexpression of FOXC1 promoted PCa cell proliferation, colony formation, cell migration, and cell invasion (Figure 4b–e). Moreover, we showed that overexpression of FOXC1 partially abolished the effects of MIR-138-5P mimic on PCa cell behaviors (Figure 4b–e).
4 | DISCUSSION

miRNAs were reported to play crucial roles in the prevention or promotion of cancer progression (Acunzo et al., 2015; Suzuki et al., 2013). The miRNAs that promote carcinogenesis are the oncomiRs, while those can inhibit tumorigenesis were termed as tumor suppressive miRNAs. To date, numerous miRNAs have been identified to be aberrantly expressed in the development of PCa. For example, MIR-301A-3P (615675) was revealed to be an elevated expression in PCa tissues along with several cell lines (Fan, Wang, Huo, & Wang, 2019). In addition, they found PCa cell proliferation and invasion can be stimulated by MIR-301A-3P through regulating the expression of runt-related transcription factor 3 (Ray et al., 2019). Besides that, MIR-138-5P was revealed to be an elevated expression in PCa tissues along with several cell lines (Fan, Wang, Huo, & Wang, 2019). MicroRNA-301a-3p overexpression promotes cell invasion and proliferation by targeting runt-related transcription factor 3 in prostate cancer. Molecular Medicine Reports, 20(4), 3755–3763. https://doi.org/10.3892/mmr.2019.10650

In this study, we showed that MIR-138-5P expression was decreased in PCa cell lines compared with the normal cell line. Previous studies demonstrated that MIR-138-5P regulates cancer migration, invasion, and epithelial–mesenchymal transition (Zhao, Ling, Li, Hou, & Zhao, 2019). Hence, we also explored the role of MIR-138-5P on cell behavior. Here we showed the restored expression of MIR-138-5P inhibits PCa cell proliferation, colony formation, cell migration, and cell invasion, indicating that MIR-138-5P functions as a tumor suppressive in PCa. Our work presented here is similar to the role of MIR-138-5P presented in other cancer types (Pan et al., 2019; Tian et al., 2017; Zhao, Zhao, et al., 2019). Several targets for MIR-138-5P have been identified in previous studies, which has helped us to understand the role of MIR-138-5P in human cancers. Hence, we also explored the potential target for MIR-138-5P in this work. Combining the results of bioinformatic analysis, luciferase activity reporter assay, and RT-qPCR analysis, we found FOXC1 was a putative target for MIR-138-5P. Functionally we showed that the overexpression of FOXC1 could promote PCa cell malignancy behavior. Importantly, rescue experiments found overexpression of FOXC1 could partially reverse the effects of MIR-138-5P on PCa cells. This work provides novel evidence regarding the mechanisms behind the progression of PCa, which could provide novel targets for cancer treatment. However, the limitation in this work was that we did not explore the function of MIR-139-5P/FOX1 axis in animal model, which we believe should be performed in the future.

Collectively, our work established the tumor suppressive role of MIR-138-5P in PCa. FOXC1 was identified as the novel target for MIR-138-5P, through which MIR-138-5P exerts the inhibitory effects on PCa cells. The validated MIR-138-5P and FOX1 axis could help us to develop novel targets for PCa treatment.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.
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