INTRODUCTION

Papillary thyroid carcinoma (PTC) is the most common (>90%) and least malignant thyroid cancer.\(^1,2\) It can develop at any age, and frequently happens to children and young women (before 40 years old). Although PTC grows slowly and is usually confined to the thyroid gland for several years, the lesions can spread from the primary site to other parts of the gland and cervical lymph nodes through the lymphatic system. It is essential to understand the underlying mechanisms to develop effective therapeutic strategies.
lymphatic vessels, leading to metastasis and poor prognosis. The incidence of PTC is increasing year by year. Investigating the mechanisms of PTC occurrence and progression may facilitate the diagnosis and treatment of patients with PTC.

In recent years, microRNAs (miRNAs) have attracted the huge attention of scientists. MiRNAs are the most abundant non-coding RNAs. They only have ~20 nucleotides. It has been well known that miRNAs can post-transcriptionally and negatively regulate the expression of target mRNAs through base pairing to the 3′-untranslated regions (UTRs). Tremendous studies have demonstrated that numerous miRNAs are involved in tumorigenesis and progression. For example, the interest miRNA in the present study, miR-203a-3p showed abnormal expression in different cancer types, such as epithelial ovarian cancer, prostate cancer, gastric cancer and non-small cell lung cancer (NSCLC), and contribute to multiple steps of cancer development. However, the function of miR-203a-3p in PTC and the underlying mechanisms remain not completely understood. The aim of our study is to prove the activity of miR-203a-3p in PTC and to uncover the possible molecular mechanisms. The mitogen-activated protein kinase (MAPK) pathway is frequently activated in cancerous cells. MAP3K1, a MAPK family member has been reported to have multiple functions in cell viability, cell death, epithelial–mesenchymal transition (EMT) and metastasis in tumors.

In the present study, following the investigation of the influence of miR-203a-3p on cell proliferation, metastatic ability, cell death and cell cycle of PTC cell lines, we further proved the direct interaction between MAP3K1 and miR-203a-3p, and the effect of MAP3K1 on cell death, viability, metastatic ability and cell cycle could be rescued by miR-203a-3p. Finally, it was demonstrated that the interest miR-203a-3p might exert its tumor-suppressive role through activating cell autophagy in PTC. Overall, our study uncovers a novel mechanism underlying the regulatory activities of miRNA-203a-3p.

2 | MATERIAL AND METHODS

2.1 | Tissue samples

From February 2017 to March 2018, we collected 54 PTC tissue pairs including the corresponding vicinal healthy tissues from Ningbo HwaMei Hospital. The detailed pathological parameters of patients with PTC were included in Table S1. The preparation and dealing with all the human specimens in the study were approved by the Ethics Review Board of the Ningbo HwaMei Hospital.

2.2 | Cell cultivation and transfection

Healthy thyroid cell line, Nthy-ori3-1, as well as five PTC cell lines: TPC-1, KTC-1, B-CPAP, 8505C and SW1736 were acquired from the Cell Bank/Stem Cell Bank (Shanghai, China) and cultured in DMEM medium containing 10% fetal bovine serum (FBS), penicillin and streptomycin in an incubator with 10% CO2 at 37°C. MiR-203a-3p mimic/inhibitor, siMAP3K1, and the corresponding negative controls were purchased from GenePharma Company, and delivered to TPC-1 and KTC-1 cells using Lipofectamine 2000 reagent (Thermo Fisher Scientific).

2.3 | cDNA synthesis and quantitative real-time polymerase chain reaction

RNAs were firstly isolated using the traditional TRIzol reagent, and quantified on a NanoDrop 2000 device (Thermo Fisher Scientific). Subsequently, for the quantification of miRNAs, cDNAs were synthesized with the M-MLV reverse transcriptase (Promega), while for the quantification of miRNAs, cDNAs were synthesized with TaqMan microRNA Reverse Transcription Kit (Applied Biosystems). Regarding quantitative real-time polymerase chain reaction (qRT-PCR), the primers of miRNA-203a-3p and the endogenous control U6, MAP3K1 and the housekeeping gene GAPDH (shown in Table S2) were purchased from Beijing Genomics Institution. The qRT-PCR system was prepared and run on a Mastercycler Gradient as previously described. The relative levels of miR-203a-3p and MAP3K1 were calculated by the 2−ΔΔCq method.

2.4 | Cell proliferation assay

In brief, TPC-1 or KTC-1 cells were transfected with miR-203a-3p mimic/inhibitor or siMAP3K1 or the corresponding negative controls in 96-well plates. Transfected cells were treated with CCK-8 solution at 4 points (24, 48, 72, and 96 h). The absorbance at 450 nm was measured with a microplate reader.

2.5 | Cell apoptosis assay

Cell apoptosis assay was conducted with the annexin V-FITC/PI apoptosis detection kit (Becton). Briefly, transfected TPC-1 or KTC-1 cells were washed with cold 1× PBS, and then resuspended with binding buffer, and finally stained with PI and Annexin V-FITC reagents in dark. The dead cells were analyzed by a flow cytometer (Beckman Coulter).

2.6 | Cell cycle evaluation

To check the influence of miR-203a-3p mimic/inhibitor and siMAP3K1 on the process of the cell cycle of TPC-1 and KTC-1 cells, cell cycle evaluation was carried out using the PI/RNase staining reagent (Becton), and were detected with a flow cytometer (Beckman Coulter) as previously described.
2.7 | Migration and invasion assays

For migration assay, TPC-1 and KTC-1 cells were transfected with miR-203a-3p mimic/inhibitor or siMAP3K1, 24 h later, the wound was created by the yellow pipette tip on 6-well plates. After incubation in an FBS-free medium for 24h, the wound was photographed. For invasion assay, transfected TPC-1 and KTC-1 cells were loaded into the upper chamber of the Transwell inserts pre-coated with and without Matrigel (BD Biosciences). The upper chamber contained an FBS-free medium, while the lower chamber contained a medium with 20% FBS. After 48 h, the invasive cells were stained with 0.1% crystal violet and were further photographed by an optical microscope.

2.8 | Protein isolation and western blot

To quantify interested protein levels, cellular proteins were first lyzed with the radioimmunoprecipitation lysis buffer. For the detection of protein expression by western blot, 20 µg of lysed cellular proteins, 10% of sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and polyvinylidene difluoride membrane, anti-rabbit secondary antibody (ab97051), anti-mouse secondary antibody (115-035-003E) and enhanced chemiluminescent substrate (Beyotime) were used. The specific procedure of western blot was as described previously. The primary antibodies used for western blot include anti-MAP3K1 (ab224628), anti-GAPDH (ab9485), anti-Bcl-2 (ab182858), anti-Bax (ab32503), anti-CDK4 (ab108357), anti-cyclin D1 (ab16663), anti-Bcl-2 (ab32124), anti-MMP-2 (#40994), anti-MMP-9 (#13667), anti-vimentin (#46173), anti-LC3 (A7198), and anti-Beclin-1 (A17028). Finally, an infrared imaging system (LI-COR) was used to visualize the signal bands. The gray-scale values of all signal bands from the western blot were evaluated by Image J 1.8.

2.9 | Luciferase reporter test

To confirm the direct interaction between miR-203a-3p and MAP3K1, the 3′-UTRs of MAP3K1-wild-type or MAP3K1-mutant were firstly cloned into pmirGLO vectors (Promega). Subsequently, TPC-1 and KTC-1 cells were co-transfected with constructed pmirGLO vectors along with miR-203a-3p mimic or inhibitor. Twenty-four hours later, the Dual-Luciferase Reporter Assay kit (Promega) was used to detect the luciferase activity as well as the Renilla luciferase activity for normalization.

2.10 | Statistical analyses

The differences between subgroups were tested by Student’s t-test or analysis of variance (ANOVA) with Tukey’s honestly significant difference test. Two-way ANOVA with Post Hoc test was used for CCK-8 data analyses. Pearson’s correlation analysis was used for association evaluation. *p < 0.05 and **p < 0.01 were considered as statistically significant.

3 | RESULT

3.1 | MiR-203a-3p was downregulated in PTC

With the purpose of exploring the function of miR-203a-3p in PTC, qRT-PCR was firstly carried out to quantify the levels of miR-203a-3p in 54 PTC tissue samples and the corresponding vicinal healthy tissues. The relative expression levels of miR-203a-3p in PTC tissue samples were notably lower than those in the vicinal healthy tissue samples (p < 0.01; Figure 1A). Moreover, the relative expression levels of miR-203a-3p in the above five PTC cell lines were obviously downregulated compared to the healthy cell line Nthy-ori3-1, in particular in the cell lines of KTC-1 and TPC-1 (Figure 1B), therefore we chose these two PTC cell lines for further investigation. MiR-203a-3p mimic was synthesized to upregulate, whereas an inhibitor was used to downregulate the miR-203a-3p levels in the PTC cell lines. The bar charts in (Figure 1C,D) show the successful delivery of mimic and inhibitor into TPC-1 as well as KTC-1 cells.

3.2 | MiR-203a-3p inhibits the oncogenic characteristics of PTC

We carried out CCK8 assay, wound healing and transwell assays, as well as flow cytometry experiments to investigate the function of miR-203a-3p in the cell lines of KTC-1 and TPC-1. As shown in Figure 2A,B, we found that miR-203a-3p mimic suppressed cell viability, and in contrast, miR-203a-3p downregulation by inhibitor promoted cell viability. In addition, the upregulation of miR-203a-3p by mimic repressed the wound healing ability (Figure 2C) and invading ability (Figure 2D) of PTC cells, and the downregulation of miR-203a-3p by inhibitor exhibited an inverse effect (Figure 2C,D). Furthermore, we carried out the transwell assay without Matrigel, and consistent with the above results, we observed similar results in the two PTC cell lines (Figure S2A). For apoptosis assay with KTC-1 and TPC-1 cells, we found miR-203a-3p mimic increased the apoptotic rate, and inversely miR-203a-3p inhibition decreased the apoptotic rate (Figure 2E). For the cell cycle analysis (Figure 2F), miR-203a-3p mimic made more cells arrested at G2/M and S phases and fewer cells at G0/G1 phases, whereas miR-203a-3p inhibitor made increasing cells enter into G0/G1 phases and decreasing cells into G2/M and S phases. The above functional assays indicate that miR-203a-3p may behave as a tumor-suppressive regulator in PTC. Inspired by the above functional results, we decided to explore the underlying mechanisms of miR-203a-3p. Since cell apoptosis, EMT and cell cycle process are important events in tumor development, apoptosis-related proteins like Bcl-2 and Bax, EMT markers such as...
MMP-2, Vimentin and MMP-9, and cell cycle-linked proteins like cyclin D1 and CDK4 were selected to be quantified by western blot. We found that in TPC-1 (Figure 2G), the levels of MMP-2, Vimentin and MMP-9, as well as CDK4 and cyclin D1, were decreased by miR-203a-3p overexpression and in contrast increased by miR-203a-3p downregulation. However, for Bcl-2 and Bax, miR-203a-3p mimic upregulated the levels of Bax, but downregulated the levels of Bcl-2. And miR-203a-3p inhibitor showed an inverse effect (Figure 2G). Consistently, almost the same phenomenon was found in KTC-1 (Figure 2H). These changes in protein levels well explain that miR-203a-3p influences PTC development via regulating related proteins.

3.3 | MiR-203a-3p directly binds to MAP3K1

It has been known that miRNAs post-transcriptionally regulate target mRNAs and inhibit protein translation. Following the investigation of the function of miR-203a-3p in PTC cells, TargetScan7.2 (Figure 3A) was used to predict the potential interacting mRNA MAP3K1 of miR-203a-3p. Subsequently, we found that different from the signature of miR-203a-3p in PTC tissues, the mRNA levels of MAP3K1 were remarkably elevated (Figure 3B). Intriguingly, the relative expression of miR-203a-3p and MAP3K1 was negatively correlated in PTC tissues (Figure 3C). Moreover, the mRNA (Figure 3D) and protein (Figure 3E) levels of MAP3K1 were much higher than those in the healthy cell line Nthy-ori3-1. The miR-203a-3p mimic could downregulate, and inversely the miR-203a-3p inhibitor upregulate the mRNA (Figure 3F,G) and protein (Figure 3H,I) levels of MAP3K1. Furthermore, it was found that the luciferase activity of MAP3K1-wt but not MAP3K1-mut could be remarkably inhibited with the co-transfection of miR-203a-3p mimic, and inversely elevated by a miR-203 inhibitor (Figure 3J,K). These results suggest that miR-203a-3p directly interacts with MAP3K1.
3.4 | The influence of MAP3K1 on PTC cells can be reversed by miR-203a-3p

Following the determination of the direct interaction of MAP3K1 and miR-203a-3p, we downregulated the MAP3K1 level by siMAP3K1, and investigated the effect of MAP3K1 on PTC development. We found that siMAP3K1 inhibited cell proliferation and at the same time the inhibition could be reversed by miR-203a-3p inhibitor in TPC-1 (Figure 4A) and KTC-1 (Figure 4B) cells. Furthermore, migration (Figure 4C) and invasion (Figure 4D) were also suppressed in the siMAP3K1 groups, and this suppression was obviously reversed by miR-203a-3p downregulation by inhibitor. Furthermore, the transwell assay without Matrigel showed similar results in TPC-1 and KTC-1 cells (Figure 5B). For apoptosis assay, siMAP3K1 enhanced cell apoptosis and the simultaneous downregulation of miR-203a-3p inhibited cell apoptosis (Figure 4E). Additionally, similar to the miR-203a-3p mimic, siMAP3K1 arrested more cells at G2/M and S phases, and miR-203a-3p downregulation by inhibitor recovered this arresting phenomenon (Figure 4F). Correspondingly, the EMT-related proteins (MMP-2, MMP-9 and Vimentin), the cell cycle-linked proteins like cyclin D1 and CDK4, and apoptosis-related protein Bcl-2 were decreased by the knockdown of MAP3K1, and the decreasing influence was weakened by the introduction of miR-203a-3p inhibitor (Figure 4G,H). The above results indicate that the influence of MAP3K1 in PTC cells might be regulated by our interest in miR-203a-3p, which proves that MAP3K1 directly interacts with miR-203a-3p.

3.5 | MiR-203a-3p activates autophagy

Since it was reported that MAP3K1 was involved in cell autophagy, we further detected the levels of autophagy-related proteins, including LC3II/LC3I and Beclin-1. It was found that in TPC-1 (Figure 5A) the upregulation of miR-203a-3p by mimic elevated, and in contrast, miR-203a-3p inhibitor decreased the amounts of LC3II/LC3I and Beclin-1. Similar to miR-203a-3p mimic, siMAP3K1 elevated the amounts of LC3II/LC3I and Beclin-1, and miR-203a-3p inhibitor weakened the increasing of LC3II/LC3I, as well as Beclin-1 (Figure 5A). And the results from TPC-1 (Figure 5A) and KTC-1 (Figure 5B) were observed alike, implying that miR-203a-3p represses PTC development by negatively regulating MAP3K1 and affecting autophagy.

4 | DISCUSSION

In PTC, numerous miRNAs have been reported to behave as tumor-suppressive miRNAs or onco-miRNAs.20,21 MiR-203 was revealed to suppress PTC occurrence and progression in vitro and in vivo
by downregulating AKT3, and therefore regarded as a tumor-suppressive miRNA.\(^{22,23}\) Also, miR-599 was reported to facilitate apoptosis and suppress cell proliferation by inhibiting the Hey2-dependent Notch signaling cascade in PTC.\(^{24}\) Conversely, miRNA-146b-5p acts as an onco-miRNA to promote PTC development by targeting CCDC6 (coiled-coil domain containing 6).\(^{25}\) From these investigations, we can get a clue that one miRNA is defined as a tumor suppressor or an onco-miRNA depending on the tumor-related mRNAs it binds to.

MiR-203 has been reported as a tumor suppressor or an onco-miRNA in PTC and also other cancer types. However, to the best of our knowledge, until now, the function of miR-203a-3p in PTC and the underlying mechanisms are not completely understood. In line with previous studies, the levels of miR-203a-3p were significantly decreased in our PTC tissue samples compared to the paired normal adjacent tissues, and this result was further confirmed with the healthy thyroid cell line, Nthy-ori3-1, as well as five PTC cell lines. Furthermore, we analyzed the data of miR-203a-3p in thyroid cancer derived from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO), and found the levels of miR-203a-3p in the tissues of thyroid cancer were significantly lower than those in normal tissues (Figure S1). Inspired by this phenomenon, we further investigated the influence of miR-203a-3p on cell viability, metastatic ability, cell cycle and cell death. We found that miR-203a-3p played a tumor repressive role in PTC via inhibiting oncogenic behavior. These findings were consistent with those in other cancer types, such as ovarian carcinoma,\(^{26}\) hepatocellular carcinoma,\(^{27}\) lung adenocarcinoma\(^{28}\) and gastric cancer.\(^{29}\)

Since miRNAs play their tumor-suppressive or tumor-promoting roles via regulating their target mRNAs in cancers, we further characterized one miR-203a-3p target MAP3K1. It has been well known that MAP3K1 exerts an oncogenic effect on different cancers through activating the downstream MAPK kinases.\(^{30}\) Besides miR-203a-3p, several other miRNAs like miR-451 and miR-302 have been reported to target MAP3K1. For instance, miR-451 targeted and inhibited MAP3K1, leading to the repression of esophageal cancer.

**FIGURE 3** MAP3K1 directly interacts with miR-203a-3p. TargetScan 7.2 screened a potential target MAP3K1 of miR-203a-3p (A). The chart shows the MAP3K1 mRNA levels in 54 PTC tissue samples and corresponding healthy specimens (B). The relative expression of MAP3K1 and miR-203a-3p was negatively associated in PTC tissue samples (C). The charts show the mRNA (D) along with protein (E) expression of MAP3K1. The charts show the mRNA as well as protein changes of MAP3K1 in two cell lines of TPC-1 (F, H) and KTC-1 (G, I). The change in luciferase activity was shown in the bar charts after TPC-1 (J) and KTC-1 (K) cells were co-transfected with MAP3K1-wt or MAP3K1-mut and miR-203a-3p mimic/inhibitor.
FIGURE 4  The effect of MAP3K1 on PTC cells is reversed by miR-203a-3p. The curves show the proliferation of TPC-1 (A) and KTC-1 (B) cells. Migration abilities (C), invasive abilities (D), apoptosis (E) and cell cycle (F) were evaluated by siMAP3K1 and siMAP3K1 along with miR-203a-3p inhibitor. The protein levels of CDK4, cyclin D1, MMP-2, Vimentin and MMP-9, Bax and Bcl-2 were detected by western blot in different experimental subgroups of TPC-1 (G) and KTC-1 (H) cell lines.

FIGURE 5  Cell autophagy is activated by a miR-203a-3p mimic or siMAP3K1. The levels of autophagy-associated proteins such as Beclin-1 and LC3II/I were tested by western blot. The gray values of western blot bands in different experimental subgroups of TPC-1 (A) and KTC-1 (B) cells were measured.
We proved that miR-203a-3p could directly target MAP3K1, and further demonstrated that miR-203a-3p prevented the occurrence and development of PTC via negatively regulating its target MAP3K1. Most importantly, we firstly revealed that miR-203a-3p might prevent PTC via activating autophagy. Since one miRNA has several target mRNAs, and at the same time each mRNA has the potential to be targeted and regulated by multiple miRNAs, the miRNA-mRNA regulatory network is incredibly complicated. The present study just proved one possible target of miR-203a-3p, and unfolded one underlying mechanism about miR-203a-3p in PTC. Therefore, more studies should be performed to further investigate the mechanisms of miR-203a-3p in the occurrence and development of PTC and other cancer types.

In conclusion, miR-203a-3p may repress the occurrence as well as the progression of PTC by directly targeting MAP3K1 and activating autophagy.

AUTHOR CONTRIBUTIONS
Lei Dai conceived the study and prepared the data and the manuscript. Weidong Zhang and Xianjiang Wu conducted the experiments. Shuihong Zhou supervised the study.

CONFLICT OF INTEREST
No conflict of competing interest is declared.

DATA AVAILABILITY STATEMENT
The published article contains all data.

ORCID
Shuihong Zhou https://orcid.org/0000-0002-7163-2289

REFERENCES
1. Baloch ZW, LiVolsi VA. Special types of thyroid carcinoma. Histopathology. 2018;72(1):40-52.
2. Song Z, Yang H, Wu X, Kong C, Xu C. microRNA-564 inhibits the aggressive phenotypes of papillary thyroid cancer by directly targeting astrocyte-elevated gene-1. OncoTargets Ther. 2019;12:4869-4881.
3. Shi D, Wang H, Ding M, et al. MicroRNA-26a-5p inhibits proliferation, invasion and metastasis by repressing the expression of Wnt5a in papillary thyroid carcinoma. OncoTargets Ther. 2019;12:6605-6616.
4. Fröhlich E, Wahl R. The current role of targeted therapies to induce radiiodine uptake in thyroid cancer. Cancer Treat Rev. 2014;40(5):665-674.
5. Shi X, Liu R, Basolo F, et al. Differential clinicopathological risk and prognosis of major papillary thyroid cancer variants. J Clin Endocrinol Metab. 2016;101(1):264-274.
6. Shi Z, Zhou H, Lu L, et al. The roles of microRNAs in spinal cord injury. Int J Neurosci. 2017;127(12):1104-1115.
7. Moi L, Braaten T, Al-Shibli K, Lund E, Busund LR. Differential expression of the mir-17-92 cluster and mir-17 family in breast cancer according to tumor type: results from the Norwegian Women and Cancer (NOWAC) study. J Transl Med. 2019;17(1):334.
8. Jin J, Zhang J, Xue Y, Luo L, Wang S, Tian H. miRNA-15a regulates the proliferation and apoptosis of papillary thyroid carcinoma via regulating AKT pathway. Oncotargets and Ther. 2019;12:6217-6226.
9. Liu H, Chen X, Lin T, Chen X, Yan J, Jiang S. MicroRNA-524-5p suppresses the progression of papillary thyroid carcinoma cells via targeting on FOXE1 and ITGA3 in cell autophagy and cycling pathways. J Cell Physiol. 2019;234(10):18382-18391.
10. Chen J, Yin J, Liu J, Zhu RX, Zheng Y, Wang XL. MiR-202-3p functions as a tumor suppressor and reduces cell migration and invasion in papillary thyroid carcinoma. Eur Rev Med Pharmacol Sci. 2019;23(3):1145-1150.
11. Ye W, Deng X, Fan Y. Exosomal miRNA423-5p mediated onco- gene activity in papillary thyroid carcinoma: a potential diagnostic and biological target for cancer therapy. Neoplasma. 2019;66(4):516-523.
12. Zhang LS, Ma HG, Sun FH, Zhao WC, Li G. MiR-203 inhibits the malignant behavior of prostate cancer cells by targeting RGS17. Eur Rev Med Pharmacol Sci. 2019;23(13):5667-5674.
13. Li J, Zhang B, Cui J, Liang Z, Liu K. miR-203 inhibits the invasion and EMT of gastric cancer cells by directly targeting annexin A4. Oncol Res. 2019;27(7):789-799.
14. Wang B, Li X, Zhao G, et al. miR-203 inhibits ovarian tumor metastasis by targeting BIRC5 and attenuating the TGFβ pathway. J Exp Clin Cancer Res. 2018;37(1):235.
15. Xue YB, Ding MQ, Xue L, Luo JH. CircAGFG1 sponges miR-203 to promote EMT and metastasis of non-small-cell lung cancer by upregulating ZNF281 expression. Thoracic Cancer. 2019;10(8):1692-1701.
16. Chen Y, Ma X, Lou C, et al. PLAG10 incorporated in exosomes could be diagnostic and prognostic biomarker for non-small cell lung cancer. Clin Chim Acta. 2022;530:55-65.
17. Rupprom K, Chavalitshewinkoon-Petmitr P, Diraphat P, Kittigul L. Evaluation of real-time RT-PCR assays for detection and quantification of norovirus genogroups I and II. Virologica Sinica. 2017;32(2):139-146.
18. Zhu LW, Li Z, Yu XC, et al. The tRNA-derived fragment 5026a inhibits the proliferation of gastric cancer cells by regulating the PTEN/Pi3K/AKT signaling pathway. Stem Cell Res Ther. 2021;12(1):418-430.
19. Shen XT, Yang YF, Chen YF, et al. Evaluation of EpCAM-specific exosomal IncRNAs as potential diagnostic biomarkers for lung cancer using droplet digital PCR. J Mol Med. 2022;100(1):87-100.
20. Zembaska A, Jawiarczyk-Przybylowska A, Wojtczak B, Bolanowski M. MicroRNA expression in the progression and aggressiveness of papillary thyroid carcinoma. Anticancer Res. 2019;39(1):33-40.
21. Zhu G, Xie L, Miller D. Expression of MicroRNAs in thyroid carcinoma. Methods Mol Biol (Clifton, N. J.). 2017;1617:261-280.
22. You A, Fu L, Li Y, Li X, You B. MicroRNA-203 restrains epithelial-mesenchymal transition, invasion and migration of papillary thyroid cancer by downregulating AKT3. Cell Cycle (Georgetown, Tex). 2020;19(10):1105-1121.
23. Wu X, Dai L, Zhang Z, Zheng J, Zhao J. Overexpression of microRNA-203 can downregulate survivin and function as a potential therapeutic target in papillary thyroid cancer. Onco Lett. 2020;19(10):61-68.
24. Wang DP, Tang XZ, Liang QK, Zeng XJ, Yang JB, Xu J. microRNA-599 promotes apoptosis and represses proliferation and epithelial-mesenchymal transition of papillary thyroid carcinoma cells via downregulation of Hey2-dependent Notch signaling pathway. J Cell Physiol. 2020;235(3):2492-2505.
25. Jia M, Shi Y, Li ZY, Lu XB, Wang JX. MicroRNA-146b-5p as an onco-R promotes papillary thyroid carcinoma development by targeting CCDC6. Cancer Lett. 2019;443:145-156.
26. Zheng ZH, Wu DM, Fan SH, et al. LncRNA AB209371 up-regulated Survivin gene by down-regulating miR-203 in ovarian carcinoma. J Ovarian Res. 2019;12(1):92.
27. Zhu Y, Liu Y, Xiao B, et al. The circular RNA PVT1/miR-203/HOXD3 pathway promotes the progression of human hepatocellular carcinoma. *Biol Open*. 2019;8(9):3687-3694.

28. Ge X, Li GY, Jiang L, et al. Long noncoding RNA CAR10 promotes lung adenocarcinoma metastasis via miR-203/30/SNAI axis. *Oncogene*. 2019;38(16):3061-3076.

29. Gong P, Qiao F, Wu H, et al. LncRNA UCA1 promotes tumor metastasis by inducing miR-203/ZEB2 axis in gastric cancer. *Cell Death Dis*. 2018;9(12):1158.

30. Liu C, Wang S, Zhu S, et al. MAP3K1-targeting therapeutic artificial miRNA suppresses the growth and invasion of breast cancer in vivo and in vitro. *SpringerPlus*. 2016;5:11.

31. Zang WQ, Yang X, Wang T, et al. MiR-451 inhibits proliferation of esophageal carcinoma cell line EC9706 by targeting CDKN2D and MAP3K1. *World J Gastroenterol*. 2015;21(19):5867-5876.

32. Zhao L, Wang Y, Jiang L, et al. MiR-302a/b/c/d cooperatively sensitizes breast cancer cells to adriamycin via suppressing P-glycoprotein (P-gp) by targeting MAP/ERK kinase kinase 1 (MEKK1). *J Exp Clin Cancer Res*. 2016;35:25.

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.

**How to cite this article:** Dai L, Zhang W, Wu X, Zhou S. MicroRNA-203a-3p may prevent the development of thyroid papillary carcinoma via repressing MAP3K1 and activating autophagy. *J Clin Lab Anal*. 2022;36:e24470. doi:10.1002/jcla.24470