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ORIGINAL ARTICLE

MicroRNA-20a Targeting LASS2 Promotes the Proliferation, Invasiveness and Migration of Bladder Cancer

Shiwei Xiao [#, 1, 2], Yujin Chen [#, 1, 3], Ting Luan [1], Yinglong Huang [1], Shi Fu [1], Yigang Zuo [1], Haifeng Wang [1], Jiansong Wang [1]

[#] *These authors contributed equally to this study*
[1] *Department of Urology, The Second Affiliated Hospital of Kunming Medical University, Yunnan Institute of Urology, Kunming, China*
[2] *Department of Urology, Guizhou Provincial People's Hospital, Guiyang, China*
[3] *Kidney Center, Yunnan Boya Hospital, Kunming, China*

SUMMARY

Background: **Abnormal expression of miR-20a is reported in various types of malignancy neoplasms. However, its function is not consistent in different tumors. This study aims to explore the potential functions of miR-20a and its underlying mechanisms in bladder cancer.**
Methods: **Ninety-six patients diagnosed with bladder cancer were recruited into the study. The expression levels of miR-20a in bladder cancer samples and adjacent non-tumor samples were investigated by qRT-PCR. Wound healing, CCK8, and transwell migration assays were carried out for determining the functions of miR20a. Bioinformatics analysis was used for predicting the downstream gene of miR-20a. Western blot, qRT-PCR, and fluorescent reporter assays were used to verify the target gene.**
Results: **MiR-20a was significantly increased in bladder cancer tissues, and its rising level was closely correlated with histological grade, clinical stage, recurrence and metastasis in bladder cancer. Exogenous up-regulation of miR-20a expression obviously enhanced the aggressive biological functions of bladder cancer *in vitro*. LASS2 was verified to be a target gene of miR-20a. Moreover, miR-20a can negatively regulate LASS2 at protein and mRNA levels.**
Conclusions: **Increasing miR-20a is closely related to aggressive clinicopathological features. MiR-20a plays an oncogenic role in bladder cancer, which contributes to target LASS2 directly.**
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Correspondence:
Jiansong Wang
Dianmian Road No. 374
Kunming
China
Phone: +86 13888256716
Fax: +86 0871-65361017
Email: jiansongwangkm@126.com

Haifeng Wang
Dianmian Road No. 374
Kunming
China
Phone: +86 18083810913
Fax: +86 0871-65361017
Email: highphone@126.com

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KEY WORDS

miR-20a, oncogene, LASS2, biological functions, clinical progress

INTRODUCTION

Bladder cancer is the most common urological malignant neoplasm, and its overall incidence is $9.6/10^5$ and the population mortality is $3.2/10^5$ according to GLOBOCAN 2018 [1]. Despite administration of surgical resection and chemotherapy, bladder cancer still has poor outcomes for its recurrence and progression [2]. Hence, the identification of genes related to bladder cancer, new prognostic biomarkers, and novel molecular therapeutic targets are crucial.

MicroRNA (miRNA) is an endogenous non-coding small RNA, which has been determined to be closely related to the progression, prognosis, and treatment response of human cancers. Furthermore, miRNAs play crucial roles in regulating transcriptional and post-transcriptional gene expression by complimentary pairing with the 3′-untranslated region (3′-UTR) and predominantly translational repression [3]. To date, accumulating evidence has revealed that microRNAs are intimately related to the tumorigenesis and progression and play a part in tumor suppressor or oncogene in different varieties of human cancers, including bladder cancer [4-6]. In our previous studies, some miRNAs were found to be related to bladder cancer using microarray analysis, including miR17-92 microRNA cluster [7-9]. MicroRNA-20a (miR-20a) is a member of miR-17-92 cluster. Recently, miR-20a was overexpressed in a wide variety of cancers. It can play a role as an oncogene, including osteosarcoma, pancreatic ductal adenocarcinoma, colorectal cancer and so on [10-13]. Li et al. [14] reported that decreasing miR-20a expression can suppress cell growth of pancreatic carcinoma *in vitro* and *in vivo*. However, several controversial studies indicated that miR-20a can possess tumor suppressor activities. For instance, Elkayam et al. [15] found that miR-20a was a part of the RNA-induced silencing complex and played a role as a tumor suppressor. Furthermore, Zhou et al. [16-18] indicated that overexpressed miR-20a can inhibit cell proliferation and metastasis in cutaneous squamous, breast cancer, and pancreatic carcinoma. Interestingly, these controversial results are shown not only in the different types of cancers, but also in the same cancers. Hence, we speculated that miR-20a can play different roles by binding to diverse target genes. Previous study indicated that miR-20a was up-regulated in bladder cancer samples [19]. However, the potential functions of miR-20a and its underlying mechanisms remain poorly elucidated in bladder cancer. On this account, this study aims to explore the potential roles of miR-20a and its underlying mechanisms in bladder cancer.

MATERIALS AND METHODS

Patients and tissue samples collection

Ninety-six patients diagnosed with bladder cancer were recruited into the study. All patients have undergone transurethral resection of bladder tumor (TURBT) or radical cystectomy and were confirmed by pathological diagnosis. Besides, the clinical data of all patients were collected. The inclusion criteria for clinical samples was a pathological result showing bladder urothelial carcinoma. Clinical samples were excluded according to the following exclusion criteria: (1) patients with other malignant tumors at present or in the past; (2) non-urothelial carcinoma; (3) incomplete data; and (4) no informed consent. The TNM staging method of Union Internationale Contre le Cancer (UICC), the 7th edition, 2009, were used for pathological classification. The WHO 2004 grading system for malignant degree of bladder urothelial carcinoma was used for tumor grade. The research protocol was approved by the Medical Ethics Committee of Kunming Medical University. All subjects signed an informed consent before enrollment. No patient underwent chemotherapy or radiotherapy before surgery. Ninety-six fresh bladder cancer samples and adjacent non-tumor samples were gathered after surgery and were immediately placed in liquid nitrogen.

Cell culture, reagents, and transfection

T24, RT4, J82, BIU-87, and UM-UC-3 human bladder cancer cells were obtained from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (Gibco, USA) in humid air at 37°C and 5% carbon dioxide, and 10% fetal bovine serum and antibiotics (Gibco, USA) were added. The cells grew on sterile petri dishes and were passaged with 0.25% trypsin (Gibco, USA) every 2 days. MiR-20a mimics and the matching negative control mimics were synthesized by RiboBio (Guangzhou, China). Transient transfection was carried out using Lipofectamine® 2000 (Invitrogen, USA). Cells were used for cell counting kit-8 (CCK8) proliferation assays at 24, 48, and 72 hours after transfection. Twenty-four hours after transfection, cells were used for wound healing assays or transwell chamber migration assay. pLUC-LAG1 longevity assurance homolog 2 (LASS2) plasmid was synthesized by JiangzongBio (Kunming, China). The cells were transfected with plasmid using Lipofectamine® 2000 (Invitrogen, USA).

RNA extraction and quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Trizol reagent (Invitrogen, USA) was used for extracting total RNA. Small nucleolar RNA U6 served as the internal standard of miR-20a for standardization. qRT-PCR was carried out using the SYBR PrimeScript miRNA RT-PCR kit (Takara Biotechnology, China). All the real-time PCR primers were synthesized by Sangon (Sangon Biotech, China). Reactions were carried out in triplicate on a LightCycler® 480 Real-Time PCR System (Roche Diagnostics GmbH, Germany). The primer sequences are as follow: miR-20a forward, 5'-TGGGTAAAGTGCTTATAGTGC-3'; U6 forward, 5'-GCTTCGGCAGCACATATACTAAAAT-3'; LASS2 forward, 5'-TCTCCTGGTTTGCCAATTACG -3'; LASS2 reverse, 5'-CCGGGCAGGGACCCTCATCA-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward, 5'-CTTAGCACCCCTGGCCAAG-3'; GAPDH reverse, 5'-GATGTTCTGGAGAGCCCCG-3'. Downstream universal PCR primer was included in the kit was used as miR-20a and U6 reverse primer. The specificity of amplification was determined by analyzing the melting curve. The cycle threshold (CT) value was calculated, and the $2^{-\Delta\Delta CT}$ method was performed to quantify the relative expression of miR-20a or LASS2 [30].

CCK8 assay

Cells (2 x 10^3/well) were plated in 96-well plates and cultured overnight. The proliferation characteristics were detected by using the CCK8 (Dojindo Laboratories, Japan) at 0 (before transfection), 24, 48, and 72 hours post-transfection. In brief, 10 µL CCK8 solution was added to each well at different time points and incubated at 37°C and at 5% CO_2 for 2 hours. The absorbance was determined at 490 nm. Relative data were acquired from triplicate wells for each condition and represented at least three independent experiments.

Wound healing assay

The cells were seeded in 6-well plates (5 x 10^5/well) and incubated overnight at 37°C with DMEM/F12 plus 10% FBS. Twenty-four hours after transfection, when the cells had spread to more than 80% of the bottom of a well, the cells were wounded. Plates were washed with PBS to remove detached cells and plates were then incubated with DMEM/F12 without FBS. The wound pictures were taken at 0 (immediately), 6, 12, and 24 hours after scraping. The cell migration speeds were measured by the degree of healing of the wound line, and the migration rates were calculated.

Transwell chamber invasion assay

Cell invasion assay was carried out using a 6-well transwell chamber (Corning, USA). To coat the inserts, 25 µL Matrigel (BD Bioscience, USA) was used. Twenty-four hours later, transfected cells were collected and were transferred to the upper Matrigel chamber, incubated for 24 hours. Then, the non-invading cells were removed. The cells that moved through the filter were fixed. Hematoxylin was used as the stain. These experiments were accomplished in triplicate.

Western blot analysis

Forty-eight hours after transfection, cells were gathered. Total proteins were extracted in RIPA lysis buffer (Solarbio Biotech, China) and quantified by means of the BCA method. 30 µg protein was separated. Samples were transferred onto 0.45 µm polyvinylidene difluoride membranes (Millipore, USA), then sealed with 5% skimmed milk and incubated overnight at 4°C with a mouse anti-LASS2 (sc-390745; 1:500) or anti-β-actin monoclonal antibody (sc-47778; 1:500). ImageJ image analysis software version 1.34 was used to quantify relative protein levels.

Luciferase reporter assay

The TargetScan Human database 7.1 (http://www.targetscan.org) and miRDB database (http://www.mirdb.org/miRDB/) were used to predict miRNA binding sites. The 3'UTR of LASS2 (NM-181746) was constructed. The sequences of constructed pLUC-LASS2-3'-UTR vector were confirmed by DNA sequencing (Jiangzong Biotech, China). The miR-20a mimic or its matching negative control (RiboBio Co, 50 nM/well) were co-transfected with pLUC-LASS2-3′-UTR plasmid (100 ng/mL) into T24 or RT4 cells. The luciferase activity was assessed 48 hours post-transfection by using a Dual-Luciferase Assay System (Promega, USA). Result assays were accomplished in triplicate.

Statistical analysis

Data are presented as means ± SD. Comparisons were accomplished by Student's *t*-test. Correlations were carried out by two-sample Student's *t*-test. One-way analysis of variance followed by Tukey's multiple comparison tests were used to assess the differences among the groups. Statistical analysis was carried out using SPSS 21.0 software (IBM, NY, USA) for Windows. $p < 0.05$ was considered to be statistically significant.

RESULTS

The expression of MiR-20a is upregulated in bladder cancer tissues

To determine the expression status of miR-20a in bladder cancer tissues and matched non-tumor tissues, 96 pairs of samples were analyzed using qRT-PCR. The correlation between miRNA-20a expression and clinicopathological features is shown in Table 1. The expression of miR-20a in bladder cancer tissues is observably higher than that in adjacent non-tumor tissues (2.42 ± 1.32 vs. 1.38 ± 0.40), with a median 1.75-fold increase ($t = 7.332$, $p < 0.001$, Figure 1A). Next, we evaluated the correlation between miR-20a and the clinicopathological parameters. As shown in Figure 1B, high expression of miR-20a in bladder cancer is clearly related to aggressive tumor phenotypes, such as histological grade ($t = -10.596$, $p < 0.001$), TNM stage ($t = -11.068$, $p < 0.001$), lymph node invasion ($t = -9.285$, $p < 0.001$), distant metastasis ($t = -7.558$, $p < 0.001$), and tumor recurrence ($t = -3.582$, $p < 0.01$). Nevertheless, miR-20a expression level is not related to other clinicopathological parameters such as age, gender, tumor size, and number (all $p > 0.05$) in bladder cancer tissue. Briefly, our data indicates that miR-20a is notably increased in bladder cancer samples. Its overexpression is closely related to the clinicopathological characteristics of tumor progression in bladder cancer patients.

MiR-20a enhances the proliferation, invasiveness, and migration of bladder cancer cells *in vitro*

To explore the underlying functions of miR-20a on tumor growth and invasiveness in bladder cancer, we selected T24 and RT4 cells transfected with miR-20a and miRNA negative control (miR-NC) mimics to perform CCK8 assay, transwell chamber invasion and wound healing assay. CCK8 assay revealed that the proliferation of T24 and RT4 cells was enhanced by miR-20a mimics ($p < 0.01$, Figure 2A). Compared with miR-NC or blank control, the wound healing assay revealed that the migration rates were significantly increased in both RT4 and T24 cell lines transfected with miR-20a mimics (50% in RT4 cell line and 68.2% in T24 cell line,

Table 1. Correlation between miRNA-20a expression and clinicopathological features.

Variables	No. of patients	Relative miR-20a level	*t* value	p-value
Age (years)				
≥ 50	**36**	**2.69 ± 1.40**		
< 50	**60**	**2.25 ± 1.26**	**1.594**	**0.114**
Gender				
Male	**74**	**2.48 ± 1.32**		
Female	**22**	**2.21 ± 1.34**	**0.841**	**0.403**
Tumor number				
Single	**42**	**2.60 ± 1.49**		
Multiple	**54**	**2.28 ± 1.18**	**1.172**	**0.244**
Tumor size (cm)				
≥ 3	**53**	**2.33 ± 1.38**		
< 3	**43**	**2.53 ± 1.27**	**-0.730**	**0.467**
Histological grade				
G1 + G2	**49**	**1.46 ± 0.55**		
G3	**47**	**3.41 ± 1.16**	**-10.596**	**< 0.001**
TNM-stage				
T1 + T2	**61**	**1.66 ± 0.70**		
T3 + T4	**35**	**3.73 ± 1.12**	**-11.068**	**< 0.001**
Lymph node invasion				
Negative	**64**	**1.77 ± 0.85**		
Positive	**32**	**3.71 ± 1.15**	**-9.285**	**< 0.001**
Distant metastasis				
Negative	**86**	**2.14 ± 1.05**		
Positive	**10**	**4.79 ± 1.03**	**-7.558**	**< 0.001**
Tumor occurrence				
Primary	**63**	**2.09 ± 1.11**		
Recurrent	**33**	**3.05 ± 1.48**	**-3.582**	**0.001**

$p < 0.05$ was considered to be statistically significant.

$p < 0.01$, Figure 2B). The same phenomenon was observed in the transwell chamber invasion assay ($p < 0.01$, Figure 2C). The data demonstrate that miR-20a may enhance the proliferation, invasiveness, and migration of bladder cancer cells *in vitro*.

MiR-20a down-regulates LASS2 expression in bladder cancer cells

To clarify the related mechanism that miR-20a regulates biological behavior of bladder cancer cells, the web tool in the TargetScan7.1 database (http://www.targetscan.org/) and miRDB database (http://www.mirdb.org/miRDB/) were utilized to predict downstream genes of miR-20a. Based upon putative target sequences at position 1602-1608 of the LASS2-3'-UTR, LASS2 was predicted to be a possible target of miR-20a (Figure 3A). Next, LASS2 was examined in bladder cancer cell lines transfected with miR-20a mimic by qRT-PCR and western blot. Compared with the miR-NC group, data from qRT-PCR indicated that mRNA levels of LASS2 were decreased in all the above five bladder cancer cell lines transfected with miR-20a mimic (Figure 3B). A similar tendency was demonstrated at the protein level (Figure 3C). Together, the results show that miR-20a can down-regulate the expression of LASS2 expression in bladder cancer.

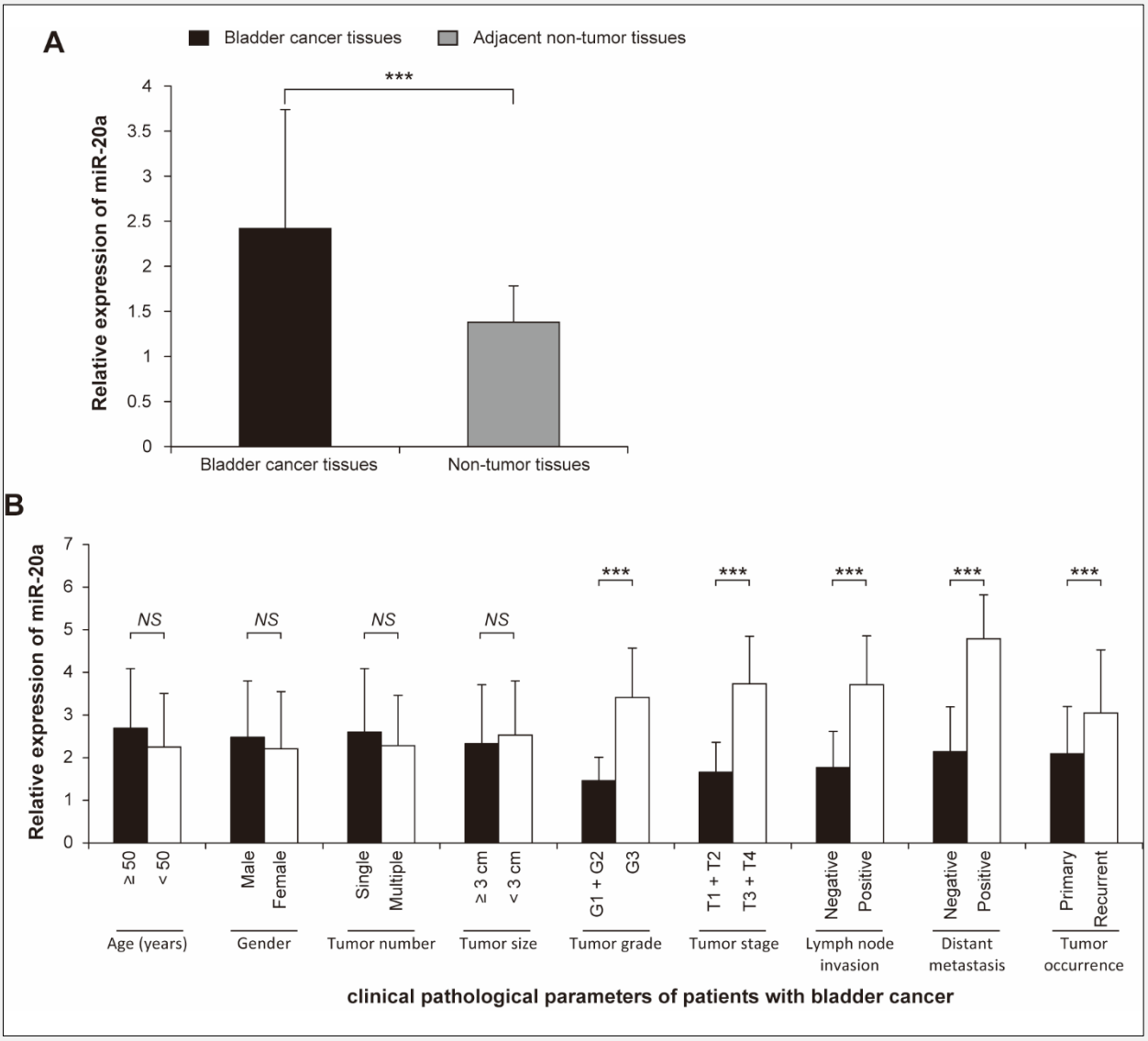

Figure 1. MiR-20a expression in bladder samples and correlation between miR-20a and clinic-pathological parameters.

A. Ninety-six pairs of samples were analyzed using qRT-PCR. MiR-20a expression in bladder cancer samples are observably higher than adjacent non-tumor tissues (2.42 ± 1.32 vs. 1.38 ± 0.40), with a median 1.75-fold increase (t = 7.332, p < 0.001).
B. Correlation between miR-20a expression and clinical pathological parameters of bladder cancers. Aggressive tumor phenotypes have a positive correlation with miR-20a, histological grade (t = -10.596, p < 0.001), TNM stage (t = -11.068, p < 0.001), lymph node invasion (t = -9.285, p < 0.001), distant metastasis (t = -7.558, p < 0.001), and tumor recurrence (t = -3.582, p < 0.01).
Data are expressed as mean ± SD. *** p < 0.001, Student's *t*-test.

MiR-20a down-regulates LASS2 expression direct targeting its 3′-UTR

To further validate whether LASS2 is the real target gene of miR-20a in bladder cancer, fluorescent reporter assays were carried out. The 3′-UTR of LASS2 was cloned into pLUC-REPORT vector, and then pLUC-LASS2-3′-UTR vector and miR-20a siRNA plasmid were co-transfected into T24 and RT4 cells. Compared with miR-NC mimics, the fluorescence intensity was reduced by miR-20a mimics in T24 and RT4 cells transfected with a vector containing pLUC-LASS2-3′-UTR vector ($p < 0.01$, Figure 3D). In a word, these results confirm that miR-20a can down-regulate LASS2 by directly targeting the 3'-UTR.

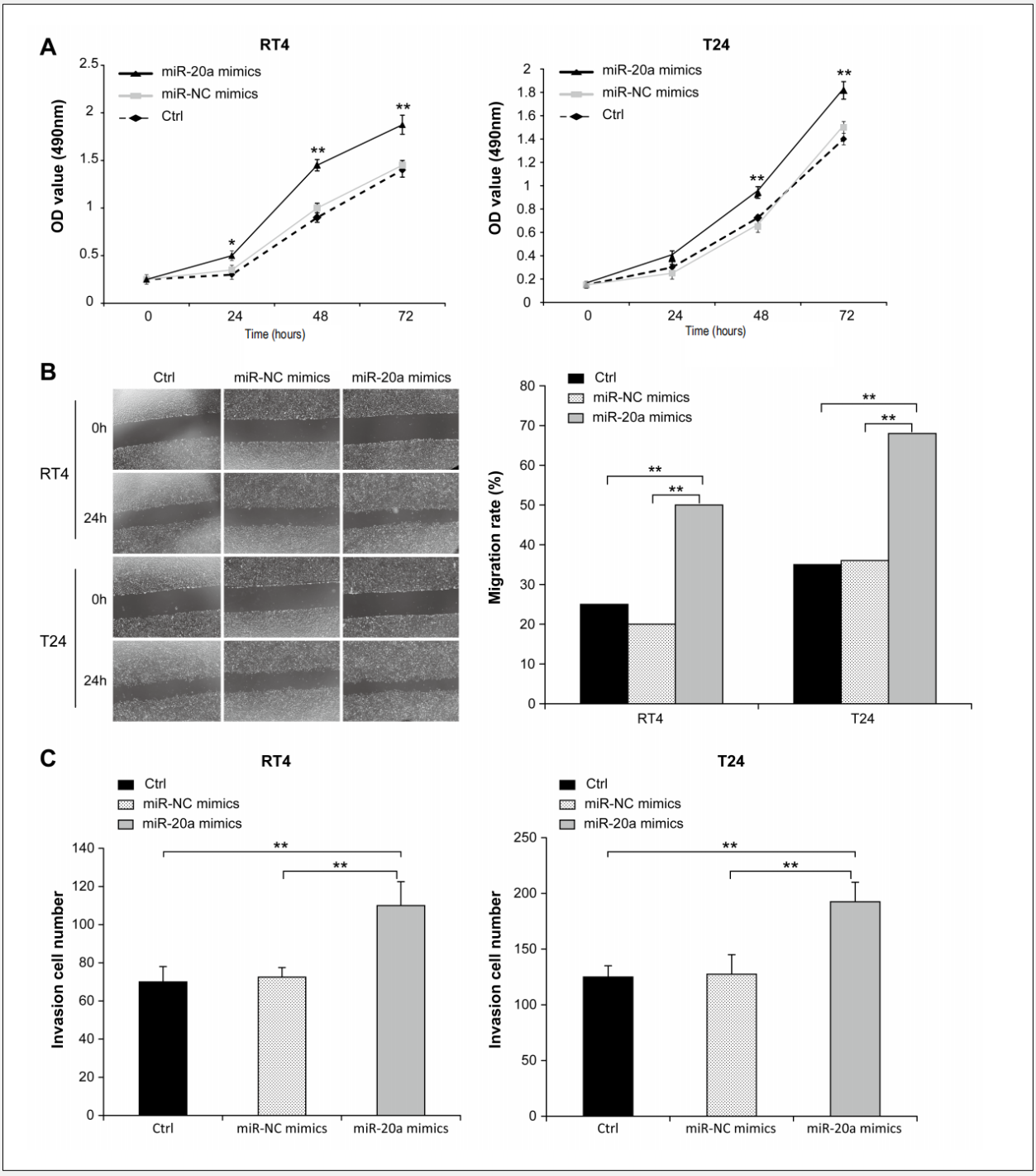

Figure 2. MiR-20a mimics enhance proliferation, metastasis, and invasion of bladder cancer.

A. The CCK8 assay shows that transfection of miR-20a mimic increases proliferation rate of RT4 and T24 cells compared with transfection of miR-NC or blank control, $p < 0.001$.
B. The wound healing assay shows that the migration rate is increased in miR-20a mimic group compared to the miR-NC group or blank control group, 50% in RT4 cell line and 68.2% in T24 cell line, $p < 0.01$.
C. Transwell chamber assay shows that the invasion cell numbers for both RT4 and T24 cell lines are significantly increased in miR-20a mimic group compared to the miR-NC group or blank control group l, $p < 0.01$.
Data are presented as mean ± SD. * - $p < 0.05$, ** - $p < 0.01$, Tukey's multiple comparison tests, experiments were done in triplicate.

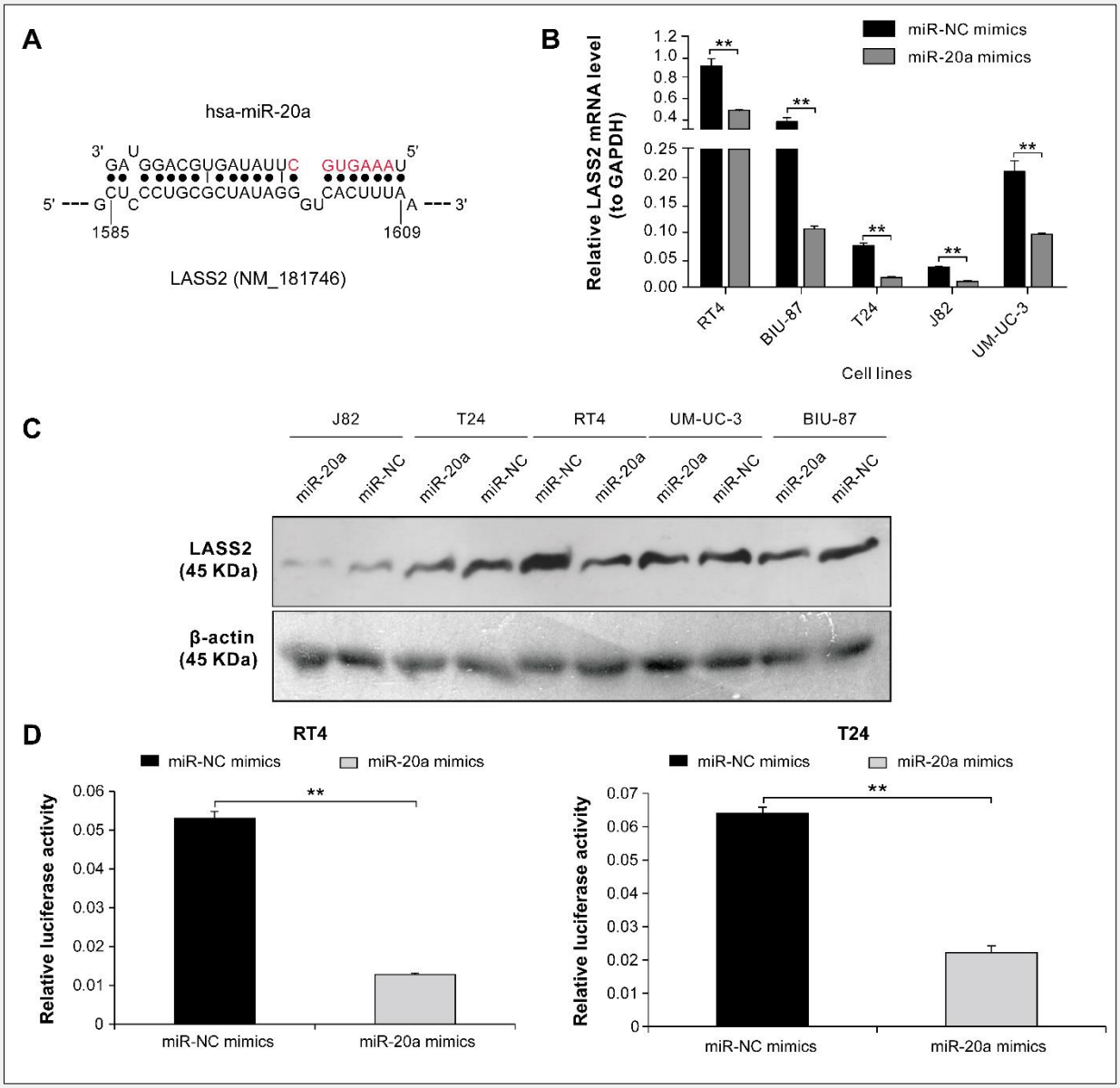

Figure 3. MiR-20a targeted LASS2 and suppressed LASS2 expression at both mRNA and protein levels.

A. Using TargetScan7.1 and miRDB database, LASS2 was predicted to be a potential target of miR- 20a based on putative target sequences at position 1,602 - 1,608 of the LASS2-3'-UTR. B. qRT-PCR shows that miR-20a mimic significantly down-regulates the mRNA relative expression of LASS2 compared with transfection of miR-NC, $p < 0.01$. C. Western blot shows that miR-20a mimics significantly decrease the protein expression of LASS2 compared with transfection of miR-NC. D. Fluorescent reporter assays on T24 and RT4 cells transfected with a vector containing pLUC-LASS2-3-UTR vector shows that the fluorescence intensity was notably reduced by miR-20a mimics, $p < 0.01$.
Data are expressed as mean ± SD. ** - $p < 0.01$, Student's *t*-test; experiments were done in triplicate.

DISCUSSION

With the progress of biotechnology, accumulating evidence has demonstrated the key roles for miRNAs whereby they participate in tumorigenesis and progression of several human tumors [20-22]. It is reported that abnormal levels of miRNA are associated with tumor grades, stages, invasiveness, poor prognosis, and increasing mortality [6,10-12]. MiRNAs are expected to become novel biomarkers of prognosis and new targets

of treatment for bladder cancer.

According to the results of our previous microarray analysis in bladder cancer [7], miR-20a was selected for further study. Compared with the matching non-tumor samples via real-time PCR analysis, our data shows that miR-20a is notably increased in bladder cancer samples, which is consistent with previous studies [19]. Moreover, it is found that increasing miR-20a expression is closely related to the clinical features of bladder cancer, such as clinical stages, high histological grade, recurrence, and metastasis. The outcomes indicate that the increased level of miR-20a may be a sign of a poor tumor phenotype of bladder cancer.

MiR-20a was overexpressed in typical bladder cancer cell lines (T24 and RT4) via transfection of hsa-miR-20a mimics for exploring its function in bladder cancer. Compared with the above two control groups, the abilities of proliferation, migration, and invasiveness were significantly enhanced in the miR-20a mimic group in both of T24 and RT4 cells. Indeed, all results demonstrate that miR-20a can act as an oncogenic microRNA, and play a vital part in the progress of bladder cancer. Furthermore, Zhong et al. proved that miR-20a acted as a ceRNA of lncRNA PTENPL and promoted bladder cancer cell proliferation and migration by regulating PDCD4 [23]. These data suggest that miR-20a can be used as a potential prognostic biomarker of bladder cancer.

Human miR-20a is located on chromosome 13q31.3 and belongs to miR-17-92 cluster, including hsa-miR-20a-5p and hsa-miR-20a-3p. The miR-17-92 cluster is composed of seven mature microRNAs. The miR-17-92 cluster is transcribed into a single pri-microRNA, followed by processing to form the individual microRNAs [24,25]. It has been reported miR-20a plays an oncogenic part in the development of various tumors by binding to its downstream target gene such as CYLD [26], TIMP-2 [27], ABL2 [28], APP [29], and RUNX3 [30]. However, many studies found that miR-20a can serve as a tumor suppressor. MiR-20a could inhabit the cell proliferation and metastasis in anaplastic thyroid cancer and cutaneous squamous cell carcinoma by targeting LIMK1 [16,31]. In addition, miR-20a plays different roles in the same cancer by targeting various genes. For instance, Chen et al. [13] proved that miR-20a overexpression can enhance hepatocellular carcinoma (HCC) cell migration and proliferation by reducing the translation of RUNX3. However, recent studies proved that miR-20a can suppress the proliferation, migration and invasiveness, and induce apoptosis in HCC cell line by directly targeting c-MYC and CCND1 respectively [32, 33]. A similar finding was also reported in breast cancer [17,34]. Moreover, Yin et al. identified hsa-miR-17, a member of miR-17-92 cluster, by bioinformatics analysis, which was associated with the prognosis of patients of bladder cancer [35]. According to these controversial results, we speculated that miR-20a can play diverse functional roles in the different kinds of tumors by binding to various target genes.

Moreover, bioinformatics analysis was used to study the potential mechanisms of miR-20a in bladder cancer and its possible target genes. Interestingly, it was found that LASS2 was a direct target gene of miR-20a. LASS2, known as ceramide synthase 2 (CERS2), a novel tumor suppression gene, is verified to play a suppressive part in proliferation and invasion in some neoplasm, such as hepatocellular carcinoma [32] and breast cancer [17]. Tang et al. [36] reported that overexpression LASS2, transfected with pCMV-HA2-LASS2 plasmid, can suppress the migration and invasion of HCCLM3 hepatocarcinoma cells. Moreover, the risk of liver cancer was significantly increased in mice with LASS2 knocked out [37]. Our previous studies demonstrated that bladder cancer with LASS2-negative is related to unfavorable prognosis. Decreasing LASS2 can promote the invasiveness and metastasis of bladder cancer cells [38]. Recent studies [34,39] suggest that LASS2 can inhabit cancer invasiveness and metastasis via the suppression of vacuolar H^+-ATPase (VPL). Meanwhile, LASS2 can bind to the V-type proton ATPase 16 kDa proteolipid subunit (the C subunit of V-ATPase). Subsequently, LASS2 can inhibit the activation of hydrogen-sensitive protease, extracellular matrix degradation, and induce tumor cell apoptosis [39-41].

In this study, the results show that LASS2 expression can be down-regulated at both mRNA and protein levels by overexpressing miR-20a in all five bladder cancer cell lines. LASS2 is primarily proved to be a target gene of miR-20a in bladder cancer. Besides, miR-20a can negatively regulate LASS2 expression *in vitro*. We further verify that miR-20a can directly bind to the LASS2 3′-UTR region using fluorescent reporter assays. These data indicated that miR-20a could reduce endogenous LASS2 expression of bladder cancer cells by binding to LASS2 3′-UTR region.

The important features of miRNAs in regulating gene expression are that miRNA can target various genes, and numerous miRNAs can target a common gene, thereby forming a complex microRNA regulation gene networks, achieving the subtle control of gene expression at the transcription and post-transcription levels [24,42]. Most recently, our group has proved that LASS2 is one target of miR-98. Furthermore, LASS2 can promote chemoresistance in bladder cancer targeting LASS2 [9]. Thus, it is considered that miR-20a can play the part of an oncogene in bladder cancer due to the following possible mechanisms: on the one hand, miR-20a can combine with other miRNAs, including miR-9 and miR-98, to tightly regulate the expression of LASS2 gene in bladder tumor by targeting the different sequence of LASS2; on the other hand, miR-20a also can combine with other multiple target genes, including LASS2, to enhance cell proliferation, metastasis and invasion in bladder cancer. Clearly, further research is required to understand the other members and the complex mechanism of both miR-20a and LASS2 in the gene regulatory network in human bladder cancer.

CONCLUSION

In conclusion, miR-20a is upregulated in bladder cancer. Increasing miR-20a is closely related to aggressive clinicopathological features. Furthermore, miR-20a, as an oncogene, plays a vital part in the occurrence and prognosis of bladder cancer by directly binding to LASS2 3-UTR region and negatively regulating LASS2 at the mRNA and protein levels. Our research provides a novel insight for the diagnosis and treatment of bladder cancer.

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Declaration of Interest:
The authors declare no conflict of interest.

References:

1. Bray F, Ferlay J, Soerjomataram I, Siegel Rl, Torre La, Jemal A. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68(6):394-424 (PMID: 30207593).

2. Audenet F, Waingankar N, Ferket BS, et al. Effectiveness of transurethral resection plus systemic chemotherapy as definitive treatment for muscle invasive bladder cancer in population level data. J Urology 2018;200(5):996-1004 (PMID: 29879397).

3. Wang Y, Xu X, Yu S, et al. Systematic characterization of A-to-I rna editing hotspots in micrornas across human cancers. Genome Res 2017;27(7):1112-25 (PMID: 28411194).

4. Zhang J, Wang L, Mao S, et al. MiR-1-3p contributes to cell proliferation and invasion by targeting Glutaminase in bladder cancer cells. Cell Physiol Biochem 2018;51(2):513-27 (PMID: 3045 8442).

5. Wang R, Sun Y, Yu W, et al. Downregulation of miRNA-214 in cancer-associated fibroblasts contributes to migration and invasion of gastric cancer cells through targeting FGF9 and inducing EMT. J Exp Clin Can Res 2019;38(1):20 (PMID: 30646925).

6. Huang F, Chen W, Peng J, et al. LncRNA pvt1 triggers cyto-protective autophagy and promotes pancreatic ductal adenocarcinoma development via the miR-20a-5p/ULK1 axis. Mol Cancer 2018;17(1):98 (PMID: 30001707).

7. Liu J, Wang H, Wang Y, et al. Repression of the miR-93-enhanced sensitivity of bladder carcinoma to chemotherapy involves the regulation of LASS2. Onco Targets Ther 2016;9:1813-22 (PMID: 27099514).

8. Fu S, Luan T, Jiang C, et al. MiR-3622a promotes proliferation and invasion of bladder cancer cells by downregulating LASS2. Gene 2019;701:23-31 (PMID: 30898713).

9. Luan T, Fu S, Huang YL, et al. MicroRNA-98 promotes drug resistance and regulates mitochondrial dynamics by targeting LASS2 in bladder cancer cells. Exp Cell Res 2018; 373(1-2):188-97 (PMID: 30463687).

10. Zhang L, He L, Zhang H, Chen Y. Knockdown of miR-20a enhances sensitivity of colorectal cancer cells to cisplatin by increasing ASK1 expression. Cell Phys Biochem 2018;47(4):1432-41 (PMID: 29940575).

11. Hoey C, Ahmed M, Fotouhi Ghiam A, et al. Circulating miRNAs as non-invasive biomarkers to predict aggressive prostate cancer after radical prostatectomy. J Transl Med 2019;17(1):173 (PMID: 31122242).

12. Liu L, He J, Wei X, et al. Microrna-20a-mediated loss of autophagy contributes to breast tumorigenesis by promoting genomic damage and instability. Oncogene 2017;36(42):5874-84 (PMID: 28628113).

13. Chen Y, Wang X, Cheng J, et al. MicroRNA-20a-5p targets RUNX3 to regulate proliferation and migration of human hepatocellular cancer cells. Oncology Rep 2016;36(6):3379-86 (PMID: 27748919).

14. Li X, Pan JH, Song B, et al. Suppression of CX43 expression by miR-20a in the progression of human prostate cancer. Cancer Biol Ther 2012;13(10):890-8 (PMID: 22785209).

15. Elkayam E, Kuhn CD, Tocilj A, et al. The structure of human argonaute-2 in complex with miR-20a. Cell 2012;150(1):100-10 (PMID: 22682761).

16. Zhou J, Liu R, Luo C, et al. MiR-20a inhibits cutaneous squamous cell carcinoma metastasis and proliferation by directly targeting LIMK1. Cancer Biol Ther 2014;15(10):1340-9 (PMID: 25 019203).

17. Meng QJ, Wang L, Lv L, Wu J, Shi W. Deletion of HNF1A-AS1 suppresses the malignant phenotypes of breast cancer cells *in vitro* and *in vivo* through targeting miRNA-20a-5p/TRIM32 axis. Cancer Biother Radiopharm 2020 (PMID: 32319789).

18. Yan H, Wu J, Liu W, et al. MicroRNA-20a overexpression inhibited proliferation and metastasis of pancreatic carcinoma cells. Hum Gene Ther 2010;21(12):1723-34 (PMID: 20583868).

19. Ratert N, Meyer HA, Jung M, et al. MiRNA profiling identifies candidate miRNAs for bladder cancer diagnosis and clinical outcome. J Mol Diag 2013;15(5):695-705 (PMID: 23945108).

20. Liu H, Lei C, He Q, Pan Z, Xiao D, Tao Y. Nuclear functions of mammalian microRNAs in gene regulation, immunity and cancer. Mol Cancer 2018;17(1):64 (PMID: 29471827).

21. Borkowska Em, Konecki T, Pietrusiński M, Borowiec M, Jabłonowski Z. MicroRNAs which can prognosticate aggressiveness of bladder cancer. Cancers (Basel) 2019;11(10):1551 (PMID: 31615 011).

22. Lai X, Eberhardt M, Schmitz U, Vera J. Systems biology-based investigation of cooperating microRNAs as monotherapy or adjuvant therapy in cancer. Nucleic Acids Res 2019;47(15):7753-66 (PMID: 31340025)

23. Zhong XL, Wang L, Yan XK, et al. MiR-20a acted as a ceRNA of lncRNA PTENPL and promoted bladder cancer cell proliferation and migration by regulating PDCD4. Eur Rev Med Pharmacol Sci 2020;24(6):295564 (PMID: 32271413).

24. Li Q, Wang H, Peng H, et al. MicroRNAs: key players in bladder cancer. Mol Diag Ther 2019; 23(5):579-601 (PMID: 31325035).

25. Yoshino H, Seki N, Itesako T, Chiyomaru T, Nakagawa M, Enokida H. Aberrant expression of microRNAs in bladder cancer. Nat Rev Urol 2013;10(7):396-404 (PMID: 23712207).

26. Zhu M, Zhou X, Du Y, et al. MiR-20a induces cisplatin resistance of a human gastric cancer cell line via targeting CYLD. Mol Med Rep 2016;14(2):1742-50 (PMID: 27357419).

27. Wang Z, Wang B, Shi Y, et al. Oncogenic miR-20a and miR-106a enhance the invasiveness of human glioma stem cells by directly targeting TIMP-2. Oncogene 2015;34(11):1407-19 (PMID: 24704830).

28. Qiang XF, Zhang ZW, Liu Q, et al. MiR-20a promotes prostate cancer invasion and migration through targeting ABL2. J Cell Biochem 2014; 115(7):1269-76 (PMID: 24464651).

29. Kondo M, Yamada H, Munetsuna E, et al. Associations of serum microRNA-20a, -27a, and -103a with cognitive function in a Japanese population: The Yakumo study. Arch Gerontol Geriat 2019;82:155-60 (PMID: 30802839).

30. Bai X, Han G, Liu Y, Jiang H, He Q. MiRNA-20a-5p promotes the growth of triple-negative breast cancer cells through targeting RUNX3. Biomed Pharmacother 2018;103:1482-9 (PMID: 29864 933).

31. Xiong Y, Zhang L, Kebebew E. MiR-20a is upregulated in anaplastic thyroid cancer and targets LIMK1. PloS One 2014; 9(5):e 96103 (PMID: 24858712).

32. Tipanee J, Di Matteo M, Tulalamba W, et al. Validation of miR-20a as a tumor suppressor gene in liver carcinoma using hepatocyte-specific hyperactive piggyBac transposons. Mol Ther Nucleic Acids 2020;19:1309-29 (PMID: 32160703).

33. Chen GS, Zhou N, Li JQ, Li T, Zhang ZQ, Si ZZ. Restoration of miR-20a expression suppresses cell proliferation, migration, and invasion in HEPG2 cells. Onco Targets Ther 2016;9:3067-76 (PMID: 27313460).

34. Si W, Shen J, Du C, et al. A miR-20a/mapk1/c-myc regulatory feedback loop regulates breast carcinogenesis and chemoresistance. Cell Death Differ 2018;25(2):406-20 (PMID: 29125598).

35. Yin XH, Jin YH, Cao Y, et al. Development of a 21-miRNA Signature Associated With the Prognosis of Patients With Bladder Cancer. Front Oncol 2019;9:729 (PMID: 31448232).

36. Tang N, Jin J, Deng Y, et al. [LASS2 interacts with V-ATPase and inhibits cell growth of hepatocellular carcinoma]. Sheng Li Xue Bao 2010; 62(3):196-202 (PMID: 20571735).

37. Lu X, Chen Y, Zeng T, Chen L, Shao Q, Qin W. Knockout of the HCC suppressor gene LASS2 downregulates the expression level of miR-694. Oncol Rep 2014;32(6):2696-702 (PMID: 25333455).

38. Chen Y J, Wang HF, Xiong T, Zou RC, Tang Z, Wang JS. The role of LASS2 in regulating bladder cancer cell tumorigenicity in a nude mouse model. Oncol Lett 2017;14(5):5149-56 (PMID: 291 13153).

39. Xu X, Liu B, Zou P, Zhang Y, You J, Pei F. Silencing of LASS2/TMSG1 enhances invasion and metastasis capacity of prostate cancer cell. J Cell Biochem 2014;115(4):731-43 (PMID: 24453 046).

40. Wang HF, Zuo YG, Ding MX, et al. LASS2 inhibits growth and invasion of bladder cancer by regulating ATPase activity. Oncol Lett 2017;13(2):661-8 (PMID: 28356943).

41. Yu W, Wang L, Wang Y, et al. A novel tumor metastasis suppressor gene LASS2/TMSG1 interacts with vacuolar ATPase through its homeodomain. J Cell Biochem 2013;114(3):570-83 (PMID: 22991218).

42. Han C, Shen JK, Hornicek FJ, Kan Q, Duan Z. Regulation of microRNA-1 (miR-1) expression in human cancer. Biochim Biophys Acta Gene Regul Mech 2017;1860(2):227-32 (PMID: 27923 712).