LNCRNA SNHG7 PROMOTES TUMOR METASTASIS OF BLADDER CANCER BY INHIBITING MIR-34A

Cuiqing Shang[#], Jinyan Zhu[#], Chuanhua Zhang, Jingdong Yuan, Juan Yang, Qian Huang, YiPeng Liu[*]
Department of Urology, The No.1 Hospital of Wuhan, Wuhan, 430000, China
[#]*Cuiqing Shang and Jinyan Zhu contributed equally to this work. They are co-first authors*

ABSTRACT

Objective: *Long non-coding RNA(lncRNA) has become a key regulatory factor for tumor progression. lncRNA SNHG7 is considered to be a carcinogenic factor, but its role and mechanism in bladder cancer (BC) need to be further elaborated. To explore the role and mechanism of lncRNA SNHG7 in BC.*

Methods: *PCR-qRT was used to detect the expression of SNHG7 and miR-34a in BC cells and normal cells. Stable and SNHG7 inhibitory vector and miR-34a over-expression vector were established and transfected into BC cells. CCK-8, traswell and flow cytometry were used to observe the proliferation and apoptosis of transfected cells. Western blot was used to detect the changes of EMT markers (E-cadherin, N-cadherin, Vimentin). The relationship between SNHG7 and miR-34a was determined using double luciferase report.*

Results: *SNHG7 was up-regulated in BC cells, while miR-34a was down-regulated. Inhibition of SNHG7 or over-expression of miR-34a on cancer cells could inhibit the proliferation and invasion of cells and increase apoptosis rate. In addition, it could up-regulate E-cadherin and down-regulate N-cadherin and Vimentin. Double luciferase reported that SNHG7 and miR-34a could be targeted combined. Rescue experiments found that inhibition of miR-34a could reverse the biological function changes of cancer cells caused by inhibition of SNHG7.*

Conclusion: *SNHG7 can promote the proliferation, invasion, EMT and inhibit apoptosis of BC cells through miR-34a.*

Keywords: *lncRNA SNHG7, miR-34a, bladder cancer, biological function.*

DOI: 10.19193/0393-6384_2020_4_401

Received *November 30, 2019;* ***Accepted*** *January 20, 2020*

Introduction

Bladder cancer (BC) is the most common urinary system tumor in clinic, with high morbidity and mortality[1]. According to the global cancer statistics in 2018, 549,393 new cases and 199,922 deaths were found in 2018, which is the 10th most common cancer in the world[2]. At present, although BC patients can be treated by surgery, radiotherapy and chemotherapy, the recurrence rate and mortality rate of patients are still very high due to the characteristics of BC with high metastasis[3, 4]. There is currently no effective treatment for patients with recurrence or metastasis. Therefore, it is very important to explore the pathogenesis of BC and find potential therapeutic targets to improve patient outcomes.

LncRNA(Long non-coding RNA) is a long-chain non-coding RNA with a length of >200 nt[5]. Although lncRNA cannot directly encode proteins, it can participate in various biological processes such as cell growth, embryonic development and tumorigenesis by regulating chromatin and transcriptional and post-transcriptional gene expression[6]. SNHG7 is a member of lncRNA, located on chromosome 9q34.3, with a length of 2157 bp[7] SNHG7 is considered as a carcinogenic gene, which can promote the progress of various tumors. For example, SNHG7 can promote tumor metastasis and invasion by up-regulating SOX4 in melanoma[8]. In renal cell carcinoma, SNHG7 can play a role in promoting cancer by negatively regulating CDKN1A expression[9].

In prostate cancer, SNHG7 can promote the proliferation and cycle development of cancer cells through miR-503/Cyclin D1 pathway[10]. SNHG7 is also considered as a carcinogenic gene in BC. Some studies have found that SNHG7 expression is up-regulated in BC tissues and cells and it is related to tumor size, metastasis and staging. In addition, inhibition of SNHG7 can inhibit the proliferation of cancer cells and promote apoptosis[11].

However, the biological mechanism of SNHG7 in BC progress still needs to be further clarified. In this paper, we found that there are targeted binding sites between SNHG7 and miR-34a through starBase3.0 gene prediction website. microRNA(miR) is a highly conserved endogenous short-chain non-coding RNA, which can participate in and regulate cell processes such as apoptosis, proliferation and differentiation[12, 13]. In tumors, miR has been widely studied as a carcinogenic or tumor suppressor gene and it is considered as a therapeutic target for tumors[14]. miR-34a is an important member of miR. The exploration of the relationship between miR-34a and BC has found that miR-34a is down-regulated in BC, and it can inhibit the invasion and migration of cancer cells by up-regulating PTEN[15].

Based on the above, we have suspected that SNHG7 can promote BC progress through miR-34a. In order to prove this conjecture, we have conducted the following research[16].

Materials and methods

Cell source and treatment

Cell sources

BC cells UMUC3, T24, RT112, SW780 and normal bladder epithelial cell SV-HUC-1 were purchased from Shanghai Institute of Cell Research, Chinese Academy of Sciences.

Cell culture

The above five cell lines were placed in RPMI1640 medium (Gibco, USA) containing 10% fetal bovine serum (PBS, Gibco, USA) and 1% penicillin-streptomycin (100X, Solarbio, USA), respectively, and cultured in an incubator at 37°C, 5% CO2, and digested with 0.25% trypsin until the cell adherence growth density reached 80%-90%.

Cell transfection

pcDNA 3.1 plasmid was used as a vector. SNHG7 inhibitory plasmid (si-SNHG7), miR-34a over-expression plasmid (miR-34a-mimics) and blank control (si-NC, miR-NC) were established, respectively. The established cell lines were transferred to 24-well plates. After 48 hours, Lipofectamine 2000(Invitrogen, USA) kit was used to transfect the cell plasmid. The operation steps were strictly carried out according to the kit instructions.

Detection methods

PCR-qRT

Total RNA was extracted from the target cells with TRIzol reagent (Invitrogen, USA). Next, the purity, concentration and integrity of the extracted total RNA were determined by UV spectrophotometer and agarose gel electrophoresis. 5 μg of total RNA was taken. Reverse Transcription was performed using the TaqMan™ Reverse Transcription reagents kit (Invitrogen, USA). The specific steps were carried out strictly according to the kit instructions. SYBR_ Premix ExTaq II (Takara, Dali, China) and ABI 7500PCR (Applied Biosystems, USA) were used for amplification. The amplification system was SYBR Premix Ex Taq II (2X) 10μL, cDNA 2μL, each 0.8μL of upstream and downstream primers, and then Sterile purified water was supplemented to 20 μL. The amplification conditions were pre-denaturation at 95°C for 30s, denaturation at 95°C for 5s, anneal and extension at 60°C for 30s, with a total of 40 cycles. $2^{-\triangle\triangle ct}$ was used to analyze the data[17]. SNHG7 used GADPH as the internal reference and miR-34a used U6 as the internal reference. The primer sequences are shown in Table 1.

Grouping	Forward	Reverse
SNHG7	5'-TTGCTGGCGTCTCGGTTAAT-3'	5'-GGAAGTC CATCACAGGCGAA-3'
GADPH	5'-AACGTGTCAGTGGTGGACCTG-3'	5'-AGTGGGTGTCGCTGTTGAAGT-3'
miR-34a	5'-CACGGACTCGGGGCATTTG-GAGATTTT-3'	5'-CTGTCTAGATCGCTTATCTTC-CCCTTGG-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTT CACGAATTTGCGT-3'

Table 1: Primer sequences.

Western blot assay

In each group, the cultured cells were collected. RIPA reagent and BCA kit (Thermo Scientifi, the United States) were used to extract the total proteins and test its concentration in each group. Subsequently, 6% SDS-PAGE was used for electrophoresis to separate the protein to be detected and transfer it to PVDF membrane.

The protein was sealed with 5% defatted milk powder for 2 hours. E-cadherin (1: 1000), N-cadherin (1: 1000), Vimentin (1: 1000) and β-catenin (1:

1000) primary antibody (Abcam, USA) were added and sealed overnight in a refrigerator at 4°C. The first antibody was removed by washing the film, and HRP-conjugated goat anti-rabbit second antibody of 1:4000 (Abcam, USA) was added, incubated at 37°C for 1h, and rinsed 3 times with PBS for 5 min each time. Then, it was developed in dark room and waste liquid on the membrane was removed. Development was carried out according to the instruction of ECL kit. The protein bands were scanned and the gray values were analyzed in Quantity One.

Detection of cell proliferation

Cells were collected at 24 hours after transfection and their concentration was adjusted to 4*104 cells. The cells were spread on 96-well plates. 10μL CCK solution (Shanghai Beyotime Biotechnology) was added at 24 hours, 48 hours, 72 hours and 96 hours after transfection, and cultured at 37°C for 2 hours. Subsequently, the absorbance of each well was detected by using enzyme-labeling instrument (Molecular Devices, USA) at 490nm and the growth curve was drawn.

Detection of cells invasion

After transfection for 24 hours, cells were collected and their concentration was adjusted to 4*104 cells. The cells were inoculated into the upper chamber. 200μL of culture solution was added into the upper chamber. 500mL of culture medium containing 20%FBS was added into the lower chamber. After culture for 48 hours at 37°C, the substrates and cells in the upper chamber that did not pass through the membrane surface were wiped off, washed with PBS for 3 times, fixed with paraformaldehyde for 10min, washed with double distilled water for 3 times, dried and stained with 0.5% crystal violet. The cell invasion was observed under a microscope.

Apoptosis detection

The suspension with 1*106 cells /mL was prepared, and the cells were fixed with precooled 70% ethanol solution for 30 min.

The ethanol solution was removed. The cells were incubated in Annexin V-FITC/7-AAD mixed solution and incubated in dark for 5 minutes. FC-500MCL flow cytometer system (BD, USA) was used for detection. The experiment was repeated for 3 times to take the average value.

Detection of target genes

There were targeted binding sites between SNHG7 and miR-34a through starBase3.0 gene prediction website. The SNHG7-3'UTR wild type (Wt) and SNHG7-3'UTR mutant (Mut), miR-34a-mimics and miR-NC were transferred into BC cells using the Lipofectamine™ 2000 kit.

After 48 hours, the luciferase activity in the cells was detected using the dual luciferase reporter gene assay kit (Promega, USA).

Statistical analysis

In this study, the data were input into the GraphPad 7 (GraphPad Software, San Diego, USA) for data statistics, analysis and drawing the required pictures. Independent sample t test was used for comparison between the two groups.

One-way anova was used for multi-group comparison. LSD-t test was used for pairwise comparison. Multiple time points were expressed by repetitive measurement and analysis of variance. Bonferroni was used for back testing. The difference was statistically significant with $P<0.05$.

Results and discussion

Expression of SNHG7 and miR-34a in BC cells

PCR-qRT was used to detect the expression of SNHG7 and miR-34a in BC cells UMUC3, T24, RT112, SW780 and normal bladder epithelial cell SV-HUC-1. It was found that SNHG7 was up-regulated and miR-34a was down-regulated in the above four BC cells. SNHG7 was expressed highest in UMUC3 cells, so it was selected for subsequent cell experiments (Figure 1).

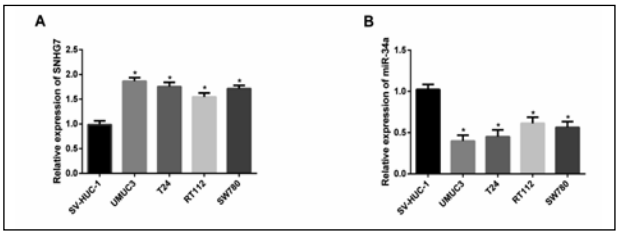

Figure 1: Expression of SNHG7 and miR-34a in BC cells. Figure A, SNHG7 up-regulated in BC cells. Figure B, miR-34a down-regulated in BC cells.
*Note: *represents p<0.05.*

Effects of SNHG7 on biological functions of BC cells

In order to explore the effect of SNHG7 on BC, UMUC3 cells were treated with SNHG7 inhibition. CCK-8, traswell and flow cytometry were used to evaluate the proliferation, invasion and apoptosis of transfected cells.

The results showed that after the treatment of UMUC3 cells with SNHG7 inhibition, SNHG7 was down-regulated, the proliferation and invasion abilities were weakened and apoptosis rate was increased. Detection of EMT marker protein showed that E-cadherin was elevated while N-cadherin and Vimentin were down-regulated (Figure 2).

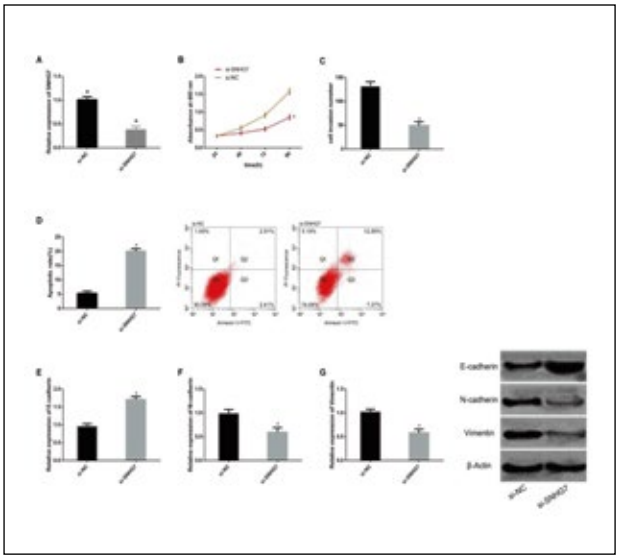

Figure 2: Effects of SNHG7 on biological functions of BC cells. Figure A, After the treatment of UMUC3 cells with SNHG7 inhibition, SNHG7 was down-regulated. Figure B, After the treatment of UMUC3 cells with SNHG7 inhibition, the proliferation ability was weakened. Figure C, After the treatment of UMUC3 cells with SNHG7 inhibition, the invasion ability was weakened. Figure D, After the treatment of UMUC3 cells with SNHG7 inhibition, the apoptosis rate was increased. Figure E/F/G: After the treatment of UMUC3 cells with SNHG7 inhibition, E-cadherin in the cells increased while N-cadherin and Vimentin decreased.
*Note: *represents p<0.05.*

Relationship between SNHG7 and miR-34a

Results 2.2 showed that SNHG7 could promote BC development, but the mechanism was not clear. According to the prediction of starBase3.0 biological website, there was a binding site between SNHG7 and miR-34a.

Therefore, the double luciferase activity test was carried out to prove the connection between them. The results showed that miR-34a-mimics could inhibit SNHG7-3'UTR Wt luciferase activity in cells and had no effect on the SNHG7-3'UTR Mut luciferase activity.

In addition, PCR-qRT showed that miR-34a was up-regulated after transfection of UMUC3 cells with si-SNHG7. This indicated that SNHG7 could target regulation of miR-34a (Figure 3).

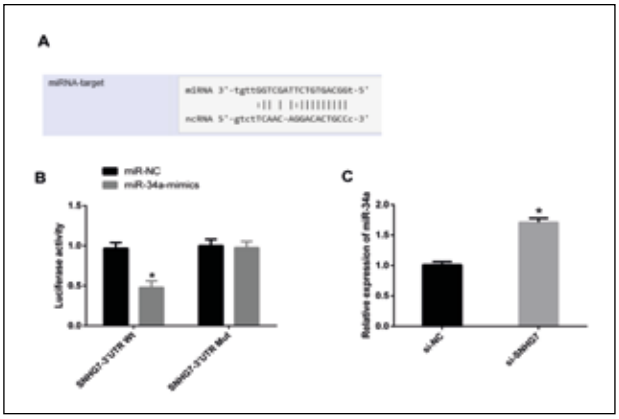

Figure 3: Relationship between SNHG7 and miR-34a. Figure A, There was a binding site between SNHG7 and miR-34a. Figure B, miR-34a-mimics could inhibit SNHG7-3'UTR Wt luciferase activity in cells and had no effect on the SNHG7-3'UTR Mut luciferase activity. Figure C, miR-34a was up-regulated after transfection of UMUC3 cells with si-SNHG7.
*Note: *represents p<0.05.*

Effect of miR-34a on biological function of BC cells

miR-34a was down-regulated in BC cells, suggesting that miR-34a might participate in BC development. In order to explore the role of miR-34a in BC, UMUC3 cells were over-expressed with miR-34a to observe the effect of such treatment on the biological function of cancer cells.

The results showed that after transfection of UMUC3 cells with miR-34a-mimics, miR-34a was up-regulated, and cell proliferation and invasion were enhanced and apoptosis rate was decreased. Detection of EMT marker protein showed that E-cadherin was elevated while N-cadherin and Vimentin were down-regulated (Figure 4).

Effects of simultaneous inhibition of SNHG7 and miR-34a on biological function of BC cells

In order to further understand the relationship between SNHG7 and miR-34a, UMUC3 cells were treated with simultaneous inhibition of SNHG7 and miR-34a to observe the changes of cell biological functions. The results showed that the proliferation, invasion and apoptosis of UMUC3 cells after transfecting si-SNHG7+miR-34a-inhibitor were equivalent to those of transfected si-NC cells. Compared with transfected si-SNHG7 cells, the proliferation and invasion ability were enhanced and apoptosis rate was decreased. Detection of EMT marker protein showed that the expression of E-cadherin, N-cadherin and Vimentin in UMUC3 cells after transfection of si-SNHG7+miR-34a-inhibitor was

not different from that of transfected si-NC cells, while E-cadherin decreased and N-cadherin and Vimentin increased compared with transfected si-SNHG7 cells (Figure 5).

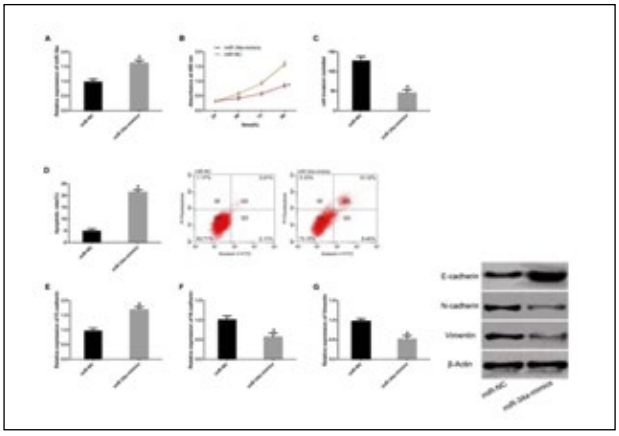

Figure 4: Effect of miR-34a on biological function of BC cells. Figure A, After transfection of UMUC3 cells with miR-34a-mimics, miR-34a was up-regulated. Figure B, After transfection of UMUC3 cells with miR-34a-mimics, the proliferation ability was decreased. Figure C, After transfection of UMUC3 cells with miR-34a-mimics, the invasion ability was decreased. Figure D, After transfection of UMUC3 cells with miR-34a-mimics, the apoptosis rate was increased. Figure E/F/G, After transfection of UMUC3 cells with miR-34a-mimics, E-cadherin increased while N-cadherin and Vimentin decreased.
*Note: *represents p<0.05.*

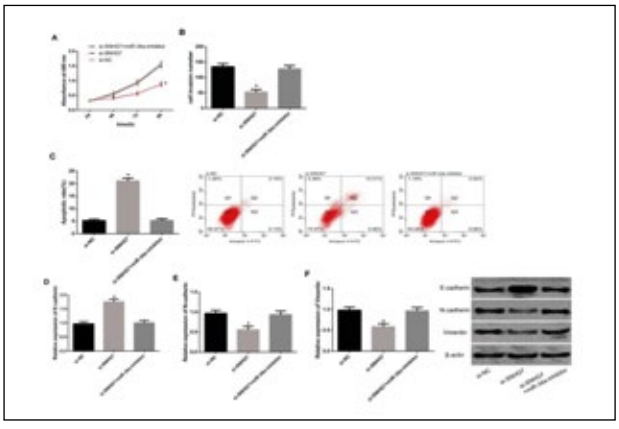

Figure 5: Effects of simultaneous inhibition of SNHG7 and miR-34a on biological function of BC cells. Figure A/B/C, The proliferation, invasion and apoptosis of cells after transfecting si-SNHG7+miR-34a-inhibitor were equivalent to those of transfected si-NC cells. Compared with transfected si-SNHG7 cells, the proliferation and invasion ability were enhanced and apoptosis rate was decreased. Figure D/E/F, The expression of E-cadherin, N-cadherin and Vimentin in UMUC3 cells after transfection of si-SNHG7+miR-34a-inhibitor was not different from that of transfected si-NC cells, while E-cadherin decreased and N-cadherin and Vimentin increased compared with transfected si-SNHG7 cells.
*Note: *represents p<0.05.*

LncRNA, as a long-chain non-coding RNA, has been regarded as a "waste product" in the transcription process due to its inability to encode proteins. However, in recent years, more and more studies have found that lncRNA can participate in biological events through various mechanisms from the transcription level to the post-transcription level[17, 18].

With the deepening understanding of lncRNA, its imbalance is considered to be one of the important factors for the development of various tumors including BC[19-21]. SNHG7 is a newly discovered lncRNA, which plays the role of carcinogenic gene in BC(11), but the specific mechanism still needs to be further explored. Therefore, we designed this study to explore the role of SNHG7 in BC and its related mechanisms. The results showed that SNHG7 was up-regulated in many BC cells. The inhibition of SNHG7 on cancer cells could inhibit cell proliferation and invasion and promote apoptosis.

More and more reports have found that lncRNAs can be used as endogenous competitive RNA(ceRNA) of miRNA, and they can be used as molecular sponge to competitively inhibit miRNA[22]. In this paper, the bioinformatics database analysis showed that miR-34a and SNHG7 had targeted binding sites. miR-34a was up-regulated after treatment of SNHG7 inhibition on cells. Dual luciferase report confirmed that miR-34a could be targeted and regulated by SNHG7. In tumors, miR-34a is considered as a tumor suppressor gene[23, 24]. Previous reports have pointed out that lncRNA can play an important role in tumor through miR-34a. For example, lncRNA Lnc-OC1 can promote the progress of the disease by inhibiting miR-34a in ovarian cancer cells[25]. In breast cancer, LncRNA UFC1 promotes cancer cell growth, invasion, migration and induces apoptosis by acting as miR-34a sponge and then regulating CXCL10 expression[26]. In esophageal squamous cell carcinoma, LncRNA MNX1-AS1 can promote the development of the disease through miR-34a/SIRT1 axis[27].

First, we found that miR-34a was down-regulated in many BC cells. Up-regulation of miR-34a expression could inhibit the proliferation and invasion of cancer cells and promote apoptosis. Subsequently, BC cells were treated with simultaneous inhibition of SNHG7 and miR-34a, which could reverse the effect of inhibition of SNHG7 on the biological function of cancer cells. This showed that SNHG7 could promote BC progress by up-regulating miR-34a.

EMT is an important factor affecting tumor growth, invasion and metastasis, of which miR is an important regulatory factor affecting EMT pro-

cess[28]. It has been reported that miR-34a can inhibit the EMT process of ovarian cancer cells[29]. As we all know, N-cadherin, Vimentin and E-cadherin are important markers of epithelial-mesenchymal transition (EMT), and the change of their expression can indicate the situation of EMT. In this paper, the effects of SNHG7 and miR-34a on EMT of BC cells were observed by detecting the expression changes of EMT markers of the three. The results showed that inhibition of SNHG7 or over-expression of miR-34a could up-regulate the expression of E-cadherin and inhibit the expression of N-cadherin and Vimentin in BC cells. In addition, inhibition of miR-34a could reverse the effect of inhibition of SNHG7 on the expression of EMT markers. This indicated that SNHG7 could also promote the process of BC cells to EMT by up-regulating miR-34a[30-38].

This article was designed to discuss the role of SNHG7 in BC and its related mechanisms at the molecular level. Our results showed that SNHG7 could promote BC cell proliferation, invasion, EMT and inhibit apoptosis through miR-34a, and SNHG7 could become a therapeutic target for BC. Previous studies have found that miR-34a can inhibit the progression of breast cancer by activating Wnt/β-catenin pathway. Therefore, it was speculated that SNHG7 could promote BC progress through miR-34a mediated Wnt /β-catenin signaling pathway in this article, and this speculation will be discussed in future experimental design. There are some deficiencies in this article. Firstly, the clinical value of SNHG7 was not discussed. Secondly, there was no in vivo experiment to observe the effect of SNHG7 on tumor growth. These deficiencies are expected to be supplemented in subsequent studies. To sum up, SNHG7 can promote the proliferation, invasion, EMT and inhibit apoptosis of BC cells through miR-34a.

References

1) Antoni S, Ferlay J, Soerjomataram I, Bladder cancer incidence and mortality: a global overview and recent trends[J]. European urology, 2017, 71(1): 96-108.
2) Bray F, Ferlay J, Soerjomataram I, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries[J]. CA: a cancer journal for clinicians, 2018, 68(6): 394-424.
3) Mohammed A A, Hani E L T, El-Khatib H M, Urinary bladder cancer: biomarkers and target therapy, new era for more attention[J]. Oncology reviews, 2016, 10(2).
4) Cao Z, Xu L, Zhao S, The functions of microRNA-124 on bladder cancer[J]. OncoTargets and therapy, 2019, 12: 3429.
5) Abdolmaleki F, Ghafoui-Fard S, Taheri M, Expression analysis of a panel of long non-coding RNAs (lncRNAs) revealed their potential as diagnostic biomarkers in bladder cancer[J]. Genomics, 2019.
6) Iyer M K, Niknafs Y S, Malik R, The landscape of long noncoding RNAs in the human transcriptome[J]. Nature genetics, 2015, 47(3): 199.
7) Cheng D, Fan J, Ma Y, LncRNA SNHG7 promotes pancreatic cancer proliferation through ID4 by sponging miR-342-3p[J]. Cell & Bioscience, 2019, 9(1): 28.
8) Zhang C, Zhu B, Li X B, Long non-coding RNA SNHG7 promotes migration and invasion of melanoma via up-regulating SOX4[J]. European review for medical and pharmacological sciences, 2019, 23(11): 4828-4834.
9) Dong J S, Wu B, Jiang B. LncRNA SNHG7 promotes the proliferation and inhibits apoptosis of renal cell cancer cells by downregulating CDKN1A[J]. European review for medical and pharmacological sciences, 2019, 23(23): 10241-10247.
10) Qi H, Wen B, Wu Q, Long noncoding RNA SNHG7 accelerates prostate cancer proliferation and cycle progression through cyclin D1 by sponging miR-503[J]. Biomedicine & Pharmacotherapy, 2018, 102: 326-332.
11) Zhong X, Long Z, Wu S, LncRNA-SNHG7 regulates proliferation, apoptosis and invasion of bladder cancer cells assurance guidelines[J]. Journal of BU ON.: official journal of the Balkan Union of Oncology, 2018, 23(3): 776-781.
12) Agarwal V, Bell G W, Nam J W, Predicting effective microRNA target sites in mammalian mRNAs[J]. elife, 2015, 4: e05005.
13) Rupaimoole R, Slack F J. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases[J]. Nature reviews Drug discovery, 2017, 16(3): 203.
14) Svoronos A A, Engelman D M, Slack F J. OncomiR or tumor suppressor? The duplicity of microRNAs in cancer[J]. Cancer research, 2016, 76(13): 3666-3670.
15) Ding Z S, He Y H, Deng Y S, MicroRNA-34a inhibits bladder cancer cell migration and invasion, and up-regulates PTEN expression[J]. Oncology letters, 2019, 18(5): 5549-5554.
16) Livak K J, Schmittgen T D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method[J]. methods, 2001, 25(4): 402-408.
17) Sullenger B A, Nair S. From the RNA world to the clinic[J]. Science, 2016, 352(6292): 1417-1420.
18) Joung J, Engreitz J M, Konermann S, Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood[J]. Nature, 2017, 548(7667): 343.
19) Nie W, Ge H, Yang X, LncRNA-UCA1 exerts oncogenic functions in non-small cell lung cancer by targeting miR-193a-3p[J]. Cancer letters, 2016, 371(1): 99-106.
20) Xu M, Wang Y, Weng W, A positive feedback loop of lncRNA-PVT1 and FOXM1 facilitates gastric cancer growth and invasion[J]. Clinical Cancer Research, 2017, 23(8): 2071-2080.
21) Wang Y, Shi F, Xia Y, LncRNA OIP5-AS1 predicts poor prognosis and regulates cell proliferation and apoptosis in bladder cancer[J]. Journal of cellular biochemistry, 2019, 120(5): 7499-7505.

22) Wieczorek E, Reszka E. mRNA, microRNA and lncRNA as novel bladder tumor markers[J]. Clinica Chimica Acta, 2018, 477: 141-153.
23) Chakravarthi B V S K, Chandrashekar D S, Agarwal S, miR-34a regulates expression of the stathmin-1 oncoprotein and prostate cancer progression[J]. Molecular Cancer Research, 2018, 16(7): 1125-1137.
24) Sun T Y, Xie H J, Li Z, miR-34a regulates HDAC1 expression to affect the proliferation and apoptosis of hepatocellular carcinoma[J]. American journal of translational research, 2017, 9(1): 103.
25) Tao F, Tian X, Lu M, A novel lncRNA, Lnc-OC1, promotes ovarian cancer cell proliferation and migration by sponging miR-34a and miR-34c[J]. Journal of Genetics and Genomics, 2018, 45(3): 137-145.
26) Xie R, Wang M, Zhou W, Long Non-Coding RNA (LncRNA) UFC1/miR-34a Contributes to Proliferation and Migration in Breast Cancer[J]. Medical science monitor: international medical journal of experimental and clinical research, 2019, 25: 7149.
27) Chu J, Li H, Xing Y, LncRNA MNX1-AS1 promotes progression of esophageal squamous cell carcinoma by regulating miR-34a/SIRT1 axis[J]. Biomedicine & Pharmacotherapy, 2019, 116: 109029.
28) Ding X M. MicroRNAs: regulators of cancer metastasis and epithelial-mesenchymal transition (EMT)[J]. Chinese journal of cancer, 2014, 33(3): 140.
29) Dong P, Xiong Y, Watari H, MiR-137 and miR-34a directly target Snail and inhibit EMT, invasion and sphere-forming ability of ovarian cancer cells[J]. Journal of Experimental & Clinical Cancer Research, 2016, 35(1): 132.
30) Wang H, An XQ, Zhang ZY, Effect of Advanced Treatment on Ammonia Nitrogen Contained in Secondary Effluent from Wastewater Treatment Plant. Fresenius Environ Bull 2018; 27(4): 2043-2050.
31) Yang F, Yang F, Wang GY, Kong T, Wang H, Zhang CS, Effects of water temperature on tissue depletion of florfenicol and its metabolite florfenicol amine in crucian carp (Carassius auratus gibelio) following multiple oral doses. Aquaculture 2020; 515: 734542-734550.
32) Zhang L, Chen JH, Lei Z, He HB, Wang YS, Li YH, Preparation of soybean oil factory sludge catalyst and its application in selective catalytic oxidation denitration process. J Clean Prod 2019; 225: 220-226.
33) Zhang L, Jia Y, Zhang L, He HB, Yang C, Luo M, Miao LT, Preparation of soybean oil factory sludge catalyst by plasma and the kinetics of selective catalytic oxidation denitrification reaction. J Clean Prod 2019; 217: 317-323.
34) Zhu BZ, Pang RZ, Chevallier J, Wei YM, Vo DT, Including intangible costs into the cost-of-illness approach: a method refinement illustrated based on the PM2.5 economic burden in China. Eur J Health Econ 2019; 20(4): 501-511.
35) Chen HX, Chen YT, Liu Y, Intelligent early structural health prognosis with nonlinear system identification for RFID signal analysis. Comput Commun 2020; 175(1): 150-161.
36) Xiong Q, Zhang X, Wang W, Gu Y, A Parallel Algorithm Framework for Feature Extraction of EEG Signals on MPI. Comput Math Method Med 2020; 1: 1-10.
37) Chen H, Zhang G, Fan D, Fang L, Huang L, Nonlinear Lamb wave analysis for microdefect identification in mechanical structural health assessment. Measurement 2020; 164: 108026-108036.
38) Yang M, Abdalrahman H, Sonia U, Mohammed A, Vestine U, Wang M, Ebadi AG, Toughani M. The application of DNA molecular markers in the study of Codonopsis species genetic variation, a review. Cell Mol Biol 2020; 2: 23-30.

Acknowledgement:
This work was supported by Scientific Research Project of Hubei Provincial Health and Family Planning Commission (WJ2015Z064).

Corresponding Author:
YiPeng Liu
Email: 1765119514@qq.com
(China)