Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

MiR-381-3p/RAB2A axis activates cell proliferation and inhibits cell apoptosis in bladder cancer

Xiaolu Sun[1], Xiufang Hu[2], Xiangtao Wang[1], Xianzhou Jiang[3*]

[1] Department of Urology, The Third Hospital of Shandong Province, Jinan, 250031, China
[2] Department of Internal Medicine, The People Hospital of Huaiyin, Jinan, 250021, China
[3] Department of Urology, Qilu Hospital of Shandong University, Jinan, 250012, China

[*]**Correspondence to:** williamson1@yeah.net

Received November 28, 2019; **Accepted** August 3, 2020; **Published** September 30, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.6.21

Abstract: Acting as a really common cancer in the world, bladder cancer has taken many people's life away. MiRNAs and mRNA have been reported can regulate the expression of cancers. In this study, the role of RAB2A and miR-381-3p was fully studied in bladder cancer. qRT-PCR assay probe the expression of RAB2A and miR-381-3p in bladder cancer cells. Meanwhile, colony formation assay, EdU assay, flow cytometry analysis, JC-1 assay and western blot assay were implemented to detect the progression of bladder cancer cells. Silenced RAB2A could reduce the cell proliferation of bladder cancer, and activate the apoptosis. Meanwhile, miR-381-3p could bind to RAB2A in bladder cancer cells and overexpressed miR-381-3p could inhibit the progression of bladder cancer cells. MiR-381-3p/RAB2A axis activates cell proliferation and inhibits cell apoptosis in bladder cancer.

Key words: miR-381-3p; RAB2A; Bladder cancer.

Introduction

Bladder cancer is a type of epithelial cell carcinoma. It is the second most common cancer of the urogenital tract. The average age for diagnosing bladder cancer is 65 years old. The incidence of bladder cancer is higher in men. This cancer is more common in whites than in blacks (1).

Bladder cancer begins when the cells lining the inner surface of the bladder change and their growth gets out of control, forming a mass called a tumor. Bladder tumors can be benign or malignant. Malignancy means that cancer cells can grow and spread to other parts of the body, a process called metastasis. But a benign tumor does not metastasize to other parts of the body. Benign bladder tumors are rare (2).

Bladder cancer is a really common tumor that happened in the mucosa of the bladder in the urogenital system. And there are no less than 420000 new bladder cancer patients found and 160000 people dead because of bladder cancer every year (3). Nearly 70% of bladder cancer patients are found without the ability of invasion, and bladder cancer can be treated with surgical therapy. However, there are 30% of bladder cancer cases have the symptom of cancer invasion and metastasis (4, 5). For patients with metastasis progression, there is no effective and satisfactory therapy in improving the life expectancy of bladder cancer patients (6). Thence, we need to explore deeper the mechanism underlying bladder cancer.

MicroRNAs (miRNAs) have been studied as a group of RNAs that are as short as 20-22 nucleotides. And miRNAs play an important function in regulating the expression of messenger RNAs (mRNAs) by target binding to mRNAs in the modulation progress of post-transcription (7). Meanwhile, miRNAs have been reported aberrantly regulated in various cancers, which have been proved their dys-regulation modulates the progression of tumor cells, such as cell growth, apoptosis, migration and invasion (8-12). Meanwhile, plenty of studies have proved that miRNAs have been involved in the progression of bladder cancer (13-16). Also, a previous study has proved that miR-381-3p can function as a tumor suppresser in many cancers, such as oral squamous cell carcinoma, papillary thyroid carcinoma and cervical cancer (17-19). However, the function of miR-381-3p was still not been scrutinized in bladder cancer. In this study, we are going to search for the function of miR-381-3p in bladder cancer.

Materials and Methods

Cell culture and transfection

Human bladder cancer cells (T24, 5637, J82, RT-4) and normal human bladder epithelial cells (SV-HUC-1), from ATCC, were cultured in DMEM with 10% FBS as supplements at 37°C in 5% CO_2. For transfection, the sh-RAB2A #1/2 and NC-shRNAs, as well as the miR-381-3p mimics and NC mimics were all procured from Genepharma Company (Shanghai, China). Transfection kit Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was utilized.

qRT-PCR

The total RNAs were isolated using Trizol reagent and then reversely transcribed into cDNA using specific reverse Transcription Kit. Expression levels of gene were measured through qRT-PCR using SYBR mix (TaKaRa, Shiga, Japan) and calculated with the $2^{-\Delta\Delta Ct}$ method.

Colony formation

Clonogenic cells were placed into 6-well plates and incubated for 14 days. After fixation, cells were stained with 0.5% crystal violet for counting.

Flow cytometry

Cell apoptosis rate was monitored by using the Annexin-V fluorescein isothiocyanate (FITC)/propidium iodide (PI) dual staining kit (BD Biosciences, San Jose, CA). The stained cells were assayed by flow cytometry (BD Biosciences).

JC-1 assay

JC-1 assay was achieved by treating cell samples with 10 mM of JC-1 dye ((Beyotime, Shanghai, China) for half an hour after centrifugation. Images were taken by a fluorescence microscope.

RNA pull-down

RNA pull-down assay was implemented by employing the Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific, Waltham, MA) as per the user manual. Protein extracts were mixed with Bio-miR-381-3p-WT/Mut and Bio-NC, then analyzed by qRT-PCR.

Luciferase reporter assay

For luciferase assay, the pmirGLO-RAB2A-WT/Mut reporter vectors were co-transfected with miR-381-3p mimics or NC mimics in cell samples. Luciferase Reporter Assay System (Promega, Madison, WI) was used for detecting luciferase activity.

Statistical analysis

All experiments were repeated thrice, results were exhibited as the means ± S.D. and assayed using GraphPad Prism V5.0 Software. Statistical analyses were performed with one-way ANOVA and Student's t-test, with $p < 0.05$ as a threshold.

Results

Silenced RAB2A could reduce the cell proliferation of bladder cancer.

RAB2A can enhance the invasiveness of breast cancer (20). Also, RAB2A can improve the stemness of breast cancer cells and enhance breast cancer progression (21). In our study, mRNA RAB2A expression was detected in bladder cancer cells (T24, 5637, J82 and RT-4) and normal human bladder epithelial cells (SV-HUC-1) by qRT-PCR assay (Figure 1A). Results found that RAB2A expression was highly increased in T24, 5637, J82 and RT-4 cells, especially in T24 and J82 cells. Thence, T24 and J82 cells were used to detect the inhibition efficiency of RAB2A by qRT-PCR assay (Figure 1B). Meanwhile, colony formation assay

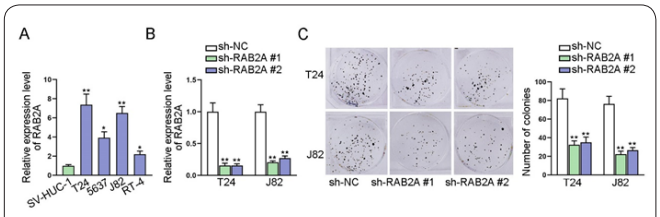

Figure 1. Silenced RAB2A could reduce the cell proliferation of bladder cancer. A: mRNA RAB2A expression was detected in bladder cancer cells (T24, 5637, J82 and RT-4) and normal human bladder epithelial cells (SV-HUC-1) by qRT-PCR assay. B: inhibition efficiency of RAB2A was examined by qRT-PCR assay. C: Colony formation assay assessed the cell proliferation of T24 and J82 cells.

(Figure 1C). We found the proliferation of T24 and J82 cells was significantly inhibited by silenced RAB2A. In conclusion, silenced RAB2A could reduce the cell proliferation of bladder cancer.

Inhibited RAB2A could activate the apoptosis of bladder cancer cells.

We further searched the function of miR-381-3p in the apoptosis of T24 and J82 cells. Flow cytometry analysis and JC-1 assay were implemented to know about cell apoptosis of T24 and J82 when RAB2A was depleted. And we detected that cell apoptosis rate increased significantly by inhibited RAB2A and the JC-1 ratio was also inhibited by silenced RAB2A (Figure 2A-B). In conclusion, inhibited RAB2A could activate the apoptosis and JNK/p38 pathway in bladder cancer cells.

MiR-381-3p could bind to RAB2A in bladder cancer cells.

miR-381-3p can inhibit oral squamous cell carcinoma by regulating the expression (17). Also, miR-381-3p can suppress the progression of papillary thyroid carcinoma (18). And, miR-381-3p can reduce the progression of cervical cancer (19). In our study, we found that miR-381-3p was down-regulated in bladder cancer cells (T24, 5637, J82 and RT-4) (Figure 3A). Meanwhile, the overexpression efficiency of miR-381-3p was verified

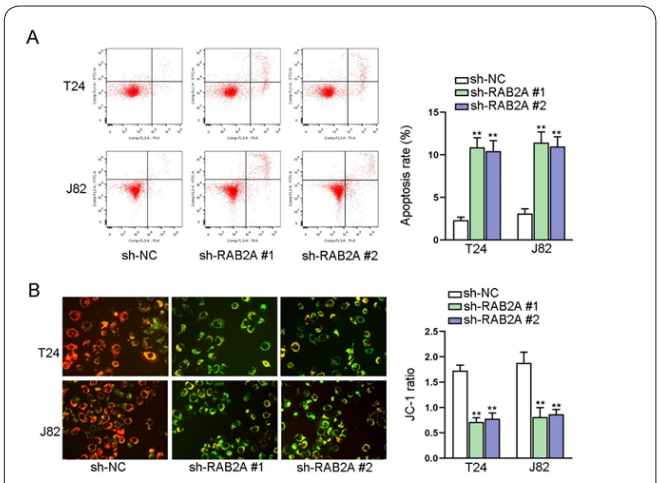

Figure 2. Inhibited RAB2A could activate the apoptosis of bladder cancer cells. A-B: Cell apoptosis of T24 and J82 was verified by flow cytometry analysis and JC-1 assay when RAB2A was depleted.

by qRT-PCR assay (Figure 3B). Also, the binding relationship between miR-381-3p and RAB2A was detected by RNA pull-down assay and luciferase reporter assay (Figure 3C-E). And we found that RAB2A was enriched in the pull-down of miR-381-3p wild type, and the luciferase activity was decreased by overexpressed miR-381-3p. At the same time, the binding site of miR-381-3p and RAB2A was presented with the information on ENCORI. In a word, miR-381-3p could bind to RAB2A in bladder cancer cells.

Overexpressed miR-381-3p could inhibit the progression of bladder cancer cells.

Then, we further verified the function of miR-381-3p in bladder cancer cells. Functional assays were implemented to investigate cell proliferation and apoptosis, such as colony formation assay, EdU assay, flow cytometry analysis and JC-1 assay. Colony formation assay and EdU assay found the cell growth was reduced by overexpressed miR-381-3p (Figure 4A-B). And Flow cytometry analysis and JC-1 assay detected that cell apoptosis was elevated by overexpressed miR-381-3p (Figure 4C). In conclusion, overexpressed miR-381-3p could inhibit cell proliferation and activate the apoptosis of bladder cancer cells.

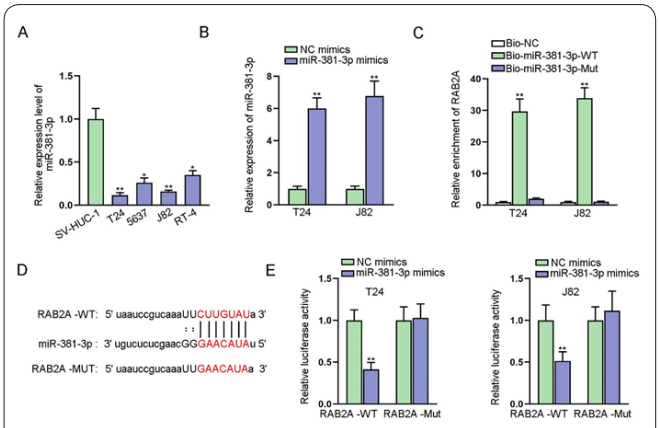

Figure 3. MiR-381-3p could bind to RAB2A in bladder cancer cells. A: miR-381-3p expression was delineated by qRT-PCR assay in bladder cancer cells (T24, 5637, J82 and RT-4) and normal human bladder epithelial cells (SV-HUC-1). B: overexpression efficiency of miR-381-3p was verified by qRT-PCR assay. C-E: The binding relationship between miR-381-3p and RAB2A was detected by RNA pull-down assay and luciferase reporter assay. And the binding site was presented with the information of ENCORI (http://starbase.sysu.edu.cn/index.php).

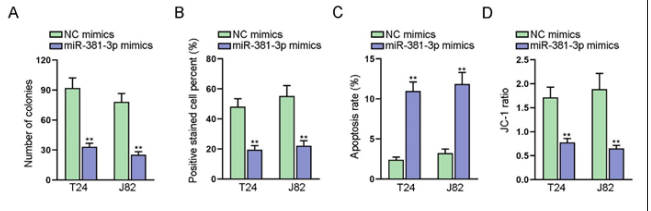

Figure 4. Overexpressed miR-381-3p could inhibit the progression of bladder cancer cells. A-B: Colony formation assay and EdU assay assessed the cell proliferation of T24 and J82 cells. C: Flow cytometry analysis and JC-1 assay verified the cell apoptosis of T24 and J82 by when RAB2A was depleted.

Discussion

RAB2A, member RAS oncogene family (RAB2A) have been studied can function as an oncogene in many cancers. For instance, RAB2A can enhance the invasiveness of breast cancer by regulating MT1-MMP and E-cadherin (20). Also, RAB2A can activate the Erk pathway to improve the stemness of breast cancer cells and to enhance breast cancer progression (21). In our study, we explored the function of RAB2A in bladder cancer. Firstly, the qRT-PCR assay found that RAB2A was highly expressed in bladder cancer cells. And meanwhile, inhibited RAB2A was detected can reduce the bladder cancer cell proliferation by colony formation assay and EdU assay. Furthermore, flow cytometry analysis and JC-1 assay were conducted and we found the cell apoptosis is also elevated by inhibited RAB2A. Meanwhile, western blot assay detected that the JNK/p38 pathway was inhibited by silenced RAB2A.

MiR-381-3p can play the inhibitory function in many cancers. For example, miR-381-3p can inhibit cell growth and activate cell apoptosis of oral squamous cell carcinoma by regulating the expression of FGFR2 (17). Also, miR-381-3p can suppress the progression of papillary thyroid carcinoma via modulating LRP6 (18). And, miR-381-3p can reduce the progression of cervical cancer by down-regulated the expression of FGF7 (19). In our study, we searched for the role of miR-381-3p in bladder cancer. MiR-381-3p was found lowly expressed in bladder cancer cells. Meanwhile, RNA pull-down assay and luciferase reporter assay found that miR-381-3p could bind to RAB2A in bladder cancer cells. Rescue assay detected that miR-381-3p/RAB2A axis could modulate the progression of bladder cancer. In conclusion, the miR-381-3p/RAB2A axis can promote the progression of bladder cancer.

In genetics, gene expression is one of the most important and essential issues that assistance a genotype to appear as a phenotype. The genetic code stored in the DNA strands is interpreted by gene expression, and the characteristics and expression of the gene will cause a phenotype in the organism (22-33). In this research, we investigated on the MiR-381-3p/RAB2A axis activates cell proliferation and inhibits cell apoptosis in bladder cancer.

References

1. Kirkali Z, Chan T, Manoharan M, Algaba F, Busch C, Cheng L, Kiemeney L, Kriegmair M, Montironi R, Murphy WM, Sesterhenn IA. Bladder cancer: epidemiology, staging and grading, and diagnosis. Urolog 2005; 66(6):4-34.

2. Neu J. The roles of tumor-suppressive microRNA-181a and CYFIP1 in cancerous proliferation and invasion of cutaneous squamous cell carcinoma (Doctoral dissertation, University of Zurich). 2017.

3. 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: a Cancer J Clinic 2018; 68(6): 394-424.

4. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, Lotan Y. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 2013; 63: 234-41.

5. Stein JP, Lieskovsky G, Cote R, Groshen S, Feng AC, Boyd S, Skinner E, Bochner B, Thangathurai D, Mikhail M, Raghavan D, Skinner DG. Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. J Clin Oncol 2001;19: 666-75.
6. Liang Z, Wang X, Xu X, Xie B, Ji A, Meng S, Li S, Zhu Y, Wu J, Hu Z, Lin Y, Zheng X, Xie, L & Liu, B. MicroRNA-608 inhibits proliferation of bladder cancer via AKT/FOXO3a signaling pathway. Mol Cancer 2017; 16: 96.
7. Bartel D P. MicroRNAs: target recognition and regulatory functions. Cell 2009: 136: 215-33.
8. Hwang HW, Mendell, JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 2006; 94: 776-80.
9. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 2007; 449: 682-8.
10. Tavazoie SF, Alarcón C, Oskarsson T, Padua D, Wang Q, Bos PD, Gerald WL, Massagué J. Endogenous human microRNAs that suppress breast cancer metastasis. Nature 2008; 451: 147-52.
11. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A. 2004; 101: 2999-3004.
12. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterol 2007; 133: 647-58.
13. Braicu C, Cojocneanu-Petric R, Chira S, Truta A, Floares A, Petrut B, Achimas-Cadariu P, Berindan-Neagoe I. Clinical and pathological implications of miRNA in bladder cancer. Int J Nanomedicine 2015; 10: 791-800.
14. Gottardo F, Liu CG, Ferracin M, Calin GA, Fassan M, Bassi P, Sevignani C, Byrne D, Negrini M, Pagano F, Gomella LG, Croce CM, Baffa R. Micro-RNA profiling in kidney and bladder cancers. Urol Oncol 2007; 25: 387-92.
15. Ichimi T, Enokida H, Okuno Y, Kunimoto R, Chiyomaru T, Kawamoto K, Kawahara K, Toki K, Kawakami K, Nishiyama K, Tsujimoto G, Nakagawa M, Seki N. Identification of novel microRNA targets based on microRNA signatures in bladder cancer. Int J Cancer 2009; 125: 345-52.
16. Adam L, Zhong M, Choi W, Qi W, Nicoloso M, Arora A, Calin G, Wang H, Siefker-Radtke A, McConkey D, Bar-Eli M, Dinney C. miR-200 expression regulates epithelial-to-mesenchymal transition in bladder cancer cells and reverses resistance to epidermal growth factor receptor therapy. Clin Cancer Res 2009; 15: 5060-72.
17. Yang X, Ruan H, Hu X, Cao A, Song L. miR-381-3p suppresses the proliferation of oral squamous cell carcinoma cells by directly targeting FGFR2. Am J Cancer Res 2017; 7: 913-922.
18. Kong W, Yang L, Li PP, Kong QQ, Wang HY, Han GX, Wang QB. MiR-381-3p inhibits proliferation, migration and invasion by targeting LRP6 in papillary thyroid carcinoma. Eur Rev Med Pharmacol Sci 2018; 22: 3804-3811.
19. Shang A, Zhou C, Bian G, Chen W, Lu W, Wang W, Li, D. miR-381-3p restrains cervical cancer progression by downregulating FGF7. J Cell Biochem 2019; 120: 778-789.
20. Kajiho H, Kajiho Y, Frittoli E, Confalonieri S, Bertalot G, Viale G, Di Fiore PP, Oldani A, Garre M, Beznoussenko GV, Palamidessi A, Vecchi M, Chavrier P, Perez F, Scita G. RAB2A controls MT1-MMP endocytic and E-cadherin polarized Golgi trafficking to promote invasive breast cancer programs. EMBO Rep 2016; 17: 1061-80.
21. Luo ML, Gong C, Chen CH, Hu H, Huang P, Zheng M, Yao Y, Wei S, Wulf G, Lieberman J, Zhou XZ, Song E. Lu KP. The Rab2A GTPase promotes breast cancer stem cells and tumorigenesis via Erk signaling activation. Cell Rep 2015; 11: 111-24.
22. Kahrizi D, Ghari SM, Ghaheri M, Fallah F, Ghorbani T, Beheshti AA, Kazemi E, Ansarypour Z. Effect of KH2PO4 on gene expression, morphological and biochemical characteristics of stevia rebaudiana Bertoni under in vitro conditions. Cell Mol Biol, 63(7): 107-111.
23. Fallah F, Nokhasi F, Ghaheri M, Kahrizi D, Beheshti Ale AA, Ghorbani T, Kazemi E, Ansarypour Z. Effect of salinity on gene expression, morphological and biochemical characteristics of Stevia rebaudiana Bertoni under in vitro conditions. Cell Mol Biol 2017; 63(7): 102-106.
24. Bordbar M, Darvishzadeh R, Pazhouhandeh M, Kahrizi D. An overview of genome editing methods based on endonucleases. Mod Genet J 2020; 15(2): 75-92.
25. Ghaheri M, Kahrizi D, Bahrami G, Mohammadi-Motlagh HR. Study of gene expression and steviol glycosides accumulation in Stevia rebaudiana Bertoni under various mannitol concentrations. Mol Biol Rep 2019; 46(1): 7-16.
26. Eruygur N, Ucar E, Akpulat HA, Shahsavari K, Safavi SM, Kahrizi D. In vitro antioxidant assessment, screening of enzyme inhibitory activities of methanol and water extracts and gene expression in Hypericum lydium. Mol Biol Rep 2019; 46(2): 2121-9.
27. Esmaeili F, Ghaheri M, Kahrizi D, Mansouri M, Safavi SM, Ghorbani T, Muhammadi S, Rahmanian E, Vaziri S. Effects of various glutamine concentrations on gene expression and steviol glycosides accumulation in Stevia rebaudiana Bertoni. Cell Mol Biol, 64(2): 1-5.
28. Akbari F, Arminian A, Kahrizi D, Fazeli A, Ghaheri M. Effect of nitrogen sources on gene expression of Stevia rebaudiana (Bertoni) under in vitro conditions. Cell Mol Biol, 64(2): 11-16.
29. Ghaheri M, Adibrad E, Safavi SM, Kahrizi D, Soroush A, Muhammadi S, Ghorbani T, Sabzevari A, Ansarypour Z, Rahmanian E. Effects of life cycle and leaves location on gene expression and glycoside biosynthesis pathway in Stevia rebaudiana Bertoni. Cell Mol Biol, 64(2): 17-22.
30. Kahrizi D, Ghaheri M, Yari Z, Yari K, Bahraminejad S. Investigation of different concentrations of MS media effects on gene expression and steviol glycosides accumulation in Stevia rebaudiana Bertoni. Cell Mol Biol, 64(2): 23-27.
31. Hashempoor S, Ghaheri M, Kahrizi D, Kazemi N, Muhammadi S, Safavi SM, Ghorbani T, Rahmanian E, Heshmatpanaah M. Effects of different concentrations of mannitol on gene expression in Stevia rebaudiana Bertoni. Cell Mol Biol, 64(2): 28-31.
32. Akbarabadi A, Ismaili A, Kahrizi D, Firouzabadi FN. Validation of expression stability of reference genes in response to herbicide stress in wild oat (Avena ludoviciana). Cell Mol Biol 2018; 64(4). 113-118.
33. Ghorbani T, Kahrizi D, Saeidi M, Arji I. Effect of sucrose concentrations on Stevia rebaudiana Bertoni tissue culture and gene expression. Cell Mol Biol, 63(11): 32-36.