World Journal of Urology (2019) 37:1889–1897
https://doi.org/10.1007/s00345-018-2596-9

ORIGINAL ARTICLE

RAB38 promotes bladder cancer growth by promoting cell proliferation and motility

Da-Wei Tian[1,2,3] · Sheng-Lai Liu[1,2,3] · Li-Ming Jiang[1] · Zhou-Liang Wu[1,2,3] · Jie Gao[1,2,3] · Hai-Long Hu[1,2,3] · Chang-Li Wu[1,2,3]

Received: 20 October 2018 / Accepted: 3 December 2018 / Published online: 10 December 2018

Abstract

Background Bladder cancer is the most common malignancy of urinary system with high morbidity and mortality. In general, the development and progression of bladder cancer are complicated pathological processes, and the treatment methods mainly include surgical resection, radiotherapy, chemotherapy, and combined therapy. In recent years, targeted therapy has made progress in the treatment of bladder cancer. Therefore, to improve survival rates of patients with advanced bladder cancer, novel therapeutic targets are still urgently needed.

Methods and results In this study, we found that RAB38 expressed in tumor tissues of patients with bladder cancer was linked to clinical features including pTNM stage and tumor recurrence, and positively correlated with the poor prognosis of bladder cancer. Notably, further results indicated that depletion of RAB38 could significantly inhibit the proliferation and motility of two types of human bladder cancer cells, T24 and 5637 cells. In addition, RAB38 ablation obviously blocked tumor growth and development in mice compared with control.

Conclusion In conclusion, this study provides significant evidence that RAB38 promotes the development of bladder cancer and provides a novel therapeutic target of bladder cancer.

Keywords Bladder cancer · RAB38 · Prognosis · Proliferation · Motility

Introduction

Bladder cancer is the most common malignant tumor of urinary system, and its morbidity occupies the fourth place in male malignant tumors and ninth in female [1–3]. In fact, early stage of bladder cancer lacks obvious symptoms, whereas advanced bladder cancer is highly metastatic, which results in high mortality of this disease [4, 5]. The development of bladder cancer is a complicated pathological process, increasing the difficulty of treatment [6]. As for bladder cancer, the main treatment methods include surgical resection, radiotherapy, chemotherapy and combined therapy [7]. In recent years, as VEGF/VEGFR, EGFR, and endostatin became therapeutic targets for bladder cancer, targeted therapy has made some advances in the treatment of bladder cancer [8–10]. However, the 5-year survival rate of bladder cancer patients did not significantly increase, whereas the annual incidence of bladder cancer increased by 36% in the last decade [11]. Therefore, novel therapeutic targets are still urgently needed.

Rabs, which consist of more than 60 members, belong to small GTPase family. Rabs are involved in multiple cellular processes, including endocytosis, signal transduction, and cellular secretion, and the formation, docking, and fusion of vesicles or organelles [12, 13]. A variety of Rabs, such as RAB25 and RAB27B, have been reported for the role in

Da-Wei Tian, Sheng-Lai Liu, and Li-Ming Jiang contributed equally to this study.

✉ Da-Wei Tian
jianshi001@126.com

✉ Chang-Li Wu
wucl2003@163.com

1 Sino-Singapore Eco-city Hospital of Tianjin Medical University, No. 3333, Hechang Road, Binhai New Area, Tianjin City 300467, China

2 Department of Urology, The Second Hospital of Tianjin Medical University, Pingjiang Road 23, Hexi District, Tianjin 300211, China

3 Tianjin Key Laboratory of Urology, Tianjin Institute of Urology, Pingjiang Road 23, Hexi District, Tianjin 300211, China

tumor growth and development [14, 15]. RAB38, a member of the RAB small G protein family, was involved in membrane trafficking, cell growth and differentiation [12–14]. A point mutation in the postulated GTP-binding domain of RAB38 has been demonstrated to lead to human Hermansky–Pudlak syndrome [15–17]. RAB38 is high-expressed in multiple specific tissues and regulates intracellular vesicle trafficking, including early vesicle transport to the endoplasmic reticulum [16]. Previous study indicated that RAB38 was associated with the synthesis, storage, and transport of melanin pigments in the biogenesis of melanosomes [16–19]. RAB38 is expressed in melanocytes and interacts with RAB32 and MYO5C, and therefore regulates melanosome biogenesis and secretion [17]. The defect of RAB38 could lead to several organ diseases and oculocutaneous albinism [18]. Previous study also provided evidence that RAB38 expression was associated with the poor prognosis of patients with glioma [19]. Furthermore, RAB38 is also over-expressed at the mRNA level in melanoma cancer [20, 21]. However, the role of RAB38 in the development of other tumors is still unknown.

Herein, we demonstrated that the expression of RAB38 was positively associated with the poor prognosis of bladder cancer patients. RAB38, additionally, was found obviously link to the tumor pTNM stage and recurrence of patients with bladder cancer. Moreover, knockdown of RAB38 in T24 and 5637 human bladder cancer cells dramatically blocked cell proliferation and motility in vitro, and inhibited tumor growth and development in mice. Collectively, RAB38 was proved to have high potential as a novel therapeutic target in the treatment of bladder cancer.

Materials and methods

Antibodies, primers, and plasmids

Rabbit anti-RAB38 (for immunohistochemical, 1:400 dilution, for immunoblot, 1:2000 dilution, bs-11244R, Bioss biotechnology), Mouse anti-β-actin (1:1000 dilution, ab8226, Abcam). The qRT-PCR primer sequences of RAB38 were as follows: forward, 5′-GTAATCGGCGACCTAGGTG-3′ and reverse, 5′-TCCATTCCCGGAACCTTCAC-3′; The qRT-PCR primer sequences of β-actin were as follows: forward, 5′-CAGCTCACCATGGATGATGATATC-3′ and reverse, 5′-AAGCCGGCCTTGCACAT-3′.

shRNA clone of vector (TRCN0000048183) was purchased from the Open Biosystems (Huntsville). Then we designated the plasmid as shRNA-targeted RAB38.

Human tissue samples and analysis

One hundred and fifteen patients clinically and pathologically diagnosed with bladder cancer at the Second Hospital of Tianjin Medical University. Tumor tissues were obtained after the surgical treatment and collected in this study. The clinical characters, such as ages, genders, pTNM stage, tumor size, and recurrence of patients were recorded.

To further explore the association between RAB38 and bladder cancer, immunohistochemical assays were then performed. Briefly, paraffin-embedded tumor tissue sections were cut, and then sample sections were deparaffinized, rehydrated with xylene and graded alcohols. Then sections were blocked with 2% BSA and incubated with RAB38 antibody for 2 h. Subsequently the sections were incubated with biotinylated secondary antibody for 1.5 h and streptavidin–biotin–peroxidase, and diaminobenzidine was used as a chromogen substrate. Finally, haematoxylin counterstaining was performed.

RAB38 was found mainly located in the cytoplasm of bladder cancer tissues. The proportion of positive tumor cells was graded as follows: 0, negative tumor cells; 1, < 5% positive tumor cells, 5–20% positive tumor cells and more than 20%, positive tumor cells. The staining intensity was evaluated on a score of 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining). The expression level of RAB38 was calculated based on the staining index: staining index was calculated as follows: staining index = staining intensity × positive tumor cell staining level. Staining index ≥ 4 was considered high expression, while staining index < 4 was considered low expression.

Cell culture and transfection

T24 and 5637 human bladder cancer cell lines were bought from ATCC. Both T24 and 5637 cells were cultured in RPMI-1640 culture medium and supplemented with 10% of fetal bovine serum. Cells were incubated at 37 °C in a 5% CO_2 incubator. The plasmids of shRNA-targeted RAB38 were transfected into bladder cancer cells by lipofectamine 3000 (Invitrogen). RAB38 stable depletion T24 cell lines were screened by shRNA lentivirus infection and used for the animal assays.

Quantitative PCR assay

Total RNA was extracted from T24 and 5637 cells using Trizol reagent (Invitrogen), respectively. Then the RNA was reverse-transcribed by M-MLV reverse transcriptase (Promega). Quantitative real-time PCR was performed using

SYBR mixture (Takara), and the relative expression level of RAB38 was normalized to the expression of β-actin.

Immunoblot assay

Total protein samples were extracted from bladder cancer cells or tumor tissues and the procedure for Immunoblot analysis was described before. The NC membranes were blocked with 5% dry milk in TBST buffer and then incubated with the primary antibodies for detection of RAB38 and β-actin for 2 h. Subsequently the membranes were incubated with HRP-conjugate secondary antibodies for 1 h. Signals were visualized with an ECL kit.

Cell proliferation assays

For colony formation assay, 2000 bladder cancer cells were added to a 6-well culture plate and transfected with RAB38 or control shRNA and cultured at 37 °C for 48 h. Cells were subsequently fixed with 70% ethanol at − 20 °C and stained with 0.1% crystal violet at room temperature for 15 min and washed with PBS twice. Cell numbers were then counted.

For MTT assays, cells were plated in 96-well plates with a density of about 1000 cells each well, transfected with shRNA and cultured for 48 days. Cells were then incubated with MTT for 2 h and removed the medium. Subsequently cells were washed with PBS buffer. MTT was extracted by 200 μL DMSO and the absorbance value was quantified with a microplate reader at a wave length of 570 nm.

Cell motility assays

For transwell assays, T24 and 5637 cells were transfected with control or RAB38-shRNA for 48 h and then re-suspended in serum-free RPMI-1640 medium. The upper chambers of filters (8.0 μm pores) were subsequently coated with 20% matrigel and incubated at 37 °C for 1 h to form a thin gel layer. A total of 105 cells in 150 μL of medium were then added to the upper chambers of the inserts and were induced to migrate toward the bottom chambers, which contained RPMI-1640 medium with 10% FBS. After 24 h, the cells that remained in the top chamber were removed, and cells on the underside were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet for 20 min, and photographs were then taken. Quantification of migrated cells was performed by dissolving crystal violet with 10% acetic acid, and the optical density of each sample was read with a microplate reader at 570 nm wave length.

For wound closure assays, bladder cancer cells were transfected and grown as confluent monolayers. Then mechanical wound was made with a 10 μL pipette tip to generate the wound. Cell debris was washed twice with PBS, and the complete culture medium was added to induce wound healing. Photographs were taken at 0 h and 24 h, and the extent of wound closure was measured and calculated.

In vivo tumor growth and metastasis assays

All animal assay procedures were approved by our Institutional Animal Care and Use Committee.

For tumor growth assay, T24 cells were stable transfected with control or RAB38 shRNA lentivirus. About 3×10^6 cells were subcutaneously inoculated into athymic nude mice. After 2 weeks, when tumors had established, the tumor was isolated, photographed, and the tumor volume was measured per week with a caliper and calculated in length × (width 2)/2.

For metastasis assay, about 106 T24/shControl, T24/shRAB38 cells in 100 μL PBS buffer was injected through the tail vein to induce the lung metastasis. After 7 weeks, all mice were sacrificed and the lungs of mice were isolated and photographed. Pulmonary metastases were then measured.

Statistics

Data are presented as the mean ± SEM, and the Student's *t* test was used for comparison between two groups. Connection between RAB3 8 expression level and patients' survival time after surgery were performed by Kaplan–Meier method. The categorical data were analyzed by Chi square test. The value of statistical significant was considered as $P < 0.05$. $^*P < 0.05$.

Results

RAB38 is positively correlated with the prognosis of patients with bladder cancer

To explore the relationship between RAB38 expression and bladder cancer growth, Immunohistochemical assays and survival analysis were performed. The expression of RAB38 in surgery samples from 115 patients with bladder cancer was detected. Immunohistochemical results indicated that RAB38 was mainly localized in plasma membrane (Fig. 1a). Actually, RAB38 was found highly expressed in the bladder cancer tissues (Fig. 1a). According to the expression of RAB38, surgery samples are classified into RAB38 low (31.3%, $n = 36$) and high- (68.7%, $n = 79$) expression groups (Table 1). Also, according the staining results of non-tumor adjacent tissues marked low RAB38 expression was detected in adjacent tissues, suggesting that RAB38 may play a critical role in bladder cancer growth (Fig. 1b).

Then we analyzed the difference of clinicopathological characteristic between low and high RAB38 expression groups. Results showed that the expression of RAB38 in

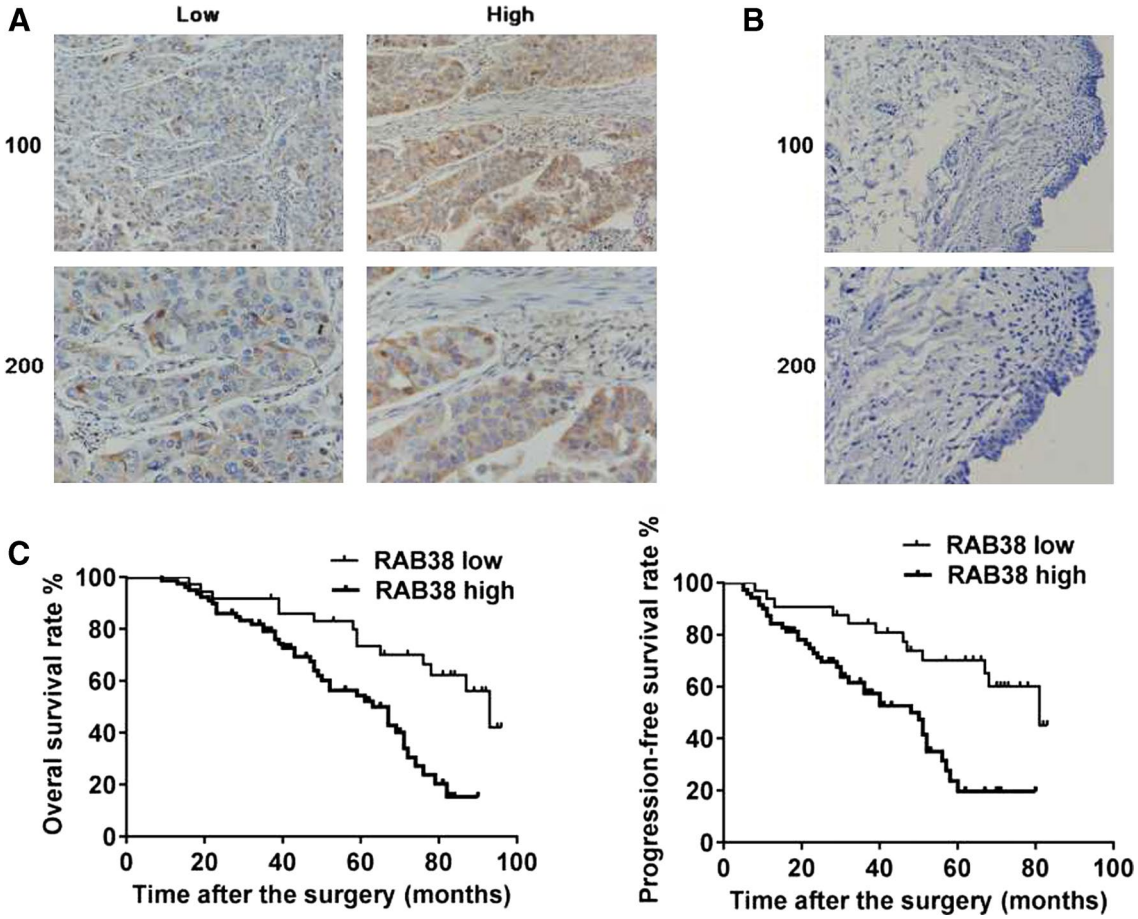

Fig. 1 RAB38 was positively associated with the poor prognosis of bladder cancer patients. **a** Immunohistochemical assays were performed, and the representive photographs of RAB38 expression level in bladder cancer tissues were exhibited (×100 and ×200 magnification, respectively). **b** Immunohistochemical assay results of RAB38 expression in the adjacent tissues are shown (×100 and ×200 magnification, respectively). **c** The KM-plot analysis of overall survival rate and disease-free survival rate between RAB38 low- and high-expression groups was performed, and the results are shown

the bladder tumor tissues was obviously associated with tumor pTNM stage ($p = 0.000$) and recurrence ($p = 0.016$), indicating a potential relationship between RAB38 and bladder cancer (Table 1). However, no significant difference was found between RAB38 high and low expression groups in other characteristics, such as patient age, gender, tumor grade, and lymph node metastasis (Table 1).

On the basis of previous results, we further investigated the prognosis of bladder cancer patients of RAB38 low and high-expressed groups, and found that patients with low expression of RAB38 had higher overall survival rate and disease-free survival rate, compared with patients in high-expression groups (Fig. 1c). In conclusion, these data firstly revealed that RAB38 was correlated with the poor prognosis of patients with bladder cancer.

RAB38 depletion inhibited the proliferation and motility of bladder cancer cells in vitro

To explore the regulation mechanism of RAB38 promoting bladder cancer, we used shRNA-targeted RAB38 to block its expression in T24 and 5637 human bladder cancer cells. The knockdown efficiency mediated by RAB38 shRNA was detected simultaneously by both quantitative PCR and Immuneblot assays. Results revealed that RAB38 shRNA was enough to obviously block RAB38 expression in both mRNA and protein level, respectively (Fig. 2a, b).

Uncontrolled proliferation and abnormal migration could result in tumor formation and metastasis [20]. Thus we then examined the effect of RAB38 on the proliferation and migration of two types of bladder cancer cells. Performing

Table 1 Relationships of RAB38 and clinicopathological characteristics in 115 patients with bladder cancer

Feature	All $n=115$	RAB38 expression		χ^2	P
		Low	High		
		$n=36$	$n=79$		
Age (year)				2.184	0.139
< 65	46	18	28		
≥ 65	69	18	51		
Gender				0.332	0.565
Male	99	30	69		
Female	16	6	10		
Tumor stage				13.431	0.000*
T2	54	26	28		
T3/T4	61	10	51		
Tumor grade				1.408	0.235
Low	33	13	20		
High	82	23	59		
Lymph node metastasis				1.669	0.196
Yes	35	8	27		
No	80	28	52		
Recurrence				5.830	0.016*
Yes	51	10	41		
No	64	26	38		
Vascular invasion				0.208	0.648
Yes	35	12	23		
No	80	24	56		

colony formation and MTT assays, the effects of RAB38 ablation on cell proliferation were detected. As we expected, the proliferation capacity was dramatically restrained by RAB38 knockdown with obviously decreased cell numbers. (Fig. 3a). In addition, the results of MTT assays showed a significant dropped absorbance value at 570 nm wavelength in T24 and 5637 cells, consistent with the results of colony formation assays (Fig. 3b).

Since cancer metastasis is partly caused by the motility of cancer cells, we then explored the effects of RAB38 depletion on bladder cancer cell migration and invasion. T24 and 5637 cells exhibited an obvious low-invasive property through the matrigel-coated membranes caused by RAB38 ablation, both cell numbers and the OD value at 570 nm wavelength significantly dropped after RAB38 knockdown (Fig. 3c). As we expected, shRNA-mediated depletion of RAB38 obviously inhibited the extent of wound closure in T24 and 5637 cells (Fig. 3d).

Collectively, we demonstrated that RAB38 was associated with the proliferation and motility regulation of bladder cancer in vitro.

Knockdown of RAB38 impaired bladder tumor growth in vivo

According to the previous results, RAB38 depletion resulted in the inhibition of proliferation and motility of bladder cancer in vitro, we then further detect the effects of RAB38 on the growth and development of bladder cancer in mice.

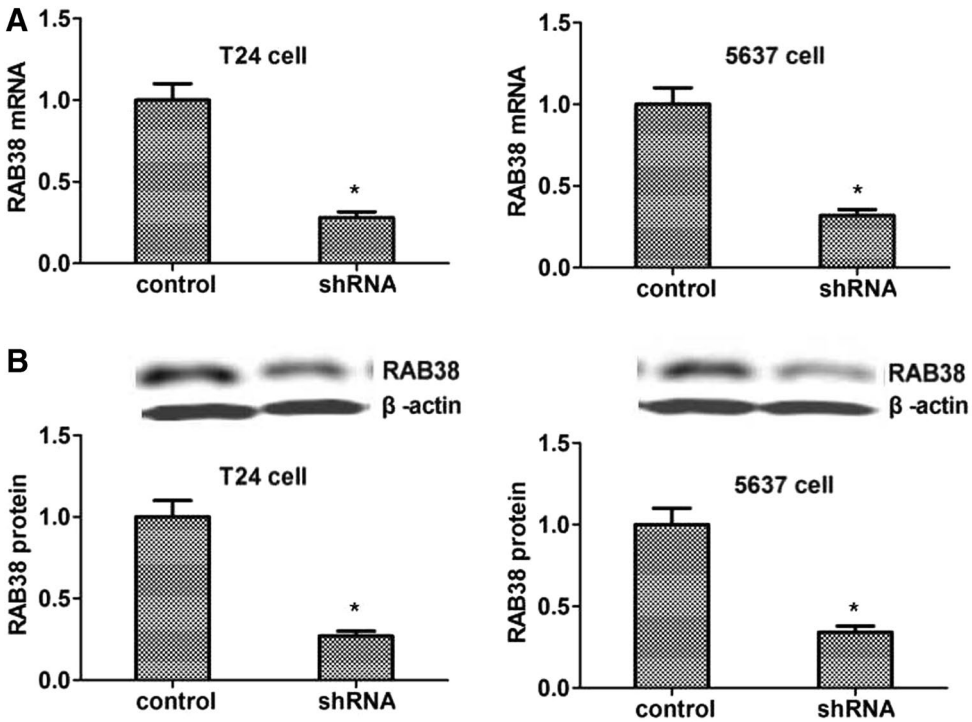

Fig. 2 The expression of RAB38 was blocked effectively in T24 and 5637 human bladder cancer cells caused by RAB38-targeted shRNA. **a** Results of quantitative PCR assays revealed the decreased expression level of RAB38 caused by its shRNA in T24 and 5637 cells, respectively. **b** Immunoblot assays confirmed the efficiently silencing of RAB38 expression caused by RAB38 shRNA in both T24 and 5637 cells. Results are presented as mean ± SEM, $*P<0.05$

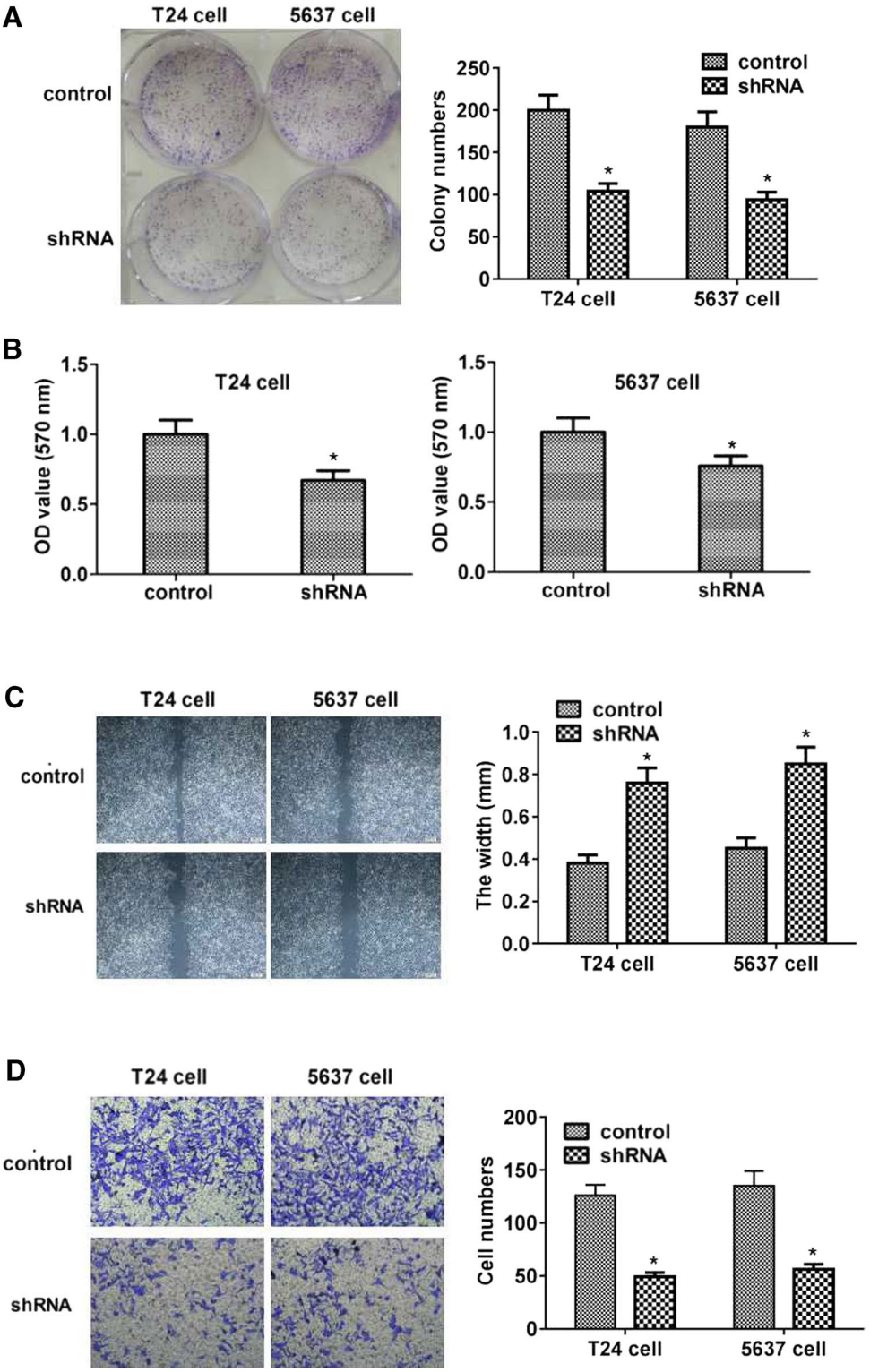

Fig. 3 Knockdown of RAB38 dramatically restrained the proliferation and motility of bladder cancer cells in vitro. **a** Representive photographs showed the results of colony formation assays of T24 (A) and 5637 cells (B) transfected with control or RAB38 shRNA. **b** The results of MTT assays showed the inhibition of proliferation in RAB38 depletion bladder cancer cells. **c** Transwell migration assays using T24 and 5637 cells transfected with control or RAB38 shRNA were performed and the extent of transwell migration was quantified by cell numbers. **d** T24 and 5637 cells were transfected with control or RAB38-shRNA, and the migration capacity was examined by wound healing assays. Results are presented as mean ± SEM, $^{*}P < 0.05$

Firstly, T24 cells were infected with shRNA lentivirus-targeted RAB38 or control and subsequently subcutaneously injected into nude mice. 2 weeks later, tumor began to grow and volume was examined each week. Representative photographs of tumors were taken and shown in Fig. 4a. Interestingly, the volume of tumors isolated from RAB38 knockdown groups was obviously smaller than that in control groups (Fig. 4a).

Additionally, we conducted lung metastasis assay in mice and found that the incidence of lung metastasis for RAB38

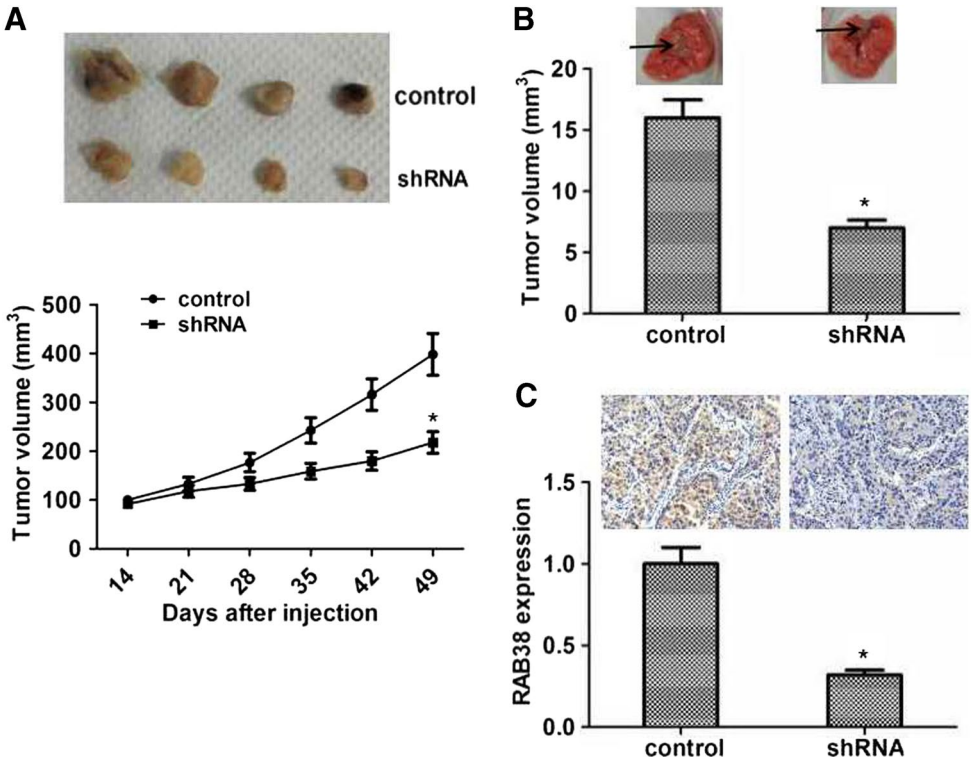

Fig. 4 RAB38 promotes bladder cancer growth and development in mice. **a** T24 cells infected with RAB38 or control shRNA lentivirus were implanted into nude mice. After 2 weeks, tumors were isolated, photographed and volume was calculated each week. ($n=6$ in each group). Tumor growth curves were calculated according to average volume of six tumors for each group. **b** Lung metastasis assays were performed, and the photographs of lung in the groups of T24 cells transfected with control or RAB38 shRNA lentivirus are shown. **c** The results of immunohistochemical assays indicated the expression level of RAB38 in control or RAB38 depletion tumor tissues isolated from mice. Results are presented as mean ± SEM, $^*P<0.05$

depletion T24 cells was significantly reduced compared with control (Fig. 4b).

Immunohistochemistry assays showed markedly decreased RAB38 expression in RAB38-depletion tumor tissues of mice (Fig. 4c). Therefore, all these results revealed that RAB38 was critical in the regulation of bladder cancer growth and development.

Discussion

Bladder cancer has a high mortality rate due to its complicated pathological process, inconspicuous early symptoms and the lack of effective treatment [1–7]. Current targeted therapy drugs and therapeutic targets for bladder cancer, such as VEGF/VEGFR, have achieved some therapeutic advances, but still cannot achieve a higher 5-year survival rate [22]. To combat bladder cancer, novel and more effective therapeutic targets should be identified. Although the results in TCGA database found that the mRNA level of the RAB38 had no change between tumor tissues and normal tissues, and the survival results between low- and high-expression RAB38 groups did not make any sense, the results from the Western Countries were not accurate for predicting the expression of RAB38 in China. Our results were based on clinical evidence. In this study, we found that a small GTPase, RAB38 was closely related to the poor prognosis of bladder cancer patients and could regulate cell proliferation and migration of bladder cancer in vivo and in vitro. This study proves that RAB38 can be a potential therapeutic target for bladder cancer. In fact, more accurate molecular mechanisms for the involvement of RAB38 in the occurrence and metastasis of bladder cancer still need further studies.

Rabs participate in a variety of biological processes. RAB8 mediates cytoskeleton remodeling [23]. RAB5 regulates the formation of early endosome [24]. Additionally, RAB8 and RAB11 are necessary for ciliogenesis through the regulation of ciliary protein transport [25]. Various studies demonstrated that Rab family could further affect the development of tumors by regulating cell migration, proliferation, apoptosis and drug resistance [26]. RAB27 was correlated with the invasive potential of breast cancer [27]. Rab25 high-expression was associated with the proliferation and invasion of renal cell carcinoma [28]. Rabs could mediate the invasion of tumor cells by affecting the formation of lamellipodia by the transport of cargos along microtubules or filaments [29]. Rabs can also interact with several cytoskeleton-associated proteins and participate in the regulation of cell division, thereby regulating tumor cell proliferation [30]. Here we found that RAB38 could affect the proliferation and motility of bladder cancer cells and further participate in the development of bladder cancer, possibly through the regulation of vesicle transport. In view of the specificity of Rab family protein function,

the molecular mechanism underlying RAB38 promoting bladder cancer development needs further study.

The depletion or mutation of RAB38 can lead to many diseases, such as platelet-dense granule storage pool disease [31]. However, there are limited studies that showed the effects of RAB38 on tumor development. RAB38 was previously thought as a melanocyte differentiation antigen, which is highly expressed in normal melanoma tissues [32]. Similarly, studies have demonstrated that RAB38 ablation affected the development of anaplastic gliomas and glioblastomas, and was also closely related to the poor prognosis of non-small cell lung cancer [19]. Interestingly, we found a significant link between RAB38 and bladder cancer, suggesting a similar regulatory mechanism underlying RAB38 promoting these types of tumors. By immune response profiling of malignant pleural mesothelioma, RAB38 was also thought as a novel prognostic bio-marker [33]. We found that RAB38 affects the proliferation and migration of bladder cancer cells, but whether RAB38 affects the occurrence and development of bladder cancer in the aspect of immune response is still worth discussing.

It is well known that the progression stage and morbidity of cancers is critical for the clinical assessment. Our studies indicated that RAB38 expression was associated with grade malignancy of bladder cancer (Table 1), which was similar to the previous study that RAB38 was correlated with the poor prognosis of patients with glioma. Interestingly, our study showed that patients with high expression of RAB38 had obvious shorter overall survival rate and disease-free survival rate and more advanced tumor stage than the patients with lower expression of RAB38.

In conclusion, we found that a small GDPase, RAB38, could act as a novel and potential therapeutic target for bladder cancer. RAB38 was associated with the poor prognosis of bladder cancer patients, and through clinical data analysis, we found that the expression of RAB38 was associated with pTNM stage and the recurrence of patients with bladder cancer. We further indicated that RAB38 affected the proliferation and invasion of bladder cancer cells, which was also confirmed by in vivo experiments.

Acknowledgements This work was supported by Tianjin natural science fund (18JCYBJC26200) and Tianjin education commission project (2017KJ207).

Author Contributions Conceived and designed the experiments: Da-Wei Tian, Chang-Li Wu, and Sheng-Lai Liu. Performed the experiments: Da-Wei Tian, Sheng-Lai Liu, Li-Ming Jiang, Zhou-Liang Wu, and Jie Gao. Contributed reagents/materials/analysis tools: Li-Ming Jiang, Zhou-Liang Wu, Jie Gao, Chang-Li Wu, Hai-Long Hu. Wrote the paper: Da-Wei Tian, Sheng-Lai Liu, Li-Ming Jiang and Chang-Li Wu.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Research involving human and animal participants All applicable international, national, and/or institutional guidelines for the care and use of human tissues and animals were followed.

References

1. Skeldon SC, Larry Goldenberg S (2015) Bladder cancer: a portal into men's health. Urol Oncol 33:40–44
2. Miremami J, Kyprianou N (2014) The promise of novel molecular markers in bladder cancer. Int J Mol Sci 15:23897–23908
3. Amling CL (2001) Diagnosis and management of superficial bladder cancer. Curr Probl Cancer 25:219–278
4. Dalbagni G (2007) The management of superficial bladder cancer. Nat Clin Pract Urol 4:254–260
5. Xia L, Guzzo TJ (2017) Preoperative anemia and low hemoglobin level are associated with worse clinical outcomes in patients with bladder cancer undergoing radical cystectomy: a meta-analysis. Clin Genitourin Cancer 15:263–272
6. Woldu SL, Bagrodia A, Lotan Y (2017) Guideline of guidelines: non-muscle-invasive bladder cancer. BJU Int 119:371–380
7. Anghel RM, Gales LN, Trifanescu OG (2016) Outcome of urinary bladder cancer after combined therapies. J Med Life 9:153–159
8. Black PC, Agarwal PK, Dinney CP (2007) Targeted therapies in bladder cancer–an update. Urol Oncol 25:433–438
9. Voutsinas GE, Stravopodis DJ (2009) Molecular targeting and gene delivery in bladder cancer therapy. J BUON 14(Suppl 1):S69–S78
10. Railkar R, Krane LS, Li QQ, Sanford T, Siddiqui MR, Haines D, Vourganti S, Brancato SJ, Choyke PL, Kobayashi H, Agarwal PK (2017) Epidermal growth factor receptor (EGFR)-targeted photoimmunotherapy (PIT) for the treatment of EGFR-expressing bladder cancer. Mol Cancer Ther 16:2201–2214
11. Cohen AJ, Packiam V, Nottingham C, Steinberg G, Smith ND, Patel S (2017) Upstaging of nonurothelial histology in bladder cancer at the time of surgical treatment in the National Cancer Data Base. Urol Oncol 35:34 e31–34 e38
12. Ohbayashi N, Fukuda M, Kanaho Y (2017) Rab32 subfamily small GTPases: pleiotropic Rabs in endosomal trafficking. J Biochem 162:65–71
13. Numrich J, Ungermann C (2014) Endocytic Rabs in membrane trafficking and signaling. Biol Chem 395:327–333
14. Wang S, Hu C, Wu F, He S (2017) Rab25 GTPase: functional roles in cancer. Oncotarget 8:64591–64599
15. Zhao H, Wang Q, Wang X, Zhu H, Zhang S, Wang W, Wang Z, Huang J (2016) Correlation between RAB27B and p53 expression and overall survival in pancreatic cancer. Pancreas 45:204–210
16. Osanai K, Takahashi K, Nakamura K, Takahashi M, Ishigaki M, Sakuma T, Toga H, Suzuki T, Voelker DR (2005) Expression and characterization of Rab38, a new member of the Rab small G protein family. Biol Chem 386:143–153
17. Bultema JJ, Boyle JA, Malenke PB, Martin FE, Dell'Angelica EC, Cheney RE, Di Pietro SM (2014) Myosin vc interacts with Rab32 and Rab38 proteins and works in the biogenesis and secretion of melanosomes. J Biol Chem 289:33513–33528
18. Osanai K (2018) Rab38 mutation and the lung phenotype. Int J Mol Sci 19:2203

19. Wang H, Jiang C (2013) RAB38 confers a poor prognosis, associated with malignant progression and subtype preference in glioma. Oncol Rep 30:2350–2356
20. Jäger D, Stockert E, Jäger E, Güre AO, Scanlan MJ, Knuth A, Old LJ, Chen YT (2000) Serological cloning of a melanocyte rab guanosine 5′-triphosphate-binding protein and a chromosome condensation protein from a melanoma complementary DNA library. Can Res 60:3584
21. Vuruputuri U, Abdelmonsef AH, Dulapalli R, Dasari T, Padmarao LS, Mukkera T (2016) Structure based drug discovery of Rab38 protein- Identification of antagonists as cancer drug candidates. Comb Chem High Throughput Screening 19:875
22. van Kessel KE, Zuiverloon TC, Alberts AR, Boormans JL, Zwarthoff EC (2015) Targeted therapies in bladder cancer: an overview of in vivo research. Nat Rev Urol 12:681–694
23. Hattula K, Peranen J (2005) Purification and functional properties of a Rab8-specific GEF (Rabin3) in action remodeling and polarized transport. Methods Enzymol 403:284–295
24. Mendoza PA, Silva P, Diaz J, Arriagada C, Canales J, Cerda O, Torres VA (2017) Calpain2 mediates Rab5-driven focal adhesion disassembly and cell migration. Cell Adh Migr 12:185–194
25. Knodler A, Feng S, Zhang J, Zhang X, Das A, Peranen J, Guo W (2010) Coordination of Rab8 and Rab11 in primary ciliogenesis. Proc Natl Acad Sci USA 107:6346–6351
26. Chia WJ, Tang BL (2009) Emerging roles for Rab family GTPases in human cancer. Biochim Biophys Acta 1795:110–116
27. Li Z, Fang R, Fang J, He S, Liu T (2018) Functional implications of Rab27 GTPases in Cancer. Cell Commun Signal 16:44
28. Li Y, Jia Q, Zhang Q, Wan Y (2015) Rab25 upregulation correlates with the proliferation, migration, and invasion of renal cell carcinoma. Biochem Biophys Res Commun 458:745–750
29. Just WW, Peranen J (1863) Small GTPases in peroxisome dynamics. Biochim Biophys Acta 2016:1006–1013
30. Geng D, Zhao W, Feng Y, Liu J (2016) Overexpression of Rab25 promotes hepatocellular carcinoma cell proliferation and invasion. Tumour Biol 37:7713–7718
31. Ninkovic I, White JG, Rangel-Filho A, Datta YH (2008) The role of Rab38 in platelet dense granule defects. J Thromb Haemost 6:2143–2151
32. Zippelius A, Gati A, Bartnick T, Walton S, Odermatt B, Jaeger E, Dummer R, Urosevic M, Filonenko V, Osanai K, Moch H, Chen YT, Old LJ, Knuth A, Jaeger D (2007) Melanocyte differentiation antigen RAB38/NY-MEL-1 induces frequent antibody responses exclusively in melanoma patients. Cancer Immunol Immunother 56:249–258
33. Creaney J, Dick IM, Musk AW, Olsen NJ, Robinson BW (2016) Immune response profiling of malignant pleural mesothelioma for diagnostic and prognostic biomarkers. Biomarkers 21:551–561