Surgical Oncology 30 (2019) 63–71

Contents lists available at ScienceDirect

Surgical Oncology

journal homepage: www.elsevier.com/locate/suronc

Ras-association domain family 1 (RASSF1A) gene regulates progression, migration and invasion of bladder cancer

Yuhai Bao[a], Xuefeng Liu[a], Yang Liu[b], Si Wang[c], Bin Wu[a,*]

[a] Department of Urology, Shengjing Hospital of China Medical University, Shenyang, China
[b] Department of Pathology, The Basic Medical Sciences of China Medical University, Shenyang, China
[c] Department of Pathogeny Biology, The Basic Medical Sciences of China Medical University, Shenyang, China

ARTICLE INFO

Keywords:
Bladder cancer
RASSF1A
Migration
Invasion
Apoptosis

ABSTRACT

Background: Bladder cancer is common malignant tumor around the world. Ras-association domain family 1 (RASSF1A) gene is inactivated in most of cancers. The aim of the study is to analyze the relationship between RASSF1A and the progression of bladder and investigate the effects of RASSF1A overexpression on bladder cancer cell *in vitro*.

Materials and methods: Immunohistochemistry assay analyzed the RASSF1A expression in bladder tissue of bladder cancer patients. Besides, the data of 138 bladder cancer patients about RASSF1A expression was analyzed. Kaplan-Meier method analyzed the connection between disease free survival (DFS) and abnormal expression of RASSF1A and E-cadherin in bladder cancer patients. RASSF1A gene transfected the bladder cancer cells by lipofectamine. Cell proliferation was determined by methyl thiazolyl tetrazolium (MTT) assay. Cell apoptosis and cell cycle was detected using flow cytometry. Scratch assay evaluated cell migration, and transwell assay evaluated cell invasion. Western blot analyzed protein expression while qRT-PCR analyzed mRNA expression.

Results: Bladder cancer tissues had lower RASSF1A expression compared with normal tissues, and the Patients with abnormal expression of RASSF1A had lower DFS and had higher possibility of recurrence and muscular layer infiltration. Besides, RASSF1A had significant difference with E-cadherin. RASSF1A overexpression inhibited the bladder cancer cell proliferation, migration and invasion, besides, it promoted apoptosis and the expression of E-cadherin and β-catenin.

Conclusion: RASSF1A may be a biomarker to evaluate the progression and recurrence of bladder cancer.

1. Introduction

Bladder cancer is one of the most common genitourinary malignant tumor in the world, and 69000 persons were diagnosed cases in 2008, and 14000 deaths because of the disease in the USA [1]. Carcinoma takes place in urothelium of bladder, and the disease is named of urothelial bladder cancer (UBC) [2], and Maximilian Burger et al. reported that approximately 180500 persons were diagnosed with UBC and 55200 patients died from UBC [3]. Risk factors can be considered to develop bladder cancer, and risk factors are classified into three main categories, such as genetic and molecular factors, chemical and environmental factors and chronic irritation [4]. Smocking is regarded as a risk factor for UBC [5], and N-acetyl transferase enzymes (NAT1, NAT2) are related to activation of UBC [6], and the frequency of activating FGFR3 mutation is higher in UBS [7]. Besides, Ki-67 and p53 are promised to be biomarker to predict the progression of bladder cancer [8].

In 1973, histopathological grade of metastatic bladder cancer was classified into grade 1–3 [9], and the grade was classified into four grades until 1998, such as papilloma, papilloma of low malignant potential, low grade bladder cancer and high grade bladder cancer [10]. Radical cystectomy for bladder cancer ensured a low recurrence rate and 56% probability of survival time although it brings a high percentage of metastase [11]. Radiation therapy in combination with chemotherapy for bladder cancer promotes that 52% patients have 5-year overall survival [12]. In the proximate dozen years, MPDL3280A (anti-PD-L1) was investigated in clinical trial [13]. Besides, durvalumab (MEDI4736) has been demonstrated that it has manageable safety and meaningful clinical activity for UBC patients who have PD-L1 positive [14].

* Corresponding author. Department of Urology, Shengjing Hospital of China Medical University, No.36 Sanhao Street, Heping District, Shenyang, 110004, China.
E-mail address: binw_wubinb@163.com (B. Wu).

https://doi.org/10.1016/j.suronc.2019.05.009
Received 9 January 2019; Received in revised form 11 April 2019; Accepted 18 May 2019

List of abbreviations

RASSF1A	Ras-association domain family 1
DFS	disease free survival
MTT	methyl thiazolyl tetrazolium
UBC	urothelial bladder cancer
DAB	diaminobenzidine
PBS	Phosphate buffered saline
FBS	fetal bovine serum
DMSO	Dimethylsulfoxide
qRT-PCR	Quantitative reverse transcription-polymerase chain reaction
SD	Standard deviation

Cellular signaling pathway about regulation of cell survival depends on Ras GTPases, and Ras-association domain family 1 (RASSF1A) gene is located in chromosomal segment of 3p21.3 [15]. RASSF1A is inactivated in most of cancer, including breast cancer, kidney cancer and thyroid cancer [15]. Recent studies supported that RASSF1A activated autophagy [16] and promoted Hippo pathway and pS127-YAP [17]. In our study, we firstly found that bladder cancer patients had lower expression of RASSF1A, and then we analyzed the relationship between RASSF1A and bladder cancer progression by database of patients, and in order to verify the relationship, we investigated that effects of RASSF1A overexpression on proliferation and characteristic of bladder cancer cell *in vitro*.

2. Materials and methods

2.1. Clinical data analysis

The data of 138 bladder cancer patients were registered using database of Shengjing Hospital of China Medical University, and the data about the patients were from February, 2007 to October, 2017. In the study, all patients underwent radical cystectomy or transurethral resection of bladder tumor. The patients signed the informed consent form before study beginning, and the process of study was approved by ethics committees and health authorities of Shengjing Hospital of China Medical University. Correlation between the demographic variable and the abnormal expression of RASSF1A and E-cadherin were analyzed by student's *t*-test in Table 2. Besides, correlation between RASSF1A abnormal expression and E-cadherin was analyzed by nonlinear regression and student's *t*-test in Table 1. All patients were followed up for 102 months, with a median follow-up of 22 months. Kaplan-Meier method analyzed the relationship between disease free survival (DFS) and abnormal expression and censor of RASSF1A and E-cadherin in bladder cancer patients.

2.2. Immunohistochemistry assay and cell transfection

Normal tissue samples were obtained 2 cm from the edge of lesion from patients with bladder cancer, bladder cancer tissues or normal tissues were made into sections by fix, dehydration and paraffin embedding. E-cadherin antibody (ab40772, Abcam, Cambridge, Massachusetts, USA) solution or RASST1A antibody (ab97749, Abcam, Cambridge, Massachusetts, USA) solution incubated the sections at 4 °C overnight. The secondary antibody solution (ab7090, Abcam, Cambridge, Massachusetts, USA) incubated the sections for 1 h at the room temperature. 100 μL diaminobenzidine (DAB) solution (Solarbio, Beijing, China) added the sections. Hematoxylin solution (Solarbio, Beijing, China) stained the sections until the cell nucleus were blue. The sections were observed by fluorescence microscope (Olympus, Tokyo, Japan).

The bladder urothelial cancer cell lines (KK47 and T24) were obtained from Southern Medical University (Guangzhou, Guangdong, China). The cells were seeded at 35 mm plate, and 4% paraformaldehyde solution (Solarbio, Beijing, China) fixed the cells, and RASST1A antibody (ab97749, Abcam, Cambridge, Massachusetts, USA) added the cells at 4 °C overnight, and secondary antibody (ab7088, Abcam, Cambridge, Massachusetts, USA) solution incubated the cells for 2 h. 10 μg/mL DAPI solution (Solarbio, Beijing, China) stained the cells for 15 min. Phosphate buffered saline (PBS) (Gibco, Carlsbad, California, USA) washed the cells, and the cells were observed under fluorescence microscope (Olympus, Tokyo, Japan).

RASSF1A gene was synthesized by MBL (Beijing, China). Lipofectamine (Invitrogen, Carlsbad, California, USA) was dissolved in RPMI 1640 medium (Gibco, Carlsbad, California, USA), and the RASSF1A gene was added into the mixed solution. The solution treated the cells for 3 h, then the complete medium (RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% 10000 units/mL penicilin-10000 μg/mL streptomycin) (Gibco, Carlsbad, California, USA) cultured the cells for 48 h or 72 h.

2.3. Methyl thiazolyl tetrazolium (MTT) assay

MTT assay evaluated the cell viability. After the cells were treated reagents, the cells were treated MTT solution (Solarbio, Beijing, China) for 2–3 h in dark room at 37 °C. 100 μL Dimethylsulfoxide (DMSO) (Solarbio, Beijing, China) added into hole and incubated the cells for 15 min in dark room at 37 °C. Multiskan (Thermo Scientific, Waltham, Massachusetts, USA) tested the absorbance ratio at a wavelength of 570 nm.

2.4. Cell apoptosis and cell cycle

After the cells were treated reagents for 48 h, all cells were digested with trypsin-EDTA (0.25%) (Gibco, Carlsbad, California, USA) and resuspended with PBS (Gibco, Carlsbad, California, USA). Cell Apoptosis Kit with Annexin V FITC and PI detected the apoptosis using flow cytometry (Invitrogen, Carlsbad, California, USA). The experimental manipulation followed the manual. Vybrant DyeCycle (Invitrogen, Carlsbad, California, USA) treated the cell, and the manipulation complied with manual, and flow cytometry measured the cell cycle.

Table 1
The correlation between RASSF1A and E-cadherin.

	RASSF1A			
	Abnormal expression	Normal expression	Correlation index	*P*
E-cadherin				
Abnormal expression	64	11	0.738	0.000
Normal expression	15	48		

Table 2
Demographic variable and abnormal expression of RASSF1A and E-cadherin among patients with bladder cancer.

	n	Abnormal expression of RASSF1A	*P*	Abnormal expression of E-cadherin	*P*
Age (years)					
≤60	64	34(53.13%)	0.363	30(46.88%)	0.454
> 60	74	45(60.81%)		34(45.95%)	
Sex					
Male	83	46(55.42%)	0.595	36(43.37%)	0.385
Female	55	33(60.00%)		28(50.91%)	
Degree of differentiation					
Middle and higher	75	36(48.00%)	0.017	31(41.33%)	0.195
lower	63	43(68.25%)		33(52.38%)	
Recurrence					
No	59	26(44.07%)	0.007	23(38.98%)	0.132
Yes	79	53(67.09%)		41(51.90%)	
Muscular layer infiltration					
No	65	31(47.69%)	0.032	20(30.77%)	0.001
Yes	73	48(65.75%)		44(60.27%)	

2.5. Scratch assay

After the cells were treated with reagents for 48 h, the living cells were digested with trypsin-EDTA (0.25%) (Gibco, Carlsbad, California, USA) and resuspended with complete medium (Gibco, Carlsbad, California, USA). The living cells were planted in 35 mm plate (Corning, Corning, New York, USA) for 24 h, and 200 μL pipette tips (Sigma-Aldrich, St. Louis, Missouri, USA) scratched the cells, and PBS softly washed the cells for three times, and complete medium contained 2% FBS cultured the cells for 12 h. The scratches were observed by microscope (Olympus, Tokyo, Japan).

2.6. Transwell assay

Cells were treated with reagents for 48 h, the living cells were digested with trypsin-EDTA (0.25%) (Gibco, Carlsbad, California, USA) and resuspended with complete medium contained 1% FBS (Gibco, Carlsbad, California, USA). Matrigel (Sigma-Aldrich, St. Louis, Missouri, USA) was diluted with RPMI 1640 medium, and the diluted solution added into top room of transwell (Corning, Corning, New York, USA), and the transwell was incubated in 37 °C incubator (Thermo Scientific, Waltham, Massachusetts, USA). Complete medium contained 20% FBS was added into bottom room of transwell. The cells solution was added into top room of transwell, and the incubator incubated this transwell for 12 h. Crystal violet solution (Solarbio, Beijing, China) stained the bottom cells after detachment of top cells. The invasion cells were observed under microscope (Olympus, Tokyo, Japan).

2.7. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

RNAs were collected from the cells with Trizol reagents (Invitrogen, Carlsbad, California, USA) in centrifuge at 4 °C 16000 rad/min for 15 min cDNA kit (Thermo Scientific, Waltham, Massachusetts, USA) synthesized cDNA with RNAs, and the experimental action complied with manual. Amplification of RNA can be realized by cDNA using polymerase chain reaction. The primers (Table 3) were used in polymerase chain reaction and synthesized by Gene Create (Wuhan, Hubei, China), and the conditions included 95 °C for 90 s, 45 cycles of 95 °C for 40 s, 60 °C for 30 s and 72 °C for 15 s, and final extension at 72 °C for 90 s. The relative expression of mRNA gene was estimated using $2^{(-\triangle\triangle Ct)}$.

2.8. Western blot

Protein was extracted from cells using lysis buffer (Thermo Scientific, Waltham, Massachusetts, USA), and the extract was centrifugalized by centrifuge (Cence, Changsha, Hunan, China) at 4 °C 12000 rad/min for 16 min, and supernatant was protein. BCA kit (Thermo Scientific, Waltham, Massachusetts, USA) confirmed the protein concentration, and the experimental manipulate was realized in agreement with the manual. Prestained protein ladder (Thermo Scientific, Waltham, Massachusetts, USA) and sample protein were separated by SDS-PAGE according to molecular size. The proteins were transferred to PVDF membranes (Sigma-Aldrich, St. Louis, Missouri, USA). 5% milk solution blocked the blank site of PVDF membranes attached the protein. Primary antibody (Table 3) solution incubated the protein membranes at 4 °C for 12 h, and the primary antibody (anti-RASSF1A, ab97749; anti-E-cadherin, ab40772; anti-β-catenin, ab32572; anti-β-actin, ab8226) was diluted in TBST solution contained Tween-20 (Solarbio, Beijing, China), and the dilute ratio was complied with manual. Secondary antibody (goat anti-rabbit IgG H&L (HRP), ab205718, Abcam, Cambridge, Massachusetts, USA) incubated the protein membranes for 2–3 h at 25 °C after the protein membranes were washed with TBST solution. ECL kit (Sigma-Aldrich, St. Louis, Missouri, USA) colored the protein membranes at dark room, and the film revealed the stain, and the Image J software (National Institutes of Health, USA) analyzed the stains.

2.9. Statistical analysis

All experiments were realized independently at least three times. Values were regarded as Mean ± Standard deviation (SD) except patient data. Kaplan-Meier method analyzed the expression of RASSF1A and E-cadherin in patients during survival time. Student's *t*-test analyzed the data, and the significant different between two groups was analyzed by one way of ANOVA with Tukey's test using SPSS (IBM, Armonk, NewYork, USA). p value is less than 0.05, which regard as statistical significance.

Table 3
Primers and primary antibodies.

Name	Forward of primer (5′-3′)	Reverse of primer (5′-3′)	Weight of antibody	Item number of antibody	Manufacturer of antibody
RASSFIA	TCCTGCAAGGAGGGTGGCTTC	GGCTGGGAACCCGCGGTG	39 kDa	ab97749	Abcam, Cambridge, Massachusetts, USA
E-cadherin	GGTAGGTGAATTTTTAGTTAATTAGTGGTA	ACCCATAACTAACCAAAAACACCA	97 kDa	ab40772	
β-catenin	AACTAAACAGGAAGGGATGGA	GCACAAGAGCCTCTATACCA	37 kDa	ab32572	
β-actin	CACACCTTCTACAATGAGCTG	GTCTCAAACATGATCTGGGTC	42 kDa	ab8227	

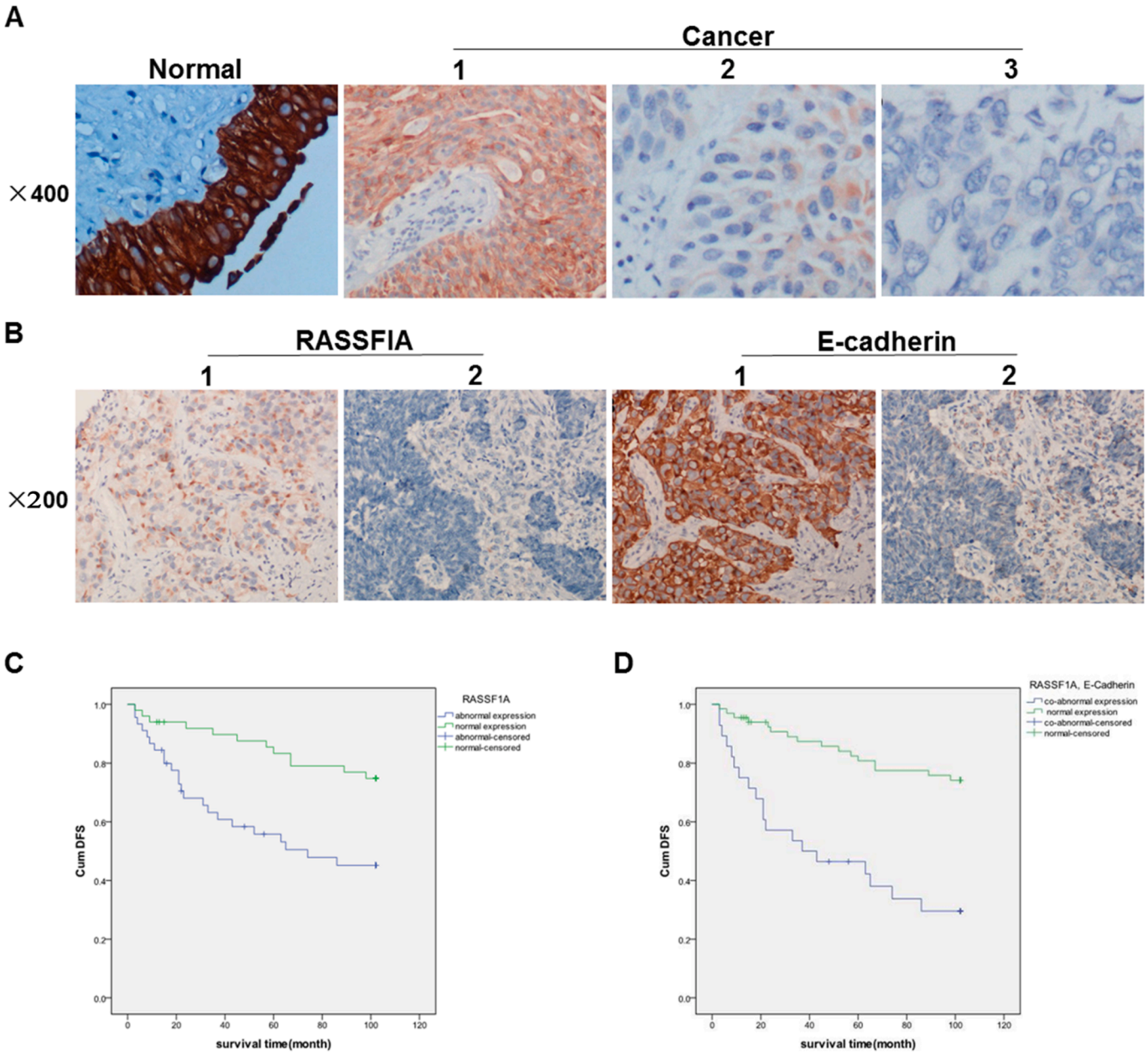

Fig. 1. Time to confired worsening event, and the expression of RASSF1A and E-cadherin in bladder cancer tissues. (A, B) Immunohistochemistry assay analyzed the RASSF1A expression in normal bladder tissue and bladder tissue of bladder cancer patients. (C, D) Kaplan-Meier method analyzed the data of bladder cancer patients with the relationship between disease free survival (DFS) and abnormal expression and censor of RASSF1A and E-cadherin. 1, 2 and 3 represent the bladder tissue of bladder cancer patients.

3. Results

3.1. Expression of RASSF1A and E-cadherin in bladder cancer patients

Normal bladder tissue had higher RASSF1A expression, and the bladder cancer tissue had lower RASSF1A expression or had not RASSF1A expression (Fig. 1A-B) in cytosol. Besides, bladder cancer tissue had lower E-cadherin expression or had not E-cadherin expression (Fig. 1B) in cytosol. Patients with abnormal expression of RASSF1A had lower DFS (Fig. 1C). Besides, patients with abnormal expression of RASSF1A and E-cadherin also had lowest DFS (Fig. 1D). The relationship between RASSF1A abnormal expression and E-cadherin abnormal expression was relevant (Table 1). Abnormal expression of RASSF1A was associated with tumor degree of differentiation, recurrent and muscular layer infiltration ($P < 0.05$), but not with age or gender; Bladder cancer patients with both of RASSF1A abnormal expression and E-cadherin abnormal expression had great chance to have recurrence and muscular layer infiltration (Table 2). Next, we investigated whether RASSF1A overexpression had an effect on characteristic of bladder cancer cell *in vitro*.

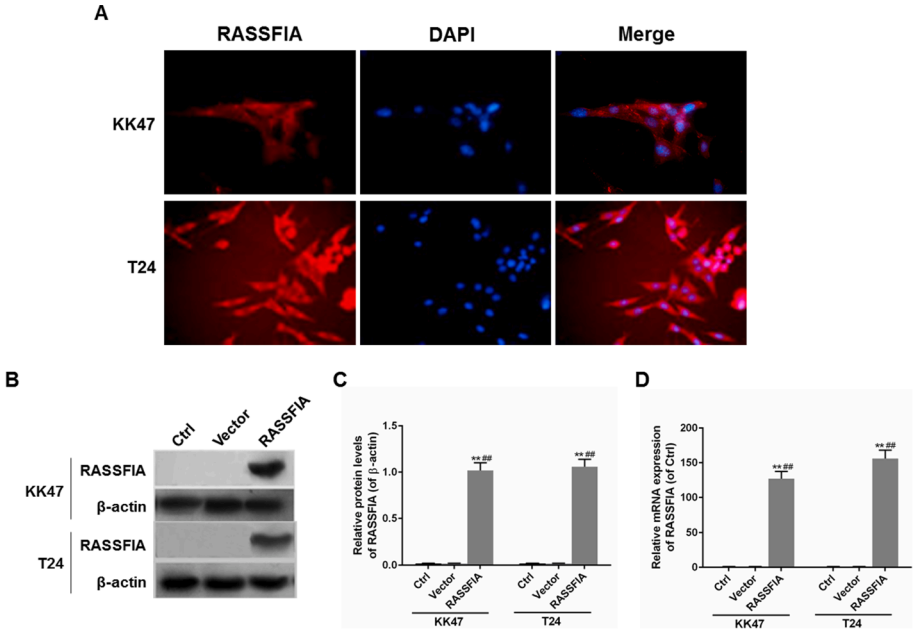

Fig. 2. RASSF1A gene transfects the bladder cancer cell and causes the RASSF1A overexpression. RASSF1A gene transfected the bladder cancer cells for 48 h. (A) Immunohistochemistry assay analyzed RASSF1A protein expression. (B, C) Western blot determined the RASSF1A protein expression. (D) qRT-PCR evaluated the RASSF1A mRNA expression. Values were regarded as Mean ± Standard deviation (SD), and one-way ANOVA with Turkey's test analyzed the significant different (* vs Ctrl group, # vs Vector group; ** = ## = $p < 0.01$). KK47 and T24 are bladder cancer lines.

3.2. RASSF1A overexpression inhibited proliferation and increased apoptosis in bladder cancer cells

The expression of RASSF1A was low in bladder cancer cells (KK47 and T24), and RASSF1A expression in RASSF1A group was significantly higher than that in control and vector group (Fig. 2A-D), indicating that RASSF1A was successfully transfected. Overexpression of RASSF1A inhibited bladder cancer cell viability (Fig. 3A-B), and promoted bladder cancer cells apoptosis (Fig. 3D). We found that increased G1 phase cells and decreased S phase cells took placed in bladder cancer cells expressed higher RASSF1A, which supported that RASSF1A overexpression promoted cell cycle G1 phase arrest, leading to inhibition of transition from G1 phase to S phase during bladder cancer cells cycle (Fig. 3C).

3.3. RASSF1A overexpression inhibited bladder cancer cells migration and invasion

Normal bladder cancer cell had higher ability of migration, and overexpression of RASSF1A inhibited ability of migration by a wide margin (Fig. 4A). Besides, overexpression of RASSF1A decreased the number of invasion cell, which suggested that RASSF1A overexpression inhibited invasion of bladder cancer cells (Fig. 4B).

3.4. RASSF1A overexpression promoted the expression of E-cadherin and β-catenin in bladder cancer cells

Bladder cancer cells expressing higher RASSF1A had high expression of E-cadherin and β-catenin, which showed that RASSF1A overexpression promoted expression of E-cadherin and β-catenin in bladder cancer cells (Fig. 5).

4. Discussion

In analysis of clinical data, we supported that over 50% bladder patients had abnormal expression of RASSF1A (Table 2). There was significant difference between abnormal expression of RASSF1A and E-cadherin (Table 1). Besides, abnormal expression of RASSF1A and E-cadherin was associated with progression of bladder cancer, including differentiation, recurrence, and muscular layer infiltration (Table 2). The results of Kaplan-Meier analysis indicated that bladder cancer with abnormal expression of RASSF1A and E-cadherin had lower DFS, which revealed that abnormal expression of RASSF1A and E-cadherin had effect on progression of bladder cancer (Fig. 1C-D). Moreover, we found that bladder cancer patients normally had lower RASSF1A expression. In following experiment, we investigated whether RASSF1A overexpression had influence on bladder cancer cell *in vitro*.

Apoptosis, cell death program, is encoded by gene and characterized by morphologic and biochemical changes [18]. Approximately 50–70 billion cells died because of apoptosis in adult every day [19]. However, potential oncogenic proliferative signals inhibit normal process of apoptosis and promote continued proliferation, survival and invasion [20]. Besides, there are gene mutations in the process of abnormal cell proliferation, such as RTKs, G-protein signal transducers and late-G1 cell-cycle checkpoint [20,21]. CDK protein family regulate cell cycle from G1 phase to S phase, and the CDK activity is controlled by CIP/KIP (CDK inhibitor) included p21, and p53 is a major transcriptional regulator of p21, and p21 regulates cancer cell apoptosis [22–24]. Therefore, cell cycle is associated with cell apoptosis. RASSF1A overexpression inhibited bladder cancer proliferation and apoptosis (Fig. 3). Besides, RASSF1A overexpression arrested transition from G1 phase to S phase (Fig. 3).

Cancer metastasis is an important progression of cancer and decreases cancer patient survival rates [25]. The process of cancer invasion and metastasis needs several sequential distinct steps, including adhesion to extracellular matrix (ECM), degradation of ECM and invasion/migration through ECM [26,27]. There are two mechanisms of cancer cell metastasis, such as passive dissemination and hematogenous metastasis. Passive dissemination is characterized by detachment from primary tumor through peritoneal fluid and ascites, and hematogenous metastasis is characterized by detachment from primary tumor through blood vessels [25]. Anyway, the invasive cancer cells firstly change cell

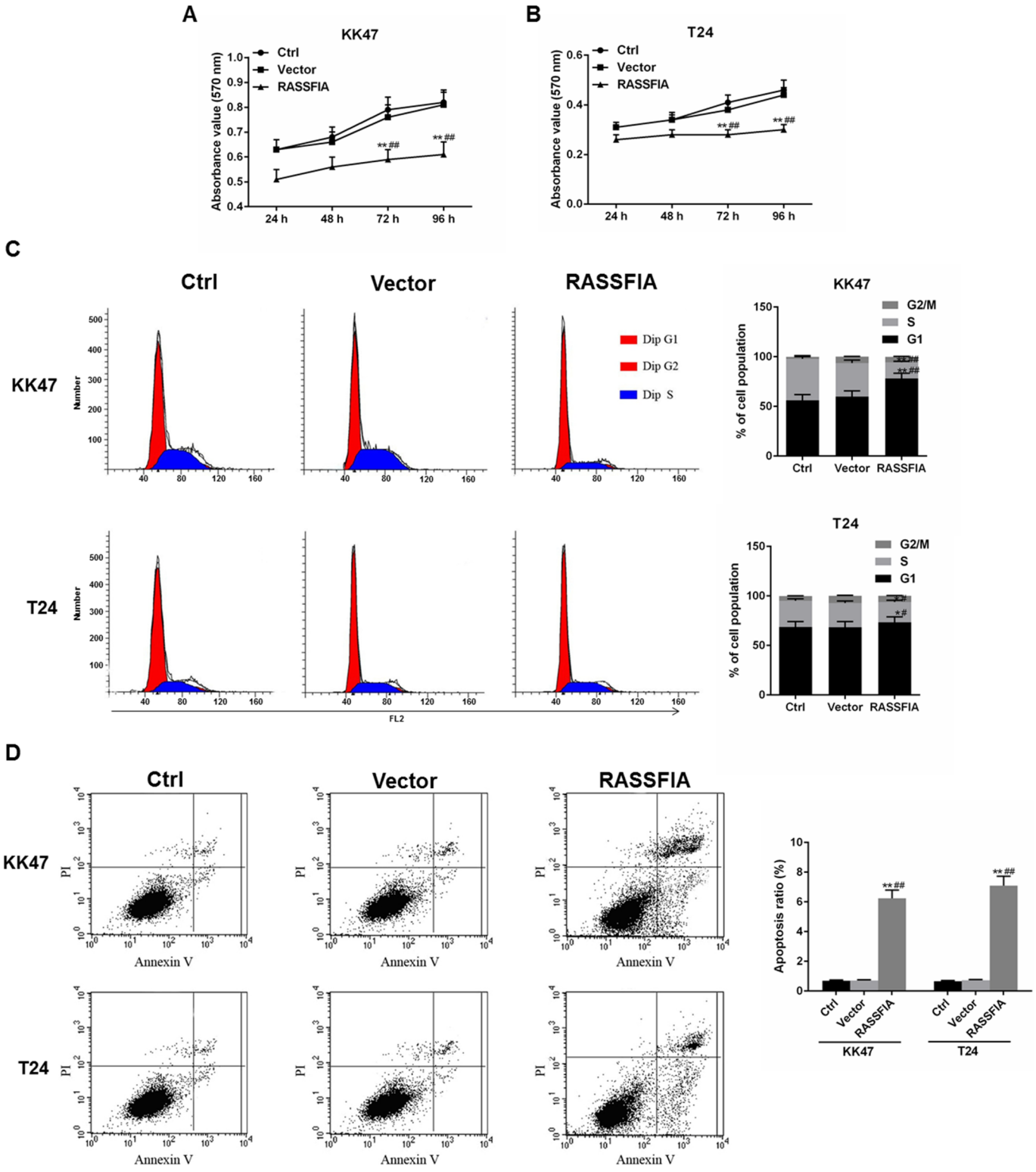

Fig. 3. RASSF1A overexpression inhibits proliferation and promoted apoptosis in bladder cancer cells. Methyl thiazolyl tetrazolium (MTT) assay determined the proliferation of KK47 (A) and T24 (B) using Multiskan at a wavelength of 570 nm RASSF1A gene transfected the bladder cancer cells for 48 h. (C) Vybrant DyeCycle added into the cells, and flow cytometry measured the cell cycle. (D) Cell Apoptosis Kit determined the apoptosis using flow cytometry. Values were regarded as Mean ± Standard deviation (SD), and one-way ANOVA with Turkey's test analyzed the significant different (* vs Ctrl group, # vs Vector group; * = # = $p < 0.05$, ** = ## = $p < 0.01$). KK47 and T24 are bladder cancer lines.

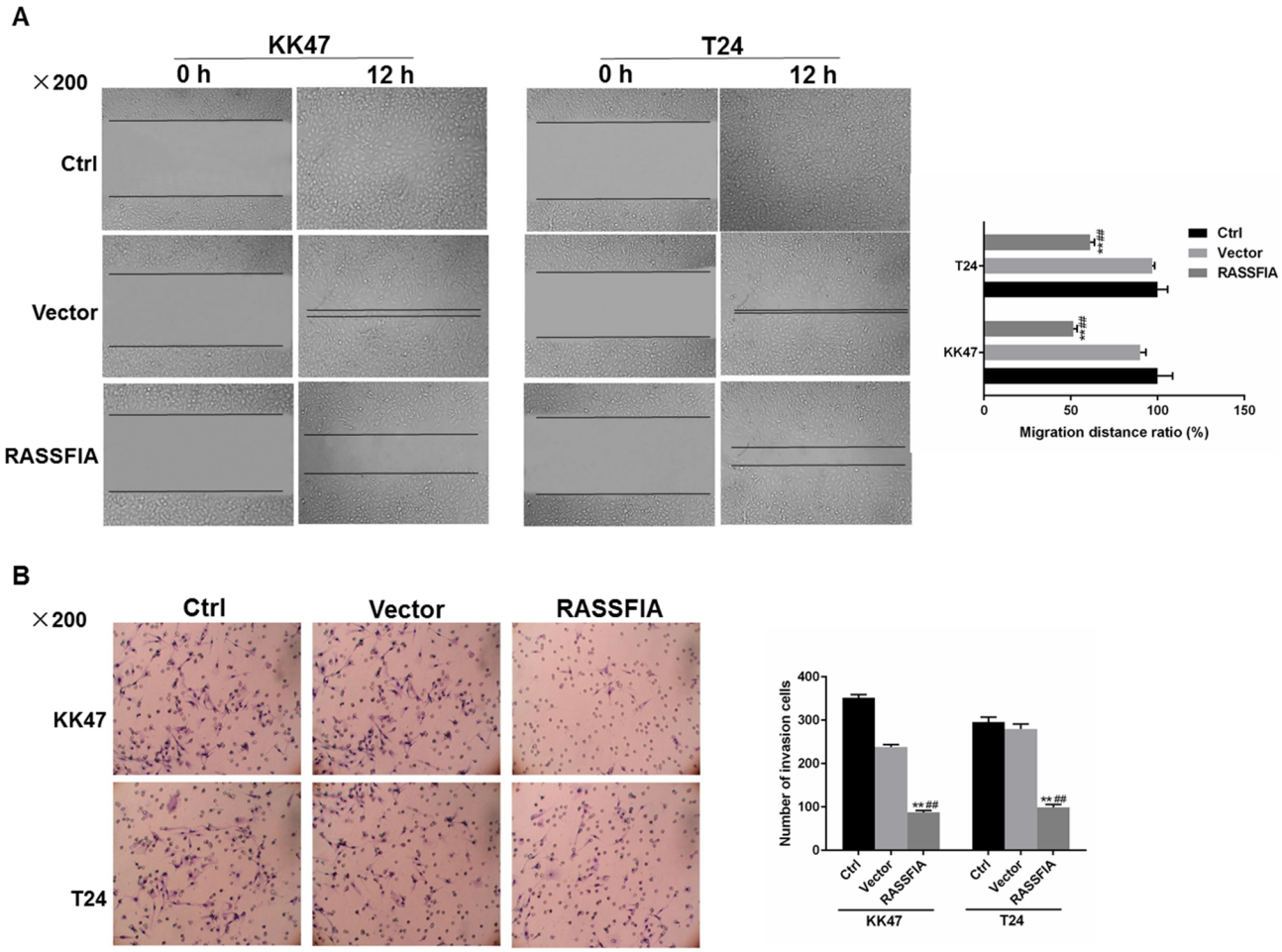

Fig. 4. RASSF1A overexpression inhibits migration and invasion of bladde cancer cells. RASSF1A gene transfected the bladder cancer cells for 48 h. (A) Scratch assay analyzed the ability of bladder cancer cell migration. (B) Transwell assay determined the ability of bladder cancer cell invasion. Values were regarded as Mean ± Standard deviation (SD), and one-way ANOVA with Turkey's test analyzed the significant different (* vs Ctrl group, # vs Vector group; ** = ## = $p < 0.01$). KK47 and T24 are bladder cancer lines.

adhesion to ECM, and the cadherin protein family plays a major role in regulation of cell adherin [28,29]. Downregulation of E-cadherin has been demonstrated to benefit cancer cell metastasis, and cadherin family is regarded as cell adhesion receptors [30]. Besides, β-catenin links cadherin family to support cell cytoskeleton [31]. Therefore, downregulation of β-catenin promotes cancer metastasis [32]. Scratch assay and transwell assay are used to evaluate the ability of cancer cell migration and invasion *in vitro* [33,34]. In our study, we demonstrated that overexpression of RASSF1A inhibited migration and invasion of bladder cancer cell (Fig. 4) and increased the expression of E-cadherin and β-catenin.

5. Conclusions

In conclusion, we found that bladder cancer patients had abnormal expression of RASSF1A and E-cadherin, and abnormal expression of RASSF1A and E-cadherin were associated with differentiation, recurrence, and muscular layer infiltration of bladder cancer. Besides, overexpression of RASSF1A decreased the bladder cancer cell proliferation and inhibited migration and invasion of bladder cancer cell by regulating expression of E-cadherin and β-catenin. The conclusion suggested that RASSF1A may be a biomarker to predict the progression and recurrence of bladder cancer.

Funding

This work was supported by Liaoning Province Scientific Projects [Grant number: 2013408001].

Authors' contributions

Yuhai Bao[1], Xuefeng Liu[1], Yang Liu[2], Si Wang[3], Bin Wu[1]

Substantial contributions to conception and design: YB, XL.

Data acquisition, data analysis and interpretation: YL, SW, BW.

Drafting the article or critically revising it for important intellectual content: BW, YB.

Final approval of the version to be published: All authors.

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved: YL, SW.

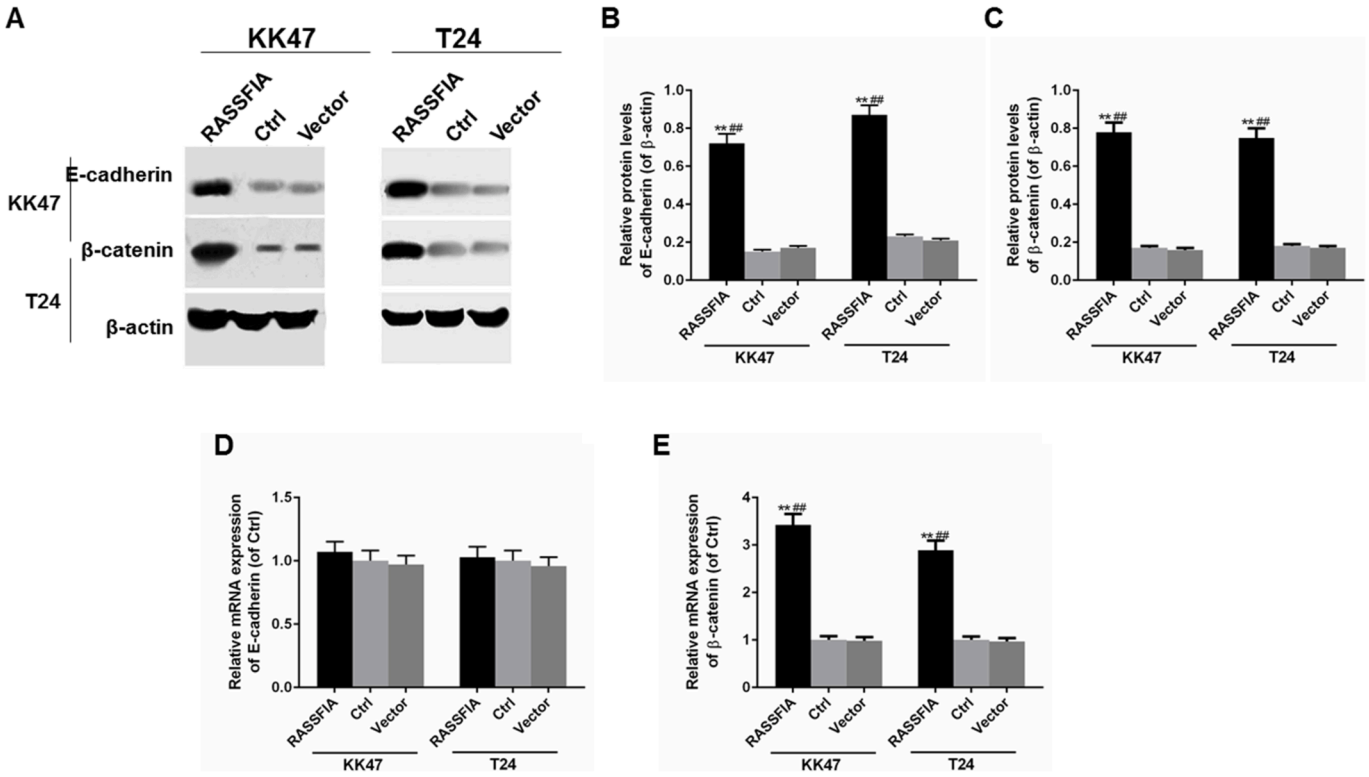

Fig. 5. RASSF1A overexpression promotes the expression of E-cadherin and β-catenin in bladder cancer cells. RASSF1A gene transfected the bladder cancer cells for 48 h. Western blot analyzed the protein levels of E-cadherin (A, B) and β-catenin (A, C). qRT-PCR determined the mRNA expression of E-cadherin (D) and β-catenin (E). KK47 and T24 are bladder cancer lines. Values were regarded as Mean ± Standard deviation (SD), and one-way ANOVA with Turkey's test analyzed the significant different (* vs Ctrl group, # vs Vector group; ** = ## = $p < 0.01$).

Competing interests

The authors declare no conflicts of interest.

References

[1] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, T. Murray, et al., Cancer statistics, CA: A Cancer J. Clin. 58 (2) (2008) 71–96 2008.

[2] N. The Cancer Genome Atlas Research, J.N. Weinstein, R. Akbani, B.M. Broom, W. Wang, R.G.W. Verhaak, et al., Comprehensive molecular characterization of urothelial bladder carcinoma, Nature 507 (2014) 315.

[3] M. Burger, J.W.F. Catto, G. Dalbagni, H.B. Grossman, H. Herr, P. Karakiewicz, et al., Epidemiology and risk factors of urothelial bladder cancer, Eur. Urol. 63 (2) (2013) 234–241.

[4] D.S. Kaufman, Shipley WU, A.S. Feldman, Bladder cancer, The Lancet 374 (9685) (2009) 239–249.

[5] N.D. Freedman, D.T. Silverman, A.R. Hollenbeck, A. Schatzkin, C.C. Abnet, Association between smoking and risk of bladder cancer among men and women, JAMA 306 (7) (2011) 737–745.

[6] J. Gu, D. Liang, Y. Wang, C. Lu, X. Wu, Effects of N-acetyl transferase 1 and 2 polymorphisms on bladder cancer risk in Caucasians, Mutat. Res. Genet. Toxicol. Environ. Mutagen 581 (1) (2005) 97–104.

[7] L.A. Kiemeney, P. Sulem, S. Besenbacher, S.H. Vermeulen, A. Sigurdsson, G. Thorleifsson, et al., A sequence variant at 4p16.3 confers susceptibility to urinary bladder cancer, Nat. Genet. 42 (2010) 415.

[8] T. Habuchi, M. Marberger, M.J. Droller, G.P. Hemstreet, H.B. Grossman, J.A. Schalken, et al., Prognostic markers for bladder cancer: international Consensus Panel on bladder tumor markers, Urology 66 (6) (2005) 64–74 Supplement 1.

[9] F.K. Mostofi, Histologic typing of urinary bladder tumors, Int. Histol. Classif. Tumors (1973) 237–256.

[10] I. Epstein J, B. Amin M, R. Reuter V, K. Mostofi F, The World Health Organization/International Society of Urological Pathology Consensus Classification of Urothelial (Transitional Cell) Neoplasms of the Urinary Bladder, (1999), pp. 1435–1448.

[11] S. Madersbacher, W. Hochreiter, F. Burkhard, G.N. Thalmann, H. Danuser, R. Markwalder, et al., Radical cystectomy for bladder cancer today—a homogeneous series without neoadjuvant therapy, J. Clin. Oncol. 21 (4) (2003) 690–696.

[12] L.A. Kachnic, D.S. Kaufman, N.M. Heney, A.F. Althausen, P.P. Griffin, A.L. Zietman, et al., Bladder preservation by combined modality therapy for invasive bladder cancer, J. Clin. Oncol. 15 (3) (1997) 1022–1029.

[13] T. Powles, J.P. Eder, G.D. Fine, F.S. Braiteh, Y. Loriot, C. Cruz, et al., MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer, Nature 515 (2014) 558.

[14] C. Massard, M.S. Gordon, S. Sharma, S. Rafii, Z.A. Wainberg, J. Luke, et al., Safety and efficacy of durvalumab (MEDI4736), an anti-programmed cell death ligand-1 immune checkpoint inhibitor, in patients with advanced urothelial bladder cancer, J. Clin. Oncol. 34 (26) (2016) 3119–3125.

[15] R. Dammann, U. Schagdarsurengin, M. Strunnikova, M. Rastetter, C. Seidel, L. Liu, et al., Epigenetic inactivation of the Ras-association domain family 1 (RASSF1A) gene and its function in human carcinogenesis, Histol. Histopathol. 18 (2) (2003) 665–677.

[16] W. Li, F. Yue, Y. Dai, B. Shi, G. Xu, X. Jiang, et al., Suppressor of hepatocellular carcinoma RASSF1A activates autophagy initiation and maturation, Cell Death Differ. (2018 Oct 12), https://doi.org/10.1038/s41418-018-0211-7 [Epub ahead of print].

[17] A. Papaspyropoulos, L. Bradley, A. Thapa, C.Y. Leung, K. Toskas, D. Koennig, et al., RASSF1A uncouples Wnt from Hippo signalling and promotes YAP mediated differentiation via p73, Nat. Commun. 9 (1) (2018) 424.

[18] D. Fisher, Apoptosis in Cancer Therapy: Crossing the Threshold, (1994), pp. 539–542.

[19] J.C. Reed, Dysregulation of apoptosis in cancer, J. Clin. Oncol. 17 (9) (1999) 2941.

[20] G.I. Evan, K.H. Vousden, Proliferation, cell cycle and apoptosis in cancer, Nature 411 (2001) 342.

[21] J.W. Harbour, D.C. Dean, The Rb/E2F pathway: expanding roles and emerging paradigms, Genes Dev. 14 (19) (2000) 2393–2409.

[22] A. Karimian, Y. Ahmadi, B. Yousefi, Multiple functions of p21 in cell cycle, apoptosis and transcriptional regulation after DNA damage, DNA Repair 42 (2016) 63–71.

[23] J.W. Roh, J.W. Kim, N.H. Park, Y.S. Song, I.A. Park, S.-Y. Park, et al., p53 and p21 genetic polymorphisms and susceptibility to endometrial cancer, Gynecol. Oncol. 93 (2) (2004) 499–505.

[24] M.R. Kang, K.H. Park, J.-O. Yang, C.W. Lee, S.J. Oh, J. Yun, et al., miR-6734 up-regulates p21 gene expression and induces cell cycle arrest and apoptosis in colon cancer cells, PLoS One 11 (8) (2016) e0160961.

[25] T.-L. Yeung, C.S. Leung, K.-P. Yip, C.L. Au Yeung, S.T.C. Wong, S.C. Mok, Cellular and molecular processes in ovarian cancer metastasis. A review in the theme: cell and molecular processes in cancer metastasis, Am. J. Physiol. Cell Physiol. 309 (7) (2015) C444–C456.

[26] A.F. Chambers, A.C. Groom, I.C. MacDonald, Dissemination and growth of cancer cells in metastatic sites, Nat. Rev. Canc. 2 (8) (2002) 563–572.
[27] C. Moncharmont, A. Levy, J.-B. Guy, A.T. Falk, M. Guilbert, J.-C. Trone, et al., Radiation-enhanced cell migration/invasion process: a review, Crit. Rev. Oncol. Hematol. 92 (2) (2014) 133–142.
[28] D.M. Li, Y.M. Feng, Signaling mechanism of cell adhesion molecules in breast cancer metastasis: potential therapeutic targets, Breast Canc. Res. Treat. 128 (1) (2011) 7–21.
[29] O.J. Scully, B.-H. Bay, G. Yip, Y. Yu, Breast cancer metastasis, Canc. Genom. - Proteom. 9 (5) (2012) 311–320.
[30] M.K. Wendt, M.A. Taylor, B.J. Schiemann, W.P. Schiemann, Down-regulation of epithelial cadherin is required to initiate metastatic outgrowth of breast cancer, Mol. Biol. Cell 22 (14) (2011) 2423–2435.
[31] M. Conacci-Sorrell, I. Simcha, T. Ben-Yedidia, J. Blechman, P. Savagner, Z. Ben, et al., Autoregulation of E-cadherin expression by cadherin–cadherin interactions, J. Cell Biol. 163 (4) (2003) 847.
[32] S.K.Y. To, A.S.C. Mak, Y.M. Eva Fung, C.M. Che, S.S. Li, W. Deng, et al., beta-catenin downregulates Dicer to promote ovarian cancer metastasis, Oncogene 36 (43) (2017) 5927–5938.
[33] N. Liu, L. Zhang, Z. Wang, Y. Cheng, P. Zhang, X. Wang, et al., MicroRNA-101 inhibits proliferation, migration and invasion of human glioblastoma by targeting SOX9, Oncotarget 8 (12) (2016) 19244–19254.
[34] K. Oono, K. Takahashi, S. Sukehara, H. Kurosawa, T. Matsumura, S. Taniguchi, et al., Inhibition of PC3 human prostate cancer cell proliferation, invasion and migration by eicosapentaenoic acid and docosahexaenoic acid, Mol. Clin. Oncol. 7 (2) (2017) 217–220.