Int J Clin Exp Med 2014;7(11):4398-4405
www.ijcem.com /ISSN:1940-5901/IJCEM0002445

Original Article

Association between polymorphisms in the integrin gene predicted microRNA binding sites and bladder cancer risk

Jiaming Liu[1*], Sihang Cheng[1*], Yonggang Zhang[2], Hong Li[1], Jin Huang[3], Peng Zhang[1]

[1]Department of Urology, West China Hospital, Sichuan University, 37 Guoxuexiang, Chengdu 610041, Sichuan, China; [2]The Periodical Press of West China Hospital, Sichuan University, 37 Guoxuexiang, Chengdu 610041, Sichuan, China; [3]West China Hospital, Sichuan University, 37 Guoxuexiang, Chengdu 610041, Sichuan, China. [*]Equal contributors.

Received September 10, 2014; Accepted November 8, 2014; Epub November 15, 2014; Published November 30, 2014

Abstract: Bladder cancer (BC) is the ninth most frequent malignancies in the world and the occurrence of this disease has dramatically increased in recent years. Integrins have been demonstrated to play an important role in the development and progression of BC. However, the association between polymorphisms in integrin genes and BC susceptibility was still unclear. A number of studies mainly focused on polymorphisms in the coding regions of integrin genes previously, while in this study, polymorphisms in the 3' untranslated regions (3'UTR) were investigated in Chinese Han population. According to previous study, seven single-nucleotide polymorphisms (SNPs) in predicted microRNA (miRNA) target sites were chosen as potential targets. And four SNPs including rs11902171, rs2675, rs17664, and rs1062484, were finally examined for their effect on BC risk and clinical prognosis. These four polymorphisms were genotyped by using the high-resolution melting method (HRM) in 317 BC patients with long-time follow-up together with 317 age-matched healthy controls. AC carriers of rs2675 in *ITGB5* were associated with an increased risk of BC (OR 1.44, 95% CI 1.02-2.03). No significant relationship was detected between these SNPs and the recurrence-free survival time of overall study population or non-muscle invasive BC subgroups in univariable analysis. In conclusion, rs2675 in miRNA binding sites of *ITGB5* might be a potential target for BC susceptibility prediction.

Keywords: Integrin gene, polymorphisms, microRNA-binding sites, bladder cancer

Introduction

Bladder cancer (BC) has been prevalent in the United States with an estimated 72,570 new cases and 15,210 deaths in 2013 [1]. The incidence rate of BC in Chinese population was rising with the average growth rate 4.60% per year during 1998-2008 [2]. Various risk factors have been demonstrated to be associated with the development of BC, including cigarette smoking, occupational and environmental exposure [3]. Recently, polymorphisms in individuals' genetic susceptibility have been revealed to be correlated with the risk of BC [4]. However, mechanisms explaining gene interactions and signal pathways in the development of BC are still unclear.

Integrins, a family of cell surface receptors, are composed of non-covalently linked type 1 transmembrane glycoprotein subunits α and β [5-8]. They mediate the connection between extracellular matrix (ECM) and intracellular actin cytoskeleton, which may lead to the activation of related signaling pathways and cause changes of cell function [9, 10]. A number of studies have confirmed the critical role of integrins in tumor development and progression [11, 12]. Integrins are highly over-expressed on different types of cancer cells. They participate in the regulation of cell cycle following specific ECM binding, and induce cellular quiescence or malignant transformation by controlling cyclin D1 and cyclin-dependent kinase inhibitor family [13]. Besides, integrins could cooperate with oncogenes or receptor tyrosine kinases to influence tumorigenesis through cross-talking various signaling pathways, including PI3K-AKT, NF-κB, and p53 pathways [14]. Therefore, integrins have profound contribution on the altera-

tion of biological behavior of tumor cells, including migration, invasion, and metastasis [15]. Recently, the possible association between integrins and BC has been identified by studies. The expression of integrin α_2 subunit was proved to be associated with the reduction of adherence between BC cells and promotion of invasive potential [16]. Abnormal expression of integrin $\alpha_2\beta_1$ and/or $\alpha_6\beta_4$ were reported to be an early event in the initiation of BC [17, 18]. Integrin $\alpha_3\beta_1$ increased a lot in BC with the progressive loss of integrin $\alpha_2\beta_1$, which was verified to contribute to migration and invasion of BC. Moreover, loss of co-expression and co-localization of integrin $\alpha_6\beta_4$ and collagen VII were identified to be related to more aggressive behavior of BC cells.

MicroRNAs (miRNAs) belong to the family of non-coding small RNA molecules, which contain 21-25 nucleotides. Gene expression was controlled by post-transcriptional regulation through strict matching between the seeding region, 2-7 nucleotides in the 5' miRNA sequence and the non-coding region, 3' untranslated region (3'UTR) of the target genes. Gene expression was down-regulated following specific mRNAs inhibition after miRNA-target interaction [19, 20]. Thus, same gene with different genotypes might result in different levels of gene expression, which was caused by miRNAs suppression. By regulating the expression of tumor suppressor genes or proto-oncogenes, miRNAs were confirmed to contribute to changes in the individual susceptibility with multiple human malignancies [21].

Polymorphisms in the miRNA binding sites of specific genes have been proved by recent studies to be associated with susceptibility of various tumors. MiRNA-target interaction between miRNAs Let-7e and Let-7f and human *IL23R* gene was disrupted by single-nucleotide polymorphism (SNP) rs10889677 in the 3'UTR in patients with breast cancer [22]. Such SNPs have also been identified to have association with other types of cancers, including colorectal cancer [23] and non-small cell lung cancer [24]. Furthermore, rs10719T > C polymorphism located in 3'UTR of *DROSHA* gene disrupted the binding site for hsa-miR-27b, which was demonstrated to be associated with the increased risk of BC [25]. Therefore, genetic variation in the miRNA binding sites of integrin genes may also contribute to the risk of BC, including the occurrence and progression. In the present hospital-based case-control study, four SNPs in the 3' UTR of four integrin genes were investigated in Chinese Han population in order to provide data for screening high-risk individuals.

Materials and methods

Study population

A total of 317 patients recruited in this study were identified from the Department of Urology, West China Hospital, Sichuan University from 2000 to 2012. All these patients were sporadic cases with the pathological diagnosis of BC (mean age 63.35 years; 242 males and 75 females). Patients with immune disease or any histopathologic diagnosis other than BC were excluded. 317 healthy individuals with no evidence of cancer or immune disease were selected in a routine check-up or health awareness campaigns as controls (mean age 64.13 years; 236 males and 81 females) from West China Hospital, Sichuan University. All controls were matched to cases by gender and age (± 5 years) at baseline. Clinical and epidemiology information were collected from all subjects, including smoking status and history, tumor grade, tumor stage, and event (recurrence/non recurrence, and death/live). 1 ml peripheral blood samples were collected from all subjects and stored at -80°C for further detection. Written informed consent was obtained from each subject.

The follow-up process was conducted in 125 patients to evaluate the potential effect of polymorphisms in the microRNA binding sites of integrin genes on the recurrence of BC. Face to face interview and telephone calls were made to collect data of these patients. Malignant urothelial tumors that have not invaded the detrusor were defined as non-muscle invasive BC, while the others were muscle invasive BC. The recurrence of BC was defined as newly growing focus in cystoscopy or imaging findings. The recurrence-free survival time was the specific period from the date of surgery to the date of recurrence or death. Follow-up lasted until May 2013.

SNP selection

SNPs were selected with detailed selection method for polymorphisms in the miRNA binding sites of integrin genes, which was provided

Table 1. Information about predicted miRNA binding sites SNPs

gene	*SNP*	*Variation*	*chromosome*	*MAF*[a]	*MiRNA/wild type allele*	*MiRNA/variant allele*
ITGAv	Rs11902171	[G/C]	2	0.166	mir-382, mir-30a-3p, mir-30e-3p,	
ITGB5	Rs2675	[A/C]	3	0.160	mir-192, mir-215, mir-449, mir-504	mir-192, mir-215, mir-449, Mir-30a-3p, mir-30e-3p.
ITGA6	Rs17664	[A/G]	2	0.404	mir-195, mir-214, mir-223	mir-195, mir-214, mir-223, mir-152.
ITGA3	Rs1062484	[C/T]	17	0.137	mir-150a, mir-187, mir-191, mir-211, mir-326, mir-339, mir-362, mir-370, mir-486, mir-501, mir-517	mir-150, mir-191, mir-145, mir-218, mir-296, mir-326, mir-339, mir-362, mir-370, mir-432, mir-433, mir-478, mir-486, mir-501, mir-517, mir-518c, mir-517a,

[a]MAF (Minor Allele Frequency) was cited from http://www.ncbi.nlm.nih.gov/SNP.

Table 2. Demographic characteristics in the cases and controls

	Variables	*Cases (n = 317) (%)*	*Controls (n = 317)*	*P*
Sex	Male	242	236	
	Female	75	81	
	Age (Mean ± SD)	63.35 ± 12.95	64.13 ± 12.38	0.443
Smoking	Non smokers	168		
	Smokers	149		
Grade	I	43		
	II	108		
	III	155		
	Mixed	11		
Stage	Superficial	138		
	Invasive	179		
Event	Recurrence/non recurrence	71/54		
	Death/live	38/87		

by Brendle *et al.* [26]. Polymorphisms in the 3'UTRs of 10 integrin genes (*ITGA3, ITGA5, ITGA6, ITGAv, ITGB1, ITGB3, ITGB4, ITGB5, ITGB6*, and *ITGB8*) and predicted putative miRNA binding sites were reported to be screened and selected through online available tools, including microInspector (http://mirna.imbb.forth.gr/microinspector/), PicTar (http://pictar.bio.nyu.edu/), and TargetScan (http://www.targetscan.org/). Seven SNPs were chosen in the previous study; however, *ITGB3* rs3809865, *ITGB4* rs743554 and *ITGB1* rs17468 were given up in this study as a result of no appropriate PCR condition. Finally, four SNPs were selected, including rs11902171, rs2675, rs17664, and rs1062484. The variations of miRNA targets are listed in **Table 1**.

DNA isolation and SNP genotyping

DNA samples were collected from peripheral blood samples of patients with EDTA as the anticoagulant. Genomic DNA was extracted with QIAamp DNA extraction kit (Qiagen) following the manufacturer's protocol. After extraction, DNA purity and concentration were determined by spectrophotometer. Genotyping of the selected microRNA binding site SNPs were done with Sequenom MassARRAY & iPLEX assay of Capitalbio Company. In brief, primers for PCR and iPLEX reaction were synthesized with the tool designed by Genotyping Tools & MassARRAY Assay Design software. PCR amplification was performed in 384-well plate and shrimp alkaline phosphatase was used to deal with the product for the purpose of dephosphorylating unincorporated dNTPs in PCR system. Then the Mass ARRAY iPLEX reaction was performed. After the iPLEX reaction, iPLEX products with different molecular weight were created by each base of SNP sites. Finally, different iPLEX product could be identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF), and then genotyping information of each SNP site for every subject could be obtained in the result of MALDI-TOF with TYPER 4.0 software.

Statistical analysis

Hardy-Weinberg equilibrium was detected in each SNP in controls. Pearson χ^2 or Fisher's exact tests was carried out to analysis the potential difference in the distribution of SNP genotypes between patients and controls. Student's t test was used to explore the difference in the distribution of age. The odds ratios (ORs) and 95% condense intervals (CIs) for association between gene miRNA binding site SNP genotype and BC risk were calculated with

Table 3. The polymorphisms of the *integrin* gene and the risk of BC

Polymorphism		Patients (n = 317)		Controls (n = 317)[a]		OR (95%)	*P value*[b]
		n	%	n	%		
rs11902171	GG (540)	268	84.54	272	85.80	1	-
	GC (91)	48	15.14	43	13.56	0.88 (0.57-1.38)	0.58
	CC (3)	1	0.32	2	0.64	1.97 (0.18-21.86)	1.00
	G (1171)	584	92.11	587	92.59	1	-
	C (97)	50	7.89	47	7.41	0.94 (0.62-1.42)	0.75
rs2675[b]	AA (412)	219	69.08	193	60.88	1	-
	AC (195)	86	27.13	109	34.38	1.44 (1.02-2.03)	0.04
	CC (27)	12	3.79	15	4.74	1.42 (0.65-3.11)	0.38
	A (1019)	524	82.65	495	78.08	1	-
	C (249)	110	17.35	139	21.92	1.34 (1.01-1.77)	0.04
rs17664	GG (453)	222	70.03	231	72.87	1	-
	GA (168)	91	28.71	77	24.29	0.81 (0.57-1.16)	0.25
	AA (13)	4	1.26	9	2.84	2.16 (0.66-7.12)	0.31
	G (1074)	535	84.28	539	85.02		
	A (195)	99	15.62	95	14.98	0.95 (0.70-1.29)	0.76
rs1062484	CC (623)	309	97.48	314	99.05	1	-
	CT (11)	8	2.52	3	0.95	0.37 (0.10-1.40)	0.22
	C (1257)	626	98.74	631	99.53		
	T (11)	8	1.26	3	0.47	0.37 (0.10-1.41)	0.23

[a]Hardy-Weinberg equilibrium calculation for control subjects: p = 0.83 for rs11902171, p = 0.94 for rs2675, p = 0.41 for rs17664, p = 0.93 for rs1062484. [b]The bold numbers mean the *p* value is < 0.05.

the risk option of crosstabs. Kaplan-Meier and Cox proportional hazards models were used to detect the association between genotypes and recurrence-free survival (RFS) time. Univariable analyses were applied throughout the whole study. All the analyses in this study were performed with the Statistical Package for Social Sciences software, v.13.0 (SPSS, Chicago, IL) and p < 0.05 in two sides was considered statistically significant.

Results

Characteristics of the study objects

Basic information of the study subjects and clinical characteristics of each patient are summarized in **Table 2**. No significant difference in the distribution of age were found between the cases (63.35 ± 12.95) and the controls (64.13 ± 12.38) (p = 0.443). Among recruited BC patients, the majority was in advanced clinical grade, including clinical grade II and III (34.1% (n = 108) and 48.9% (n = 155), respectively). The percentage of patients in clinical grade I was 13.6% (n = 43), and the rest 3.4% (n = 11) were mixed grade tumor. In addition, patients with superficial BC accounted for 43.5% (n = 138), while the remaining 56.5% (n = 179) were invasive BC patients.

Association of SNPs with BC susceptibility

The genotype and allele frequencies of 4 selected SNPs in all subjects are shown in **Table 3**. All the SNPs were in Hardy-Weinberg equilibrium (P > 0.05). Genotype and allele distributions of *ITGB5* gene polymorphism rs2675 exhibited significant difference between cases and controls. Compared with the AA genotype, AC genotype was strongly associated with an increased risk of BC (OR = 1.44; 95% CI = 1.02, 2.03; p = 0.04). The frequency of C allele is significantly higher than the A allele in the cancer subjects than the controls (OR = 1.34; 95% CI = 1.01, 1.77; p = 0.04). However, for the other three SNPs, the genotype and allele frequencies did not differ significantly between the patients and the controls (P > 0.05).

No association between SNPs and BC recurrence-free survival

Among 317 BC patients, 125 patients completed the follow-up and 71 patients suffered from BC recurrence. According to the collected infor-

Table 4. Recurrence-free survival analysis of 125 bladder cancer patients

polymorphisms	*genotype*	*N (all, n = 125)*	*N (reoccurence, n = 71)*	*HR (95% CI)*
Rs2675	AA	83 (66.4%)	48 (57.8%)	1
	AC	37 (29.6%)	20 (54.1%)	0.858 (0.393, 1.871)
	CC	5(4.0%)	3 (60.0%)	1.094 (0.173, 6.897)
Rs1062484	CC	120 (96.0%)	70 (58.3%)	1
	CT	5 (4.0%)	1 (20.0%)	0.179 (0.019, 1.646)
Rs11902171	GG	99 (79.2%)	56 (56.6%)	1
	GC	25 (20.0%)	14 (56.0%)	0.977 (0.404, 2.366)
	CC	1 (0.8%)	1 (100.0%)	—
Rs17664	GG	91 (72.8%)	53 (58.2%)	1
	GA	32 (25.6%)	17 (53.1%)	0.813 (0.362, 1.826)
	AA	2 (1.6%)	1 (50.0%)	0.717 (0.043, 11.825)

Table 5. Recurrence-free survival analysis of 50 non-muscle invasive bladder cancer patients

non-muscle invasive bladder cancer				
polymorphisms	*genotype*	*N (all, n = 50)*	*N (reoccurence, n = 29)*	*HR (95% CI)*
RS2675	AA	32 (64.0%)	21 (65.6%)	1
	AC	15 (30.0%)	7 (46.7%)	0.458 (0.131, 1.599)
	CC	3 (6.0%)	1 (33.3%)	0.262 (0.021, 3.219)
RS1062484	CC	49 (98.0%)	29 (59.25%)	-
	CT	1 (2.0%)	0 (0.0%)	-
RS11902171	GG	39 (78.0%)	20 (51.3%)	1
	GC	10 (10.0%)	8 (80.0%)	3.800 (0.714, 20.224)
	CC	1 (2.0%)	1 (100.0%)	-
RS17664	GG	36 (72.0%)	20 (55.6%)	1
	GA	14 (28.0%)	9 (64.3%)	1.440 (0.402, 5.157)

mation, the mean and median follow-up time of this study was 38.25 ± 3.62 months and 24 months, and the mean and median recurrence-free survival time for the 125 patients was 38.87 ± 2.79 months and 25 months, respectively. No association was detected between the four polymorphisms and BC recurrence-free survival time among the 125 patients (**Table 4**). In the subgroup of non-muscle invasive BC cases, no relationship was found either (**Table 5**).

Discussion

Previous studies mainly focused on the relationship between polymorphisms in the coding region of integrin genes and BC susceptibility. In the present study, polymorphism rs2675 in the predicted microRNA binding site of *ITGB5* gene was identified to be associated with the occurrence and progress of BC, which might provide a potential predicted target for the evaluation of BC susceptibility.

Integrin β_5, encoded by the *ITGB5* gene [27], was proved to play a critical role in the process of tumor angiogenesis, proliferation, invasion and migration. Blockage of integrin αvβ5 was identified to result in the reduction in tumor angiogenesis, which was caused by the suppression of phosphoinositide-3-kinase-dependent pathway [28]. In addition, integrin β_5 participated in promoting tumor cell invasion through cooperation with protease activated receptor-1 [29]. And tumor cell migration could be facilitated by integrin β_5 via reactive oxygen species-induced mitochondrial dysfunction [30]. Integrin β_5 was also reported to have the potential to promote tumorigenesis by contributing to the TGF-β-induced epithelial-mesenchymal transition, which was mediated by the Src-focal adhesion kinase and MEK-extracellular signal-regulated kinase induced signaling pathways independently [31]. Moreover, a function research showed that depletion of integrin β_5 in tumor cells resulted in decreased tumor intake and angiogenesis, which led to the

reduction in proliferative capacities and migration of tumor. However, this phenotype could be recovered after re-expression of integrin β_5 [32]. So integrin β_5 might be functionally involved in the BC development and progression. In this study, our results indicated that rs2675 in *ITGB5* might be correlate with BC risk in Chinese Han population. Due to the lack of validated studies investigating the relationship between various genotypes of this SNP and diverse *ITGB5* mRNA/protein expression, it is too early to draw a conclusion about the application of this SNP to predict BC risk. In addition, the recruitment of large samples in different population is required in the future.

Furthermore, the accessibility of miRNA-target combination might be changed as a result of the base transition from A to C in rs2675 (*ITGB5*), which led to the loss of miRNA targets for mir-504. MiR-504 was demonstrated to promote the tumorigenicity of cells in vivo [33]. As reported by Ma X *et al.* [34], up-regulation of miR-504 was associated with primary glioblastoma, while ectopic miR-504 increased invasion and migration in oral squamous cell carcinoma [35]. New miRNA targets in *ITGB5* gene appeared for mir-30a-3p and mir-30e-3p at the same time. The emergence of mir-30a-3p and mir-30e-3p might have the opposite effect against miR-504. These two miRNAs inhibited tumor proliferation, invasiveness and metastasis, which were identified to be downregulated in hepatocellular carcinoma and clear renal cell carcinoma [36, 37]. But it is still unclear about the accurate regulation mechanisms of these miRNAs on rs2675 in BC susceptibility. Moreover, whether this predicted miRNA binding site is the exact miRNA binding site needs further analysis.

No association was detected between the other three polymorphisms and BC risk in this study. For rs17664 in *ITGA6* gene and rs1062484 in *ITGA3* gene, no relationship between these polymorphisms and the risk of other tumor types was demonstrated by existing studies as well. However, polymorphism rs11902171 in *ITGAv* gene was reported to be associated with the occurrence and progression of prostate cancer [38] and gastric cancer [39], which might be relevant with the characteristics of tumor itself or the limitation caused by the sample size.

Integrins were proved to play an important role in the process of tumor recurrence. Previous studies identified that integrins could predict the intravesical recurrence of non-muscle invasive bladder cancer (NMIBC) [40]. However, none of these four SNPs in the integrin gene predicted miRNA binding sites mediates BC recurrence-free survival of 125 patients who completed the follow-up. Treatment and prognosis for muscle invasive bladder cancer (MIBC) and NMIBC are quite different. The majority of patients with MIBC received radical cystectomy or radiotherapy, and distant metastases were the most common cause of death [41]. For the treatment of NMIBC, although a combination of transurethral resection (TURBT) and intravesical therapy was widely applied clinically, the recurrence rate was rather high in both low-and high-grade disease, which fluctuated between 50% and 70% [42, 43]. In addition, the degree of malignancy increases with the raised frequency of recurrence. No exact indicators have been found for the prediction of NMIBC recurrence. Therefore, subgroup analysis in NMIBC patients is necessary to verify the prognostic role of these polymorphisms. However, no significant association was detected between these polymorphisms and the recurrence-free survival of NMIBC, which might be caused by survival index selection or limited sample size.

In conclusion, our data proved the association between polymorphism rs2675 in *ITGB5* gene and the risk of BC. This finding will be of benefit to the screening of high BC risk population and the prediction of prognosis in BC patients. However, regional population recruitment, SNPs selection limitation, and lack of functional analysis contributed to the defect of the study. Therefore, further well-designed studies in a large scale are needed to confirm the results in our study.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 81101939).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jin Huang, West China Hospital, Sichuan University, 37 Guoxuexiang,

Chengdu 610041, Sichuan, China. Tel: 86-189-8060-1852; Fax: 86-028-8542-2642; E-mail: huangjinscu@gmai.com; Dr. Peng Zhang, Department of Urology, West China Hospital, Sichuan University, 37 Guoxuexiang, Chengdu 610041, Sichuan, China. Tel: 86-189-8060-5795; Fax: 86-028-8542-2642; E-mail: zhangpengscu@gmail.com

References

[1] Siegel R, Naishadham D and Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013; 63: 11-30.

[2] Han S, Zhang S, Chen W and Li C. Analysis of the status and trends of bladder cancer incidence in China. Oncology Process 2013; 11: 89-95.

[3] Kiriluk KJ, Prasad SM, Patel AR, Steinberg GD and Smith ND. Bladder cancer risk from occupational and environmental exposures. Urol Oncol 2012; 30: 199-211.

[4] Ma XP, Zhang T and Peng B. Association between microRNA Polymorphisms and Cancer Risk Based on the Findings of 66 Case-Control Studies. PLoS One 2013; 8: e79584.

[5] Damsky CH, Knudsen KA, Bradley D, Buck CA and Horwitz AF. Distribution of the cell substratum attachment (CSAT) antigen on myogenic and fibroblastic cells in culture. J Cell Biol 1985; 100: 1528-1539.

[6] Hemler ME, Huang C and Schwarz L. The VLA protein family. Characterization of five distinct cell surface heterodimers each with a common 130,000 molecular weight beta subunit. J Biol Chem 1987; 262: 3300-3309.

[7] Taddei I, Faraldo MM, Teulière J, Deugnier MA, Thiery JP and Glukhova MA. Integrins in mammary gland development and differentiation of mammary epithelium. J Mammary Gland Biol Neoplasia 2003; 8: 383-394.

[8] Takada Y, Ye X and Simon S. The integrins. Genome Biol 2007; 8: 215.

[9] Hood JD and Cheresh DA. Role of integrins in cell invasion and migration. Nat Rev Cancer 2002; 2: 91-100.

[10] Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002; 110: 673-687.

[11] Dedhar S. Integrin mediated signal transduction in oncogenesis: an overview. Cancer Metastasis Rev 1995; 14: 165-172.

[12] Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SF, Csiszar K, Giaccia A, Weninger W, Yamauchi M, Gasser DL and Weaver VM. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 2009; 139: 891-906.

[13] Fournier AK, Campbell LE, Castagnino P, Liu WF, Chung BM, Weaver VM, Chen CS and Assoian RK. Rac-dependent cyclin D1 gene expression regulated by cadherin- and integrin-mediated adhesion. J Cell Sci 2008; 121: 226-233.

[14] Desgrosellier JS and Cheresh DA. Integrins in cancer: Biological implications and therapeutic opportunities. Nat Rev Cancer 2010; 10: 9-22.

[15] Moschos SJ, Drogowski LM, Reppert SL and Kirkwood JM. Integrins and cancer. Oncology 2007; 21: 13-20.

[16] Liebert M, Washington R, Stein J, Wedemeyer G and Grossman HB. Expression of the VLA beta 1 integrin family in bladder cancer. Am J Pathol 1994; 144: 1016-1022.

[17] Grossman HB, Washington RW Jr, Carey TE and Liebert M. Alterations in antigen expression in superficial bladder cancer. J Cell Biochem Suppl 1992; 161: 63-68.

[18] Liebert M, Washington R, Wedemeyer G, Carey TE and Grossman HB. Loss of co-localization of alpha 6 beta 4 integrin and collagen VII in bladder cancer. Am J Pathol 1994; 144: 787-795.

[19] Boyd SD. Everything you wanted to know about small RNA but were afraid to ask. Lab Invest 2008; 88: 569-578.

[20] Brennecke J, Stark A, Russell RB and Cohen SM. Principles of microRNA-target recognition. PLoS Biol 2005; 3: e85.

[21] Calin GA and Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.

[22] Wang L, Liu W, Jiang W, Lin J, Jiang Y, Li B and Pang D. A miRNA Binding Site Single-Nucleotide Polymorphism in the 39-UTR Region of the IL23R Gene Is Associated with Breast Cancer. PLoS One 2012; 7: e49823.

[23] Landi D, Gemignani F, Naccarati A, Pardini B, Vodicka P, Vodickova L, Novotny J, Försti A, Hemminki K, Canzian F and Landi S. Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer. Carcinogenesis 2008; 29: 579-584.

[24] Chin LJ, Ratner E, Leng S, Zhai R, Nallur S, Babar I, Muller RU, Straka E, Su L, Burki EA, Crowell RE, Patel R, Kulkarni T, Homer R, Zelterman D, Kidd KK, Zhu Y, Christiani DC, Belinsky SA, Slack FJ and Weidhaas JB. A SNP in a let-7 microRNA complementary site in the KRAS30 untranslated region increases non-small cell lung cancer risk. Cancer Res 2008; 68: 8535-8540.

[25] Yuan L, Chu H, Wang M, Gu X, Shi D, Ma L, Zhong D, Du M, Li P, Tong N, Fu G, Qin C, Yin C and Zhang Z. Genetic variation in DROSHA 3'UTR regulated by hsa-miR-27b is associated with bladder cancer risk. PLoS One 2013; 8: e81524.

[26] Brendle A, Lei H, Brandt A, Johansson R, Enquist K, Henriksson R, Hemminki K, Lenner P and Försti A. Polymorphisms in predicted mi-

croRNA-binding sites in integrin genes and breast cancer: ITGB4 as prognostic marker. Carcinogenesis 2008; 29: 1394-1399.
[27] McLean JW, Vestal DJ, Cheresh DA and Bodary SC. cDNA sequence of the human integrin beta 5 subunit. J Biol Chem 1990; 265: 17126-17131.
[28] Desch A, Strozyk EA, Bauer AT, Huck V, Niemeyer V, Wieland T and Schneider SW. Highly invasive melanoma cells activate the vascular endothelium via an MMP-2/integrin αvβ5-induced secretion of VEGF-A. Am J Pathol 2012; 181: 693-705.
[29] Even-Ram SC, Maoz M, Pokroy E, Reich R, Katz BZ, Gutwein P, Altevogt P and Bar-Shavit R. Tumor cell invasion is promoted by activation of protease activated receptor-1 in cooperation with the alpha vbeta 5 integrin. J Biol Chem 2001; 276: 10952-10962.
[30] Hung WY, Huang KH, Wu CW, Chi CW, Kao HL, Li AF, Yin PH and Lee HC. Mitochondrial dysfunction promotes cell migration via reactive oxygen species-enhanced β5-integrin expression in human gastric cancer SC-M1 cells. Biochim Biophys Acta 2012; 1820: 1102-1110.
[31] Bianchi A, Gervasi ME and Bakin A. Role of β5-integrin in epithelial-mesenchymal transition in response to TGF-β. Cell Cycle 2010; 9: 1647-1659.
[32] Bianchi-Smiraglia A, Paesante S and Bakin AV. Integrin β5 contributes to the tumorigenic potential of breast cancer cells through the Src-FAK and MEK-ERK signaling pathways. Oncogene 2013; 32: 3049-3058.
[33] Hu W, Chan CS, Wu R, Zhang C, Sun Y, Song JS, Tang LH, Levine AJ and Feng Z. Negative regulation of tumor suppressor p53 by microRNA miR-504. Mol Cell 2010; 38: 689-699.
[34] Ma X, Yoshimoto K, Guan Y, Hata N, Mizoguchi M, Sagata N, Murata H, Kuga D, Amano T, Nakamizo A and Sasaki T. Associations between microRNA expression and mesenchymal marker gene expression in glioblastoma. Neuro Oncol 2012; 14: 1153-1162.
[35] Yang MH, Lin BR, Chang CH, Chen ST, Lin SK, Kuo MY, Jeng YM, Kuo ML and Chang CC. Connective tissue growth factor modulates oral squamous cell carcinoma invasion by activating a miR-504/FOXP1 signalling. Oncogene 2012; 31: 2401-2411.
[36] Wang W, Lin H, Zhou L, Zhu Q, Gao S, Xie H, Liu Z, Xu Z, Wei J, Huang X and Zheng S. MicroRNA-30a-3p inhibits tumor proliferation, invasiveness and metastasis and is downregulated in hepatocellular carcinoma. Eur J Surg Oncol 2013; 13: 912-918.
[37] Mathew LK, Lee SS, Skuli N, Rao S, Keith B, Nathanson KL, Lal P and Simon MC. Restricted expression of miR-30c-2-3p and miR-30a-3p in Clear Cell Renal Carcinomas enhances HIF2α activity. Cancer Discov 2013; 4: 53-60.
[38] Liu J, Huang J, He Y, Liu J, Liao B and Liao G. Genetic variants in the integrin gene predicted microRNA-binding sites were associated with the risk of prostate cancer. Mol Carcinog 2014; 53: 280-285.
[39] Scartozzi M, Loretelli C, Bearzi I, Mandolesi A, Galizia E, Onofri A, Pistelli M, Bittoni A, Berardi R and Cascinu S. Allele polymorphisms of tumor integrins correlate with peritoneal carcinosis capability of gastric cancer cells in radically resected patients. Ann Oncol 2011; 22: 897-902.
[40] Behnsawy HM, Miyake H, Abdalla MA, Sayed MA, Ahmed Ael-F and Fujisawa M. Expression of integrin proteins in non-muscle-invasive bladder cancer: significance of intravesical recurrence after transurethral resection. BJU Int 2011; 107: 240-246.
[41] Booth CM, Siemens DR, Li G, Peng Y, Tannock IF, Kong W, Berman DM and Mackillop WJ. Perioperative chemotherapy for muscle-invasive bladder cancer: A population-based outcomes study. Cancer 2014; 120: 1630-1638.
[42] Weintraub MD, Li QQ and Agarwal PK. Advances in intravesical therapy for the treatment of non-muscle invasive bladder cancer (Review). Mol Clin Oncol 2014; 2: 656-660.
[43] Feng J, He W, Song Y, Wang Y, Simpson RJ, Zhang X, Luo G, Wu J and Huang C. Platelet-derived growth factor receptor beta: a novel urinary biomarker for recurrence of non-muscle-invasive bladder cancer. PLoS One 2014; 9: e96671.