Int J Clin Exp Pathol 2018;11(2):831-838
www.ijcep.com /ISSN:1936-2625/IJCEP0064508

Original Article

Focal adhesion kinase (FAK) is associated with poor prognosis in urinary bladder carcinoma

Qi Zhang[1,2,3], Huiju Wang[2,3], Haibing Wei[1,2], Dahong Zhang[1,2,3]

[1]Department of Urology, Zhejiang Provincial People's Hospital, Hangzhou, China; [2]People's Hospital of Hangzhou Medical College, Hangzhou, China; [3]Key Laboratory of Tumor Molecular Diagnosis and Individualized Medicine of Zhejiang Province, Hangzhou, China

Received August 27, 2017; Accepted November 13, 2017; Epub February 1, 2018; Published February 15, 2018

Abstract: Objective: Overexpression of the enhancer of focal adhesion kinase (FAK) protein, an intracellular tyrosine kinase protein, has been reported to be associated with biological malignancy of gastric cancer and several other tumors. The purpose of this study was to examine the expression of FAK and analyze its correlation with the clinicopathological features of human urinary bladder carcinoma. Methods: 315 archived cases of urinary bladder carcinoma were reviewed and TMAs were developed as per established procedures. Immunohistochemical staining for FAK was performed to assess the correlation between the expression profiles and the clinicopathological parameters and clinical outcome. Results: Protein level of FAK was up-regulated in urinary bladder carcinoma compared with adjacent non-tumor tissues. Overexpression of FAK was significantly associated with high histologic grade, angiolymphatic invasion, lymph node metastasis, myometrial invasion and cervical involvement ($P < 0.05$). Further multivariate analysis suggested that expression of FAK was independent prognostic indicator for urinary bladder carcinoma. These alterations in expression were also associated with greater risk of disease progression and decreased chance of carcinoma-specific survival. Kaplan-Meier analysis demonstrates that overexpression of FAK was significantly associated with decreased overall survival. Conclusion: Overexpression of FAK corelates with well established pathologic risk factors and may predict more aggressive biologic behavior in urinary bladder carcinoma. The expression patterns of FAK correlated well with the pathologic stage, disease progression, and carcinoma-specific survival. This finding may aid in identifying more biologically aggressive carcinomas and thus patients who could benefit from more intensive adjuvant therapy.

Keywords: Urinary bladder carcinoma, FAK, prognostic marker, prognosis

Introduction

Urinary bladder carcinoma is the most common type of cancer worldwide occurs in the urinary tract and an estimated 290,000 new cases are diagnosed each year in men and 88,000 in women [1]. Standard treatment for the muscle invasive bladder cancer is radical cystectomy and bilateral pelvic lymph-node dissection [2]. Although surgery could be curative, about 50% of patients with muscle-invasive transitional-cell carcinoma (TCC) get metastases within 2 years of cystectomy and subsequently die of this disease [3, 4]. For patients with advanced or metastatic disease, systemic combination chemotherapy is commonly used as the main viable option. The overall 5-year survival rate for patients with advanced or metastatic diease is between 13 to 15%, but current pathological classification schema cannot adequately predict tumor behavior nor permit patient seletion for early aggressive treatment or long-term surveillance as appropriate. Therefore, specific diagnostic and prognostic molecular markers for urinary bladder carcinoma diagnosis and novel therapeutic targets for treatment are needed.

Focal adhesion kinase (FAK), an intracellular tyrosine kinase protein, is known to be involved in many critical cellular events including adhesion, migration, proliferation, and survival [5]. FAK has been proved to regulate cell migration and invasion through distinct mechanisms by promoting the dynamic regulation of focal adhesion and peripheral actin structures, as same as the matrix metalloproteinases (MMP)-mediated matrix degradation [6]. Increased

FAK expression and its tyrosine phosphorylation in malignant cells showed good correlation with the progression to an invasive cell phenotype [7]. Increased FAK expression was positively related with poor survival and tumor progression in a variety of invasive and metastatic tumors, including gastric cancer, pancreatic cancer, colon cancer, and breast cancer [8-10].

The primary purpose of the present work was to determine the expression pattern of FAK and to evaluate its prognostic value in patients with transitional cell urinary bladder carcinoma who had undergone radical cystectomy.

Material and methods

Patient population and tissue microarray

From January 1998 to October 2008, 315 consecutive patients diagnosed by urinary bladder carcinoma (BC) underwent radical cystectomy at Zhejiang Provincial People's Hospital were included in this retrospective study; only specimens of primary diagnoses obtained by radical cystectomy were included. The patient group consisted of 232 males and 83 females, with median age of 62.1 years (range 35-78) at the time of surgery. All patients had follow-up records more than 5 years. The follow-up dealine was May 2008. The survival time was figured from the date of surgery to the date of death or follow-up deadline. Death was caused mainly by cancer recurrence or metastasis. Sixty three non-tumorous human bladder tisues were obtained from radical cystectomy of adjacent urinary bladder carcinoma margins > 5 cm. Lymphovascular invasion was defined according to the presence of carcinoma cells wit in the endothelium space. Carcinoma cells which had merely invaded vascular lumen were considered negative. The 2009 TNM classification was used for pathologic staging, and the World Health Organization classification was used for pathologic grading. Written informed consent was obtained from all participants involved. We obtained ethics approval from the ethics committees at Zhejiang Provincial People's Hospital.

TMAs were made according to our former standard protocol [11]. Briefly, Core areas (2 mm in diameter) contains the most characteristic features of pathologic processes, which on a whole tissue section with hematoxylin eosin (H&E) stained slides were identified by two pathologists. The areas of the slides which contains the center of the tumor tissue are identified and marked. Areas on the block corresponding to the marked slides were arranged in recipient paraffin blocks (tissue array blocks) using TM instrument; in small specimens, 1-3 cores were obtained, based on tissue amount. Staining results from different intratumoral areas in various tumors show reliable consistency, therefore, a single core was sampled in each case. A qualified sample was defined as tumor occupying more than 10% of the core area. Each block contained six noncarcinomaous bladder mucosa as internal controls. The design of each block was detailed in a TMA map, indicating the position and identification of each core. Consecutive 4 μm-thick sections were cut from each tissue array block, deparaffinized and dehydrated.

Immunohistochemical analysis

Immunohistochemical staining was performed by the standard method in 63 noncarcinomaous human bladder tissue samples and 315 human urinary bladder carcinoma tissues. Briefly, 4 μm sections from the TMAs were baked at 60°C for 2 h. Then, the sections were deparaffinized in xylene, rehydrated using a gradient of ethanol concentrations, microwaved in 10 mM citrate buffer for 15 min to retrieve antigen, blocked with 3% hydrogen peroxide for 10 min to inhibit endogenous peroxidase activity and incubated with 10% goat non-immune serum for 20 min to reduce background nospecific staining. After that, the sections were incubated with mouse anti-FAK (Santa Cruz Biotechnology Inc, Delaware Avenue, California, USA) overnight at 4°C for 16 hours. Secondary antibody was performed with the use of Envision (Rabbit, Dako, Denmark). After washing, tissue sections were treated with secondary antibody. Finally, the sections were counte stained with hematoxylin, dehydrated, cleared and mounted.

The degree of immunostaining was reviewed and scored independently by two pathologist based on the intensity of staining who was unaware of the clinical and pathologic data. In the cases of discrepancy, a consensus score was selected for evaluation. The staining was graded based on intensity (no staining = 0, weak staining = light yellow = 1, moderate staining = yellowish brown = 2, and strong

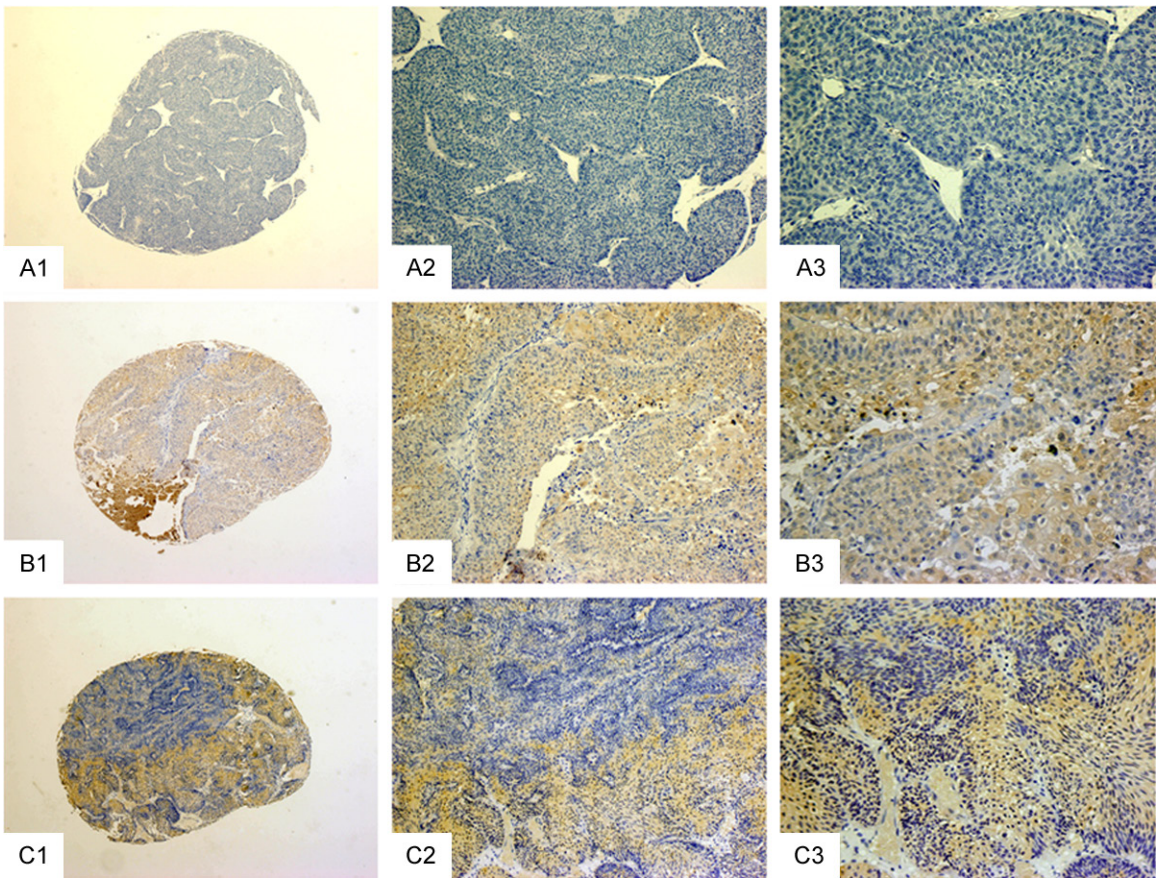

Figure 1. Immunohistochemical staining for FAK in normal and cancerous bladder tissue. A1-A3: Immunostaining of FAK in normal bladder tissue. B1-B3: Strong staining (yellow-brown granules, mainly in the cytoplasm) in non-muscle-invasive urinary bladder carcinoma. C1-C3: Strong staining in muscle-invasive urinary bladder carcinoma. Magnification: the original magnification ×40 (A1-C1), ×100 (A2-C2), and ×400 (A3-C3).

staining = brown = 3) and distribution (< 5% = 0, 6-25% = 1, 26-50% = 2, > 51% = 3) and the staining index was calculated. We use this method to evaluate FAK expression by determining the staining index with scores of 0, 1, 2, 3, 4, 6, or 9. Optimal cut-off value was identified as: index score of ≥ 4 was used to qualified tumors with high FAK expression, and index score of ≤ 3 was used to indicate low FAK expression.

Statistical analysis

All statistical analyses were performed using the Statistical Package of Social Sciences (SPSS V.16.0 for Windows). To evaluate the relationships between the expression of FAK and the clinicopathological parameter of the patients with urinary bladder carcinoma, measurement data were studied using Student's t test, while categorical data were anlyzed using the X^2 or Fisher's exact test. Survival rate curves were drawn according to the Kaplan-Meier method, and differences between the curves were analyzed by applying the log-rank test. Multivariate survival analysis using the Cox proportional hazards regression model was performed to evaluate the prognostic values of protein expression. Correlation coefficients between protein expression and clinicopathological findings were measured using the Spearsman correlation method. *P* value < 0.05 was considered statistically sinificant and all *P* values were two-sided.

Results

Expression of FAK in urinary bladder carcinoma and non-tumor mucosa

The immunostaining of FAK was mainly detected in the cytoplasm of the tumor cells (**Figure 1**). The expression of FAK protein was detected in 5 of 63 (7.93%) human non-tumor mucosa; all samples expressed the protein at low levels. FAK protein was detected in 207 of 315

Table 1. Relationship of FAK expression with pathological parameters of tumor

Clinical parameters	FAK			
	Low	High	t/χ^2	P
Gender			1.498	0.221
Male	75 (69.4%)	157 (75.8%)		
Female	33 (30.6%)	50 (24.2%)		
Age (yrs)			1.195	0.274
< 60	36 (33.3%)	82 (39.6%)		
≥ 60	72 (66.7%)	125 (60.4%)		
Size			0.047	0.828
< 3 cm	67 (62.0%)	131 (63.3%)		
≥ 3 cm	41 (38.0%)	76 (36.7%)		
Number of tumor			9.166	0.002
Single	35 (32.4%)	36 (17.4%)		
Multiple	73 (67.6%)	171 (82.6%)		
Invasion depth			7.311	0.007
Ta-T_1	46 (42.6%)	57 (27.5%)		
T_2-T_4	62 (57.4%)	150 (72.5%)		
Lymph node metastasis			6.611	0.010
No	90 (83.3%)	145 (70.0%)		
Yes	18 (16.7%)	62 (30.0%)		
Distant metastasis			7.579	0.006
No	105 (97.2%)	182 (87.9%)		
Yes	3 (2.8%)	25 (12.1%)		
Lymphovascular invasion			0.546	0.460
Negative	97 (89.8%)	191 (92.3%)		
Positive	11 (10.2%)	16 (7.7%)		
Histological grade			8.447	0.015
PUNLMP+ Low grade	48 (44.4%)	60 (55.6%)		
High grade	73 (35.3%)	134 (64.7%)		

PUNLMP = Papillary urothelial neoplasm of low malignant potential.

(65.71%) human urinary bladder carcinoma cases. The FAK expression in urinary bladder carcinoma was significantly higher than that in non-tumor bladder mucosa ($P < 0.01$).

Correlation between FAK and clinicopathological features

The expression of FAK in urinary bladder carcinoma was significantly related to number of tumor, invasion depth, lymph node metastasis and distant metastasis, and not related to age, gender, tumor size, histological grade and lymphovascular invasion (**Table 1**). Urinary bladder carcinoma patients with multiple tumor, deep tumor invasion (T_2-T_4), lymphnode metastasis, and distant metastasis had significantly higher expression of FAK than those with single tumor, superficial tumor invasion (Ta-T_1), without lymphnode metastasis and without distant metastasis (**Table 1**). The detection rate of FAK was 69.9% (172/246) in urinary bladder carcinoma specimens of multiple tumor, which was higher than that in specimens of single tumor (50.7%, 35/69, P < 0.01). FAK was detected in 70.8% (150/212) of deep tumor invasion (T_2-T_4), which was lower than in superficial tumor invasion (Ta-T_1) samples (61.7%, 57/103, $P < 0.01$). FAK was detected in 77.5% (62/80) cases of bladder cancer specimens with lymphnode metastasis, which was higher than in specimens without lymphnode metastasis (57.1%, 145/235, $P < 0.05$). The detection rate of FAK was 89.3% (25/28) in specimens with distant metastasis, which was higher than in specimens without distant metastasis (63.4%, 182/287, $P < 0.01$). The Spearman correlation coefficient of FAK expression with number of

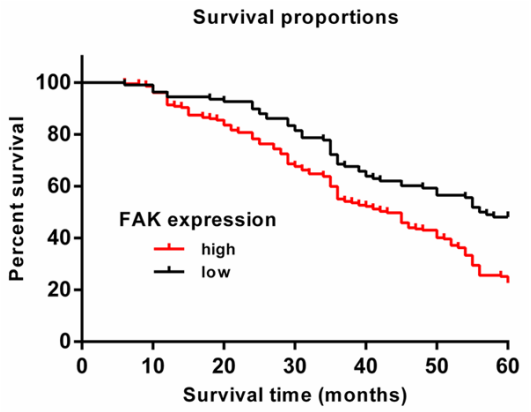

Figure 2. Kaplan-Meier survival curves of urinary bladder carcinoma patients with positive and negative FAK expression.

tumor, depth of invasion, lymphnode metastasis, and distant metastasis of tumor were 0.167, 0.152, 0.145 and 0.155 ($P < 0.05$), respectively.

Patient prognosis and survival analysis

The mean survival time in patients with high expression of FAK was 40.60 ± 1.19 months, which was significantly lower than in patients with low expression of FAK (47.10 ± 1.52 months, $P < 0.001$). Kaplan-Meier survival statistics showed that high expression of FAK was associated with worse overall survival when compared to low expression of FAK ($P < 0.001$, **Figure 2**). Factors with possible prognostic effects in urinary bladder carcinoma were analyzed by multivariate Cox proportional hazard regression analysis, and the results showed that depth of invasion ($P = 0.002$), lymph node metastasis ($P = 0.022$), distant metastasis ($P = 0.035$), and expression of FAK ($P < 0.001$) were independent prognostic factors in patients with urinary bladder carcinoma. However, age, size of tumor, number of tumor, lymphovascular invasion, and histological grade had no significant prognostic value ($P > 0.05$, **Table 2**).

Discussion

Focal adhesion kinase (FAK) is a tyrosine phosphorylated protein which becomes phosphorylated and activated subsequently in response to integrin clustering, cellular adhesion to the extracellular matrix, or cellular transformation with the v-Src oncogene [12]. It is known to control a number of biological processes including cell spreading, proliferation, cell survival and motility [8]. Under normal conditions, FAK deregulation of several of these processes is associated with malignancy. These studies identified FAK gene amplification in cancer cells in vitro, and researched differences in the FAK gene status between normal cells and cancer cells, however, the relationships between FAK protein and clinicopathological parameters have not been reported in urinary bladder carcinoma yet.

Up to now, invasion depth, lymphnode metastasis and distant metastasis are considered to be the prognostic factors for urinary bladder carcinoma. In the present work, we found out that high expression of FAK was significantly related to poor prognosis of patients with urinary bladder carcinoma, and expression of FAK was shown to be an independent prognostic factor. Other factors which significantly correlated with the survival of the patients included depth of invasion, lymph node metastasis and distant metastasis. Further analysis revealed that patients with low FAK expression had a significantly longer mean survival time than those with high expression. It suggests that FAK can serve as an effective and objective indicator for the identification of urinary bladder carcinoma patients who are at high-risk of tumor invasion and progression.

In agreement with our results, previous studies also showed that increased FAK expression was correlated with poor progression and prognosis in numerous cancers. For example, elevated FAK expression has been associated with advanced stage in breast and colon tumors, and in metastatic prostate carcinoma copared with normal tissue [13, 14]. Patients with high FAK expression had shorter overall survival time than those with low expression, and the prognostic effect of FAK expression was independent prognostic elements for urinary bladder carcinoma. Increased FAK expression has been shown to interact with invasion and to play a crucial role in malignant and shorter survival in colorectal cancer [15, 16]. FAK could present as a marker for metastatic or advanced disease in a lot kind of solid tumors including breast and prostate carcinoma [14, 17]. In NSCLC, a remarkable correlation was found between FAK overexpression and advanced disease stage [18, 19]. Moreover,

Table 2. Multivariate analysis of the correlation between clinicopathological parameters and survival time of patients with bladder cancer

Covariates	Coeffcient	Standarderror	Hazard ratio (HR)	95.0% CI for HR	*P*
Age range (>60 vs ≤60)	0.069	0.178	1.072	0.756-1.518	0.698
Tumor size (≥3cm vs <3cm)	0.058	0.150	1.059	0.789-1.422	0.702
Number of tumor (single vs multiple)	0.108	0.189	1.114	0.769-1.612	0.569
Lymph node metastasis (positive vs negative)	0.377	0.164	1.458	1.056-2.013	0.022
Lymphovascular invasion (positive vs negative)	0.055	0.171	1.057	0.756-1.477	0.746
Distant metastasis (positive vs negative)	0.330	0.156	1.391	1.024-1.890	0.035
FAK expression (high vs low)	0.785	0.180	2.192	1.539-3.122	0.000
Depth of invasion (T_2-T_4 vs Ta-T_1)	0.572	0.183	1.771	1.237-2.537	0.002
Histological grade	0.188	0.233	1.207	0.765-1.905	0.419

in an IHC study of 91 patients with esophageal squamous cell cancer, increased FAK expression was associated with tumor invasiveness, lymphnode metastasis, and advanced disease stage; the survival time was significantly lower in patients with FAK overexpression [20].

Early diagnosis, conventional clinicopathological parameters and constant disease monitoring remain the prerequisites for effective treatment against urinary bladder carcinoma [21]. As current biomarkers like P53 lack sensitivity and specificity, there is a pressing need for novel urinary bladder carcinoma diagnosis. Although how FAK expression regulates the progression of these tumors has not been clarified, former studies advised that FAK was a point of convergence to various models of cell signaling pathways related to cancer progression [22]. FAK interacts with Src family kinases has been proposed to allow autophosphorylation, which adjusts the trans-phosphorylation of FAK in the kinase domain activation loop, meanwhile promoting maximal FAK catalytic activation [23-25]. Interaction of FAK with these signaling molecules may lead to the activation of the binding associates necessary for triggering downstream signaling pathways [26]. Alternatively, such interaction may recruit these FAK binding molecules to focal contacts which promote the downstream signaling events. Combined targeting of FAK and Src could be beneficial for the outcome of colorectal cancer and could afford an chance for therapeutic intervention [23]. Paxillin is one of the FAK/Src protein complex targets, and phosphorylation of the latter is crucial for cell migration processes [23, 27]. Although FAK is known as a cytoplasmic protein, it also interacts with membranous protein such as HER-2 and c-MET [22, 28].

In summary, the present study showed that high correlation of FAK expression relate to decreased overall survival, which suggests that the capacity of FAK to predict poor prognosis and also help us understand more molecular or carcinogenesis mechanisms in urinary bladder carcinoma. Thus, FAK may act an important role in the progression of urinary bladder carcinoma and could be an effective indicator to predict local invasion and prognosis for urinary bladder carcinoma. Based on the TNM stage, the expression of FAK in urinary bladder carcinoma will help to identify patients with high potential for tumor metastasis, while predicting the prognosis of the patients with urinary bladder carcinoma.

Acknowledgements

This work was supported by Medicine and Health Research Foundation of Zhejiang Province (Grant No. 2015106257), Science and Technology Agency Research Foundation of Zhejiang Province (Grant No. 2013C03017-5), Science and Technology Agency Research Foundation of Zhejiang Province Medicine (Grant No. 2013C03017-5), and National Natural Science Foundation of China (Grant No. 81502541). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Dahong Zhang, Department of Urology, Zhejiang Provincial People's Hospital, 158 Shangtang Road, Hangzhou 310014, Zhejiang, China; People's Hospital of Hangzhou Medical College, 158 Shangtang Road, Hangzhou 310014, Zhejiang, China. Tel: +86-571-85893312; Fax: +86-571-85893587; E-mail: urology@zju.edu.cn

References

[1] Gui Y, Guo G, Huang Y, Hu X, Tang A, Gao S, Wu R, Chen C, Li X, Zhou L, He M, Li Z, Sun X, Jia W, Chen J, Yang S, Zhou F, Zhao X, Wan S, Ye R, Liang C, Liu Z, Huang P, Liu C, Jiang H, Wang Y, Zheng H, Sun L, Liu X, Jiang Z, Feng D, Chen J, Wu S, Zou J, Zhang Z, Yang R, Zhao J, Xu C, Yin W, Guan Z, Ye J, Zhang H, Li J, Kristiansen K, Nickerson ML, Theodorescu D, Li Y, Zhang X, Li S, Wang J, Yang H, Wang J, Cai Z. Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. Nat Genet 2011; 43: 875-8.

[2] Stenzl A, Cowan NC, De Santis M, Kuczyk MA, Merseburger AS, Ribal MJ, Sherif A, Witjes JA, Lebret T. Treatment of muscle-invasive and metastatic bladder cancer: update of the EAU guidelines. Eur Urol 2011; 59: 1009-18.

[3] Hussain SA, James ND. The systemic treatment of advanced and metastatic bladder cancer. Lancet Oncol 2003; 4: 489-97.

[4] Liu C, Zheng S, Shen H, Xu K, Chen J, Li H, Xu Y, Xu A, Chen B, Kaku H, Nasu Y, Kumon H, Huang P, Watanabe M. Clinical significance of CD24 as a predictor of bladder cancer recurrence. Oncol Lett 2013; 6: 96-100.

[5] Sorenson CM, Sheibani N. Focal adhesion kinase, paxillin, and bcl-2: analysis of expression, phosphorylation, and association during morphogenesis. Dev Dyn 1999; 215: 371-82.

[6] Siesser PM, Hanks SK. The signaling and biological implications of FAK overexpression in cancer. Clin Cancer Res 2006; 12: 3233-7.

[7] Mon NN, Ito S, Senga T, Hamaguchi M. FAK signaling in neoplastic disorders: a linkage between inflammation and cancer. Ann N Y Acad Sci 2006; 1086: 199-212.

[8] Park JH, Lee BL, Yoon J, Kim J, Kim MA, Yang HK, Kim WH. Focal adhesion kinase (FAK) gene amplification and its clinical implications in gastric cancer. Hum Pathol 2010; 41: 1664-73.

[9] Lark AL, Livasy CA, Calvo B, Caskey L, Moore DT, Yang X, Cance WG. Overexpression of focal adhesion kinase in primary colorectal carcinomas and colorectal liver metastases: immunohistochemistry and real-time PCR analyses. Clin Cancer Res 2003; 9: 215-22.

[10] Sawai H, Okada Y, Funahashi H, Matsuo Y, Takahashi H, Takeyama H, Manabe T. Activation of focal adhesion kinase enhances the adhesion and invasion of pancreatic cancer cells via extracellular signal-regulated kinase-1/2 signaling pathway activation. Mol Cancer 2005; 4: 37.

[11] Zhang Q, Wang HJ, Zhang DH, Ru GQ, He XJ, Ma YY. High expression of HEF1 is associated with poor prognosis in urinary bladder carcinoma. Onco Targets Ther 2014; 7: 1319-26.

[12] McLean GW, Carragher NO, Avizienyte E, Evans J, Brunton VG, Frame MC. The role of focal-adhesion kinase in cancer - a new therapeutic opportunity. Nat Rev Cancer 2005; 5: 505-15.

[13] Ocak S, Chen H, Callison C, Gonzalez AL, Massion PP. Expression of focal adhesion kinase in small-cell lung carcinoma. Cancer 2012; 118: 1293-301.

[14] Rovin JD, Frierson HF Jr, Ledinh W, Parsons JT, Adams RB. Expression of focal adhesion kinase in normal and pathologic human prostate tissues. Prostate 2002; 53: 124-32.

[15] Owens LV, Xu L, Craven RJ, Dent GA, Weiner TM, Kornberg L, Liu ET, Cance WG. Overexpression of the focal adhesion kinase (p125FAK) in invasive human tumors. Cancer Res 1995; 55: 2752-5.

[16] Yu HG, Nam JO, Miller NL, Tanjoni I, Walsh C, Shi L, Kim L, Chen XL, Tomar A, Lim ST, Schlaepfer DD. p190RhoGEF (Rgnef) promotes colon carcinoma tumor progression via interaction with focal adhesion kinase. Cancer Res 2011; 71: 360-70.

[17] Lightfoot HM Jr, Lark A, Livasy CA, Moore DT, Cowan D, Dressler L, Craven RJ, Cance WG. Upregulation of focal adhesion kinase (FAK) expression in ductal carcinoma in situ (DCIS) is an early event in breast tumorigenesis. Breast Cancer Res Treat 2004; 88: 109-16.

[18] Carelli S, Zadra G, Vaira V, Falleni M, Bottiglieri L, Nosotti M, Di Giulio AM, Gorio A, Bosari S. Up-regulation of focal adhesion kinase in non-small cell lung cancer. Lung Cancer 2006; 53: 263-71.

[19] Ji HF, Pang D, Fu SB, Jin Y, Yao L, Qi JP, Bai J. Overexpression of focal adhesion kinase correlates with increased lymph node metastasis and poor prognosis in non-small-cell lung cancer. J Cancer Res Clin Oncol 2013; 139: 429-35.

[20] Miyazaki T, Kato H, Nakajima M, Sohda M, Fukai Y, Masuda N, Manda R, Fukuchi M, Tsukada K, Kuwano H. FAK overexpression is correlated with tumour invasiveness and lymph node metastasis in oesophageal squamous cell carcinoma. Br J Cancer 2003; 89: 140-5.

[21] Yang GL, Zhang LH, Bo JJ, Huo XJ, Chen HG, Cao M, Liu DM, Huang YR. Increased expression of HMGB1 is associated with poor prognosis in human bladder cancer. J Surg Oncol 2012; 106: 57-61.

[22] Meng XN, Jin Y, Yu Y, Bai J, Liu GY, Zhu J, Zhao YZ, Wang Z, Chen F, Lee KY, Fu SB. Characterisation of fibronectin-mediated FAK signalling pathways in lung cancer cell migration and invasion. Br J Cancer 2009; 101: 327-34.

[23] de Heer P, Koudijs MM, van de Velde CJ, Aalbers RI, Tollenaar RA, Putter H, Morreau J, van de Water B, Kuppen PJ. Combined expression of the non-receptor protein tyrosine kinases FAK and Src in primary colorectal cancer is associated with tumor recurrence and metastasis formation. Eur J Surg Oncol 2008; 34: 1253-61.

[24] Hanks SK, Ryzhova L, Shin NY, Brabek J. Focal adhesion kinase signaling activities and their implications in the control of cell survival and motility. Front Biosci 2003; 8: d982-96.

[25] Zheng Y, Lu Z. Paradoxical roles of FAK in tumor cell migration and metastasis. Cell Cycle 2009; 8: 3474-9.

[26] Shen TL, Guan JL. Differential regulation of cell migration and cell cycle progression by FAK complexes with Src, PI3K, Grb7 and Grb2 in focal contacts. FEBS Lett 2001; 499: 176-81.

[27] van Nimwegen MJ, Verkoeijen S, van Buren L, Burg D, van de Water B. Requirement for focal adhesion kinase in the early phase of mammary adenocarcinoma lung metastasis formation. Cancer Res 2005; 65: 4698-706.

[28] Chiang YY, Chow KC, Lin TY, Chiang IP, Fang HY. Hepatocyte growth factor and HER2/neu downregulate expression of apoptosis-inducing factor in non-small cell lung cancer. Oncol Rep 2014; 31: 597-604.