Pathol. Oncol. Res. (2012) 18:471–477
DOI 10.1007/s12253-011-9470-z

RESEARCH

Prognostic Value of Raf Kinase Inhibitor Protein in Esophageal Squamous Cell Carcinoma

Chengcheng Gao · Liqun Pang · Chengcheng Ren · Tianheng Ma

Received: 3 September 2011 / Accepted: 26 October 2011 / Published online: 19 November 2011

Abstract Raf kinase inhibitory protein (RKIP, also PEBP1) is involved in regulation of multiple cellular signaling processes and suppressing metastasis in animal models. Downregulation of RKIP expression has been shown to promote tumor progression in a variety of human cancers. However, its role and clinical significance in resectable esophageal squamous cell carcinoma (ESCC) is still scanty. The purpose of this study was to investigate the prognostic significance of RKIP expression by immunohistochemistry in a group of patients with ESCC treated with surgical resection. RKIP expression in 233 surgically resected ESCC specimens and 49 cases of adjacent normal tissues was detected by using immunohistochemical staining. The clinical and prognostic significance of RKIP expression was statistically analyzed. Kaplan-Meier analysis was used to compare the postoperative survival between groups. Significant downregulation was noted for RKIP protein in ESCCs, compared to adjacent normal tissues ($p<0.001$). A lower disease-free survival and overall survival of ESCC was found in patients whose tissues had low RKIP expression (both $P<0.001$). In addition, RKIP expression could stratify the patient survival (disease-free survival/ overall survival) in stage II ($P=0.01$ and 0.02, repectively). The Cox proportionate hazard regression model also established that low expression of RKIP was significantly correlated with increased risk (RR=3.572) of recurrence compared with high RKIP expression ($P<0.001$). Furthermore, the results of multivariate analysis suggested that RKIP expression ($P<0.001$) was an independent factor that affected overall survival. These findings suggest that the low expression of RKIP be associated with poor survival in resectable ESCC patients.

Keywords Esophageal squamous cell carcinoma · Raf kinase inhibitor protein · Prognosis

C. Gao · L. Pang · C. Ren (✉) · T. Ma
Department of Gastroenterology, Huai'an No.1 Hospital, Affiliated to Nanjing Medical University, Huai'an, Jiangsu 223300, China
e-mail: gao.jsha@gmail.com

Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most malignant diseases of the digestive tract. It has a high incidence in China. ESCC is characterized by late clinical presentation, rapid progression and poor survival [1]. Surgical resection is still the major therapeutic strategy used for operable ESCC. Nevertheless, disease recurrence often occurs even after curative resection, and the overall survival remains low. The tumor-node-metastases (TNM) classification based on pathologic examination of resected specimens is the most reliable information to predict clinical outcome [2]. However, substantial differences in survival are observed for the same TNM stage, and these differences are likely attributable to the inherent heterogeneity in the biologic behavior of the tumors [3]. Thus, prediction of tumor carcinogenesis by the analysis of novel molecular prognostic markers might aid in developing more effective therapeutic strategies for better prognosis.

Raf kinase inhibitor protein (RKIP), also known as phosphatidylethanolamine-binding protein 1 (PEBP1), is a member of the PEBP family [4]. It is a conserved, small, cytosolic protein firstly purified from bovine brain by Bernier group [5]. It has wide tissue expression in a variety of different mammalian species such as monkey, rat, chicken and human. RKIP was originally identified as an endogenous inhibitor of Raf and it negatively regulates the

Raf/MEK/ERK-signaling cascade [6]. It has been well-established that RKIP suppresses the metastatic spread of tumor cells, moreover, the down-regulated expression of RKIP is observed in a number of human cancers including highly metastatic prostate carcinoma, lung cancer, breast carcinoma, colon cancer and hepatocellular carcinoma [7–10]. The importance of RKIP in metastasis was demonstrated by the finding that the restoration of RKIP expression inhibits prostate cancer metastasis in a murine model and, hence, RKIP was identified as a metastasis suppressor gene [11]. In addition, over-expression of RKIP reverses tumor cell resistance to apoptosis by both chemotherapeutic drugs and by TRAIL. RKIP has also been implicated as an immune surveillance cancer gene in these studies.

However, the significance of RKIP expression in clinical settings and in the prognosis of ESCC is still elusive. Therefore, we performed the present study to investigate the clinical/prognostic implication of RKIP expression in 233 ESCC patients treated with surgical resection.

Materials and Methods

Patients and Tissue Samples

Prior informed consent was obtained from the patients for the collection of specimens in accordance with the guidelines of Department of Gastroenterology, Huai'an No.1 Hospital, Affiliated to Nanjing Medical University, P.R.China, and the study protocols were approved by the Ethics Committee of Department of Gastroenterology, Huai'an No.1 Hospital, Affiliated to Nanjing Medical University, P.R.China. All specimens were handled and made anonymous according to the ethical and legal standards.

A total of 65 females and 168 males, aged from 31 to 80 years (median 56.8 years), were included in the study. The patients underwent surgery at the Department of Gastroenterology, Huai'an No.1 Hospital,Affiliated to Nanjing Medical University, between October 2001 and April 2008. The cases selected in this study fulfilled these criteria: (i) newly diagnosed cancer of the esophagus without previous treatment; (ii) histologically confirmed primary ESCC; (iii) underwent a complete surgical resection (R0) at our cancer center; (iv) adequate clinical information and follow-up data were available. The histologic grade and clinical stage of the tumors were defined according to the 7th edition of the TNM classification of the International Union Against Cancer (2009) [12]. Patients with noncurative resection (R1) or died from postoperative complications were excluded from the study. Patients with neoadjuvant or adjuvant therapy were also excluded. The tumor specimens and adjacent normal tissue samples were obtained as paraffin blocks from the Bank of Tumor Source at our cancer center. Clinical data were obtained from hospital records after surgery. All the patients were followed up in March 2011 to determine their current status. The clinicopathological characteristics of the 100 patients studied are summarized in Table 1.

Immunohistochemistry Analysis

The specimens were fixed in 10% neutral buffered formalin and subsequently embedded in paraffin. The paraffin-embedded tissues were cut at 3 μm and stained following being dried on ProbeOn Plus (Fisher Scientific International, Hampton, NH, USA). Staining was done using avidin- biotin complex with a microprobe manual stainer (Fisher Scientific International). The slide to which a paraffin section was attached went through deparaffinization and hydration, and was then treated with a solution of Peroxidase-blocking reagent (Dako, Glostrup, Denmark) to exhaust endogenous peroxidase activity. It was put in citric acid solution and heated for 10 min in a microwave and then left at room

Table 1 Correlation between immunoreactivity of RKIP and clinical features of patients with ESCC

Features	Cases	RKIP expression pattern (n,%)		
		High	Low	P *
Age (years)				
≤55 *	139	31 (22.3)	108 (77.7)	0.7
>55	94	23 (24.5)	71 (75.5)	
Sex				
Male	168	38 (22.6)	130 (77.4)	0.7
Femal	65	16 (24.6)	49 (75.4)	
Location				
Upper	12	3 (25.0)	9 (75.0)	0.9
Middle	160	38 (23.8)	122 (76.2)	
Lower	61	13 (21.3)	48 (78.7)	
WHO grade				
G1	56	11 (19.6)	45 (80.4)	0.6
G2	147	34 (23.1)	113 (76.9)	
G3~4	30	9 (30.0)	21 (70.0)	
T status				
T2~3	230	52 (22.6)	178 (77.4)	0.9
T4	3	2 (67.7)	1 (33.3)	
N status				
N0	123	28 (22.8)	95 (77.2)	0.3
N1	110	26 (23.6)	84 (76.4)	
TNM stage				
I	8	1 (12.5)	7 (87.5)	0.4
II	133	28 (21.1)	105 (78.9)	
III	92	25 (27.2)	67 (72.8)	

* Chi-square test

temperature for 20 min to expose antigen hidden inside the tissue due to formalin fixation, and the process was repeated three times. To inhibit non-specific antigen-antibody reactions possible in immunohistochemical staining, reaction was done using a protein blocker (Research Genetics, Huntsville, AL, USA) for 5 min and the slide was washed thoroughly with water. The slides were incubated overnight with polyclonal rabbit RKIP antibody (1:160 dilution; Santa Cruz Biotechnology) at 4°C. Secondary antibodies for the detection of primary antibodies were reacted for 10 min using anti- rabbit IgG (Sigma, St. Louis, MO, USA) to which biotin was attached, and then washed with buffer solution and reacted with horseradish peroxidase for 10 min. It was washed thoroughly with buffer solution; chromogen AEC (3-amino-9-ethylcarvazole; Zymed, San Francisco, CA, USA) was then applied and reddish brown response was examined. After hematoxylin contrast staining, the slide was enclosed with Universal Mount (Research Genetics) and examined. In each immunohistochemistry run, negative controls were carried out by replacing the primary antibody with mouse IgG. Known immunostaining-positive slides were used as positive controls.

Following a hematoxylin counterstaining, immunostaining was scored by two independent experienced pathologists, who were blinded to the clinicopathological parameters and clinical outcomes of the patients. The staining results were scored based on these criteria: (i) percentage of positive tumor cells in the tumor tissue: 0 (<10%), 1 (11 ~ 25%), 2 (26 ~ 50%), 3 (51 ~ 75%), and 4 (76 ~ 100%); and (ii) staining intensity: 0 (no signal), 1 (weak), 2 (moderate), 3 (marked). The immunoreactivity score (IRS) was calculated by multiplying the score for the percentage of positive cells by the intensity score (range 0 ~ 12). The average IRS for each case was assigned as the staining result for the patient. The specimens were rescored if the difference between the scores determined by the 2 pathologists was greater than 3. The final score was stratified as - (0 score, absent), + (1–4 score, weak), ++ (5–8 score, moderate), +++ (9–12 score, strong), in this study, - to + was considered low expression, and ++ to +++ was considered high expression. To evaluate the reliability of the scoring method, all sections were reviewed three times.

Statistical Analysis

The software of SPSS version 16.0 for Windows (SPSS Inc, IL, USA) and SAS 9.1 (SAS Institute, Cary, NC) was used for statistical analysis. Continuous variables were expressed as $\overline{X} \pm s$. The associations between protein expression and different clinical parameters were evaluated using Fisher's exact test or X^2 test. Disease-free survival (DFS) was defined as the time from surgery to regional relapse or distant metastasis. Overall survival (OS) was defined as the time from surgery to death. DFS and OS were assessed using the Kaplan-Meier method and compared by the log-rank test. Multivariate survival analysis was performed for all of the parameters that were significant in the univariate analysis using the Cox regression model. Differences were considered statistically significant when p was less than 0.05.

Results

Immunohistochemical Expression and Cellular Distribution of RKIP

In the present study, the protein expression of RKIP was examined by immunohistochemistry in 233 cases of primary ESCC and in 49 cases of adjacent normal esophageal mucosa. The positive expression of RKIP in normal esophageal mucosa cells and ESCC exhibited a primarily cytoplasm pattern (Fig. 1). Using the criteria described previously, low expression (− to +) of RKIP was observed in 76.8% (179 of 233) and high expression (++ to +++) of RKIP was 23.2% (54 of 233) of the ESCC. In addition, the low expression (− to +) of RKIP was observed in 18.4% (9 of 49) and high expression (++ to +++) of RKIP was 81.6% (40 of 49) of the adjacent normal esophageal mucosa. The RKIP expression was significantly down-regulated in tumors compared to that in the adjacent normal esophageal mucosa ($p<0.001$) (Fig. 2).

There was no significant statistical correlation between RKIP expression and gender, age, tumor location, WHO grade, tumor status, nodal status, and TNM staging, as seen in Table 1.

Correlation of RKIP Expression with ESCC Patient Survival

Of the 233 ESCC patients, none were lost to follow-up. The median observation period was 65.0 months (6–112 months), and 122 patients were deceased and 111 were alive at the end of the follow-up. The 5-year DFS and OS for the entire cohort were 48.6% and 51.3%, with median survival times of 51.0 and 65.0 months, respectively.

Patients with low expression of RKIP showed shorter OS compared with those with high expression of RKIP ($P=0.001$, Fig. 1a, Table 2). A similar result was obtained for DFS ($P<0.001$, Fig. 1b, Table 2). In the subgroup analysis, RKIP expression distinguished the OS and DFS well for pathological stage II patients (Fig. 1c and d, $P=0.01$ and 0.02, respectively), but not in stage III patients ($P=0.10$ and 0.22, respectively).

Univariate analysis using Cox's proportional hazard model showed that the following parameters correlated

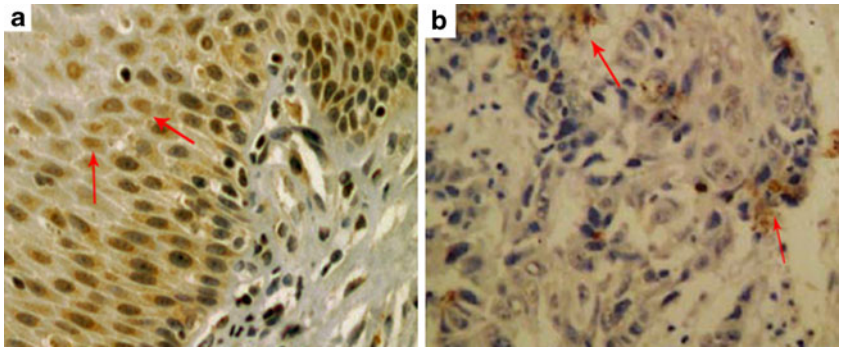

Fig. 1 RKIP expression by immunohistochemical staining (magnification: 400×). **a** Normal esophageal mucosa demonstrated high expression of RKIP protein in the cytoplasm of esophageal squamous cells. **b** Low expression level of RKIP was detected in ESCC

significantly with DFS and OS: T status (both $P<0.001$), N status (both $P<0.001$), WHO grade ($P=0.01$ and 0.03, respectively), TNM stage (both $P=0.001$) and RKIP expression (both $P<0.001$) (Table 2). When the aforementioned parameters were included in multivariate analysis, the results suggested that N status ($P=0.02$) and RKIP expression ($P<0.001$) were independent factors that affected OS (Table 3).

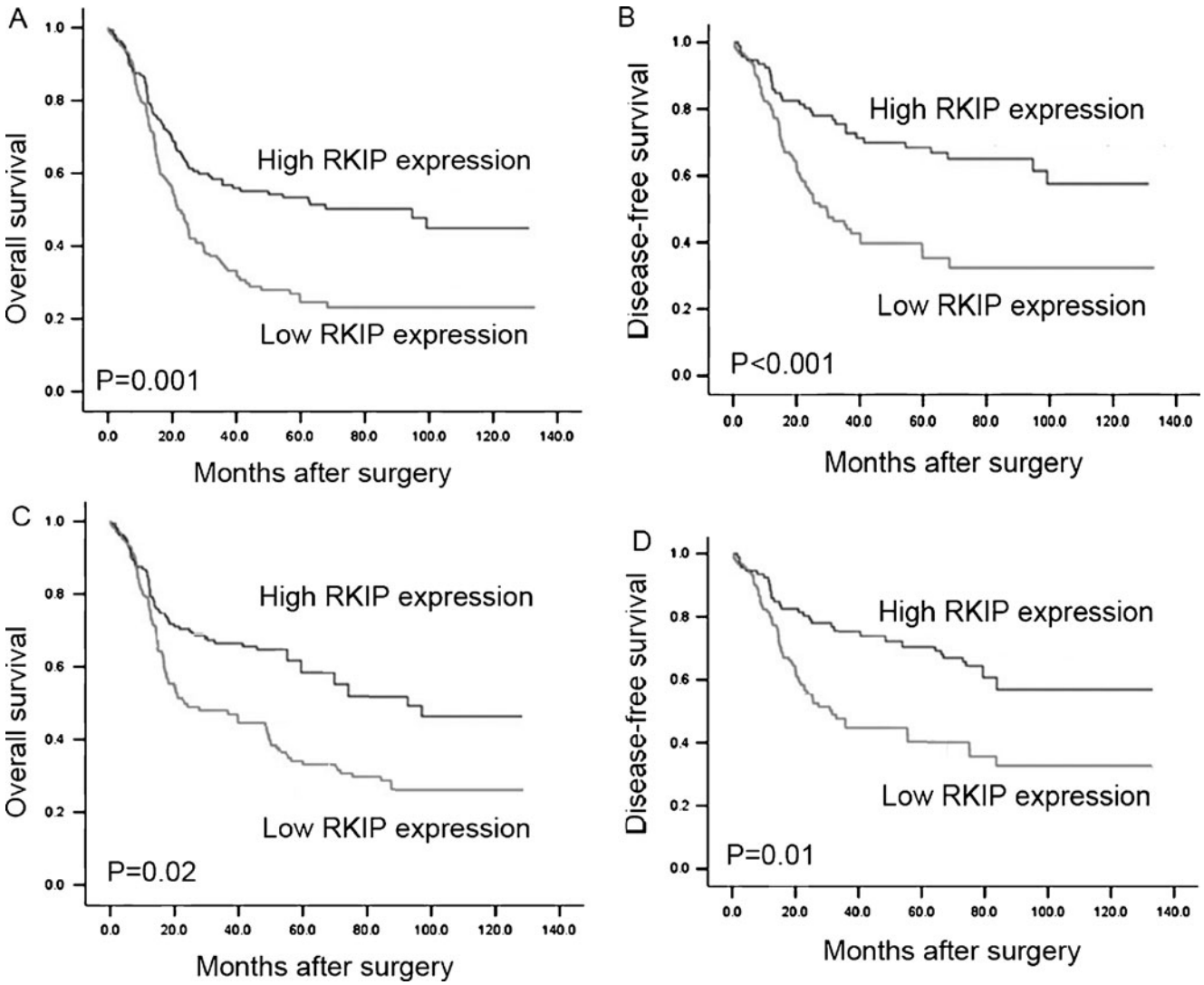

Fig. 2 Disease-free survival (DFS) and overall survival (OS) curves for esophageal squamous cell carcinoma patients according to their RKIP expression status. A, B DFS and OS curves: patients with low and high expression levels of RKIP. C, D DFS and OS curves: patients with low and high expression levels of RKIP at stage II

Table 2 Predictive variables for patient survival in ESCC

Features	Cases	DFS (Months)		OS (Months)	
		Median	P	Median	P
Age (years)					
≤55 *	139	52.6	0.7	63.8	0.7
>55	94	41.9		55.6	
Sex					
Male	168	40.2	0.3	55.3	0.2
Femal	65	65.6		63.9	
Location					
Upper	12	19.8	0.5	25.7	0.5
Middle	160	55.2		63.9	
Lower	61	53.6		67.8	
WHO grade					
G1	56	66.3	0.01	68.0	0.03
G2	147	52.2		54.8	
G3~4	30	19.7		26.6	
T status					
T2~3	230	54.6	<0.001	58.9	<0.001
T4	3	2.9		6.7	
N status					
N0	123	NR	<0.001	NR	<0.001
N1	110	15.2		18.3	
TNM stage					
I	8	66.9	0.001	70.3	0.001
II	133	42.9		45.1	
III	92	9.7		11.2	
RKIP expression					
Low	179	18.0	<0.001	22.5	<0.001
High	54	67.0		72.9	

Discussion

This is the first study to analyze RKIP expression in human ESCC tissues using immunohistochemical methods. Here, the staining pattern of RKIP was mainly cytoplasmic in both ESCC tissues and adjacent normal esophageal mucosa. Studies conducted in hepatocellular carcinoma, lung cancer, prostate cancer, gastric cancer and urinary bladder cancer have shown the same results with us [7–10, 13, 14]. In addition, the analyses of the expression levels of RKIP revealed its prognostic significance in patients with ESCC. Specifically, individuals whose tumors had relatively higher RKIP expression survive longer as compared to patients with relatively lower levels. It is also interesting to note that the prognostic power of RKIP is somewhat stronger in early stage cancer (stage II). Although we do not yet know the mechanistic basis for this subgroup bias, we do acknowledge that such stratification might be relevant for future targeted therapy and/or early predictions of ESCC patients' survival.

Table 3 Multivariate Cox regression analysis for patient survival

Features	DFS		OS	
	Relative risk	P	Relative risk	P
T status	1.376	0.08	1.393	0.08
N status	1.482	0.06	2.011	0.02
TNM stage	1.539	0.06	1.135	0.10
WHO grade	1.241	0.10	1.546	0.06
RKIP expression	3.572	<0.001	3.496	<0.001

RKIP, a member of the PEBP family, was originally identified as a negative regulator of the Raf and nuclear factor-kB (NF-kB) survival signaling pathways [15]. It is a highly species-conserved ubiquitously expressed protein involved in growth and differentiation signaling regulation. RKIP disrupts the Raf-1-MEK1/2-ERK1/2 and NF-kB signaling pathways, via physical interaction with Raf-1-MEK1/2 and NIK or TAK1, respectively, thereby, abrogating the survival and antiapoptotic properties of these signaling pathways either by promoting or by inhibiting the formation of productive signaling complexes through protein-protein interactions [16]. In addition, RKIP expression in many different organs enables it to play a role in a variety of processes sucha as reproduction, cardiology and neurology. Moreover, it also involves in the progression of human cancers. In 2003, Fu et al. [17] identified a novel anti-metastasis function for RKIP in prostate cancer. They compared levels of RKIP expression in non-metastatic prostate cancer cell lines and metastatic prostate cancer cell lines. The metastatic prostate cancer cells had much less RKIP expression than non-metastatic prostate cancer cells. Biewenga et al. [18] showed differential expression of RKIP between tumour samples from patients of early stage cervical cancer with and without lymph node metastasis. Ruan et al. [19] reported that downregulation of RKIP was significantly correlated with advanced clinical stage, lymph node metastasis and radioresistance of patients with nasopharyngeal carcinoma. In human breast cancer, Li et al. [9] demonstrated that the expression level of RKIP is decreased in invasive breast carcinoma and significantly reduced or lost in the metastasis lymph node matched to the invasive breast carcinoma compared with the normal breast epithelia, benign breast epithelia, or in situ ductal carcinoma. To explore the potential role of RKIP in breast cancer metastasis, they also studied the effect of RKIP on the malignant phenotypes of the breast cancer cells with ectopically overexpression or knockdown of RKIP. Consequently, RKIP has no effect on in vitro proliferation rate or colony-forming ability of breast cancer cell lines. In vitro cell invasion and migration assays indicated that the RKIP expression was inversely associated with the invasiveness of cancer cells. Consistent with these results, in the

orthotopic murine models, they observed that overexpression of RKIP in breast cancer cells impaired invasiveness and metastasis, whereas down-regulation of RKIP expression promoted invasiveness and metastasis. These observations define RKIP as a metastasis suppressor gene which, by definition, suppress metastasis without affecting tumorigenicity. Although, increasing evidences that RKIP is lost during tumour progression and especially in metastasis, the mechanism responsible for the down-regulation of RKIP remains to be elucidated. One study reported that in colorectal cancer completely lacking RKIP expression, the promoter region of RKIP was methylated, suggesting that CpG methylation of the RKIP promoter may be a possible mechanism of silencing of the RKIP gene [20]. However, another study failed to find RKIP promoter methylation in all 28 colorectal carcinoma cases examined [21]. In a study using hepatocellular carcinoma samples, RKIP expression was not induced by exposure to the demethylating agent, 5-azacytidine, indicating that promoter methylation does not account for RKIP down-regulation [22]. Therefore, further investigation on the mechanism for the abnormal expression of RKIP and its function in cancer progression should be continued.

Lack of RKIP expression is associated with poor survival in hepatocellular carcinoma, gastrointestinal stromal tumors, rectal cancer, and gastric cancer, and its expression has been shown to inhibit metastasis in prostate and breast cancer [7–10, 13, 14]. It has also been shown to promote invasiveness and migration of breast cancer cells [23]. In malignant melanoma cells, downregulation of RKIP correlates with enhanced invasion and progression of the disease [24]. Furthermore, up-regulated RKIP sensitizes cancer cells to drug-induced apoptosis and its loss induces radioresistance, most probably by protection against radiation-associated apoptosis. As a surgical oncologist, a pervasive question on the minds is how to identify patients with locoregional ESCC patients who could benefit from surgical resection. In this study, our results showed that high expression of RKIP was significantly correlated with a shorter survival time, accounting for a higher recurrence rate in ESCC patients. In this regard, RKIP can stratify DFS and OS in different subsets of the patients, especially with stage II patients, but not with stage III. This finding suggests that the prognosis with stage II patients may be predominantly affected by tumor malignant potentiality, whereas that of stage III may be rather influenced by anatomical staging. Therefore, adjuvant therapy should be recommended to these patients with high expression of RKIP in stage II. Meanwhile, it may help us understand the heterogeneity in the prognosis of ESCC patients, even with the same stage. The examination of RKIP expression by IHC, therefore, could be used as a biomarker to aid selection of the most appropriate therapy to improve clinical outcome and minimize ESCC recurrence.

In conclusion, in our study, we describe for the first time the the downregulation of RKIP expression in resectable ESCC is unfavorable for survival, indicating that RKIP could play an important role in tumor aggressiveness; thus RKIP may be a new therapeutic target.

References

1. Landis SH, Murray T, Bolden S, Wingo PA (1999) Cancer statistics. CA Cancer J Clin 49:8–31
2. Yu WW, Guo YM, Zhu M, Cai XW, Zhu ZF, Zhao WX, Fu XL (2011) Clinicopathological and prognostic significance of EGFR over-expression in esophageal squamous cell carcinoma: a meta-analysis. Hepatogastroenterology 58:426–431
3. Liu J, Hu Y, Hu W, Xie X, Ela Bella A, Fu J, Rao D. (2011) Expression and prognostic relevance of p21(WAF1) in stage III esophageal squamous cell carcinoma. Dis Esophagus. In press.
4. Keller ET, Fu Z, Brennan M (2004) The role of Raf kinase inhibitor protein (RKIP) in health and disease. Biochem Pharmacol 68:1049–1053
5. Keller ET (2004) Metastasis suppressor genes: a role for raf kinase inhibitor protein (RKIP). Anticancer Drugs 15:663–669
6. Trakul N, Rosner MR (2005) Modulation of the MAP kinase signaling cascade by Raf kinase inhibitory protein. Cell Res 15:19–23
7. Bernier I, Jolles P (1984) Purification and characterization of a basic 23 kDa cytosolic protein from bovine brain. Biochim Biophys Acta 790:174–181
8. Khamis ZI, Iczkowski KA, Sang QX. (2011) Metastasis suppressors in human benign prostate, intraepithelial neoplasia, and invasive cancer: their prospects as therapeutic agents. Med Res Rev. In press.
9. Li HZ, Gao Y, Zhao XL, Liu YX, Sun BC, Yang J, Yao Z (2009) Effects of raf kinase inhibitor protein expression on metastasis and progression of human breast cancer. Mol Cancer Res 7:832–840
10. Xu YF, Yi Y, Qiu SJ, Gao Q, Li YW, Dai CX, Cai MY, Ju MJ, Zhou J, Zhang BH, Fan J (2010) PEBP1 downregulation is associated to poor prognosis in HCC related to hepatitis B infection. J Hepatol 53:872–879
11. Baritaki S, Katsman A, Chatterjee D, Yeung KC, Spandidos DA, Bonavida B (2007) Regulation of tumor cell sensitivity to TRAIL-induced apoptosis by the metastatic suppressor Raf kinase inhibitor protein via Yin Yang 1 inhibition and death receptor 5 up-regulation. J Immunol 179:5441–5453
12. Rice TW, Blackstone EH, Rusch VW (2010) 7th edition of the AJCC Cancer Staging Manual: esophagus and esophagogastric junction. Ann Surg Oncol 17:1721–1724
13. Wang J, Yang YH, Wang AQ, Yao B, Xie G, Feng G, Zhang Y, Cheng ZS, Hui L, Dai TZ, Du XB, Wang D (2010) Immunohistochemical detection of the Raf kinase inhibitor protein in nonneoplastic gastric tissue and gastric cancer tissue. Med Oncol 27:219–223
14. Zaravinos A, Chatziioannou M, Lambrou GI, Boulalas I, Delakas D, Spandidos DA (2011) Implication of RAF and RKIP Genes in Urinary Bladder Cancer. Pathol Oncol Res 17:181–190
15. Baritaki S, Bonavida B (2010) Viral infection and cancer: the NF-kappaB/Snail/RKIP loop regulates target cell sensitivity to apoptosis by cytotoxic lymphocytes. Crit Rev Immunol 30:31–46
16. Wu K, Bonavida B (2009) The activated NF-kappaB-Snail-RKIP circuitry in cancer regulates both the metastatic cascade and resistance to apoptosis by cytotoxic drugs. Crit Rev Immunol 29:241–254

17. Fu Z, Smith PC, Zhang L, Rubin MA, Dunn RL, Yao Z, Keller ET (2003) Effects of raf kinase inhibitor protein expression on suppression of prostate cancer metastasis. J Natl Cancer Inst 95:878–889
18. Biewenga P, Buist MR, Moerland PD, Loren V, van Themaat E, van Kampen AH, ten Kate FJ, Baas F (2008) Gene expression in early stage cervical cancer. Gynecol Oncol 108:520–526
19. Ruan L, Wang GL, Yi H, Chen Y, Tang CE, Zhang PF, Li MY, Li C, Peng F, Li JL, Chen ZC, Xiao ZQ (2010) Raf kinase inhibitor protein correlates with sensitivity of nasopharyngeal carcinoma to radiotherapy. J Cell Biochem 110:975–981
20. Al-Mulla F, Hagan S, Al-Ali W et al (2008) Raf kinase inhibitor protein: mechanism of loss of expression and association with genomic instability. J Clin Pathol 61:524–529
21. Minoo P, Zlobec I, Baker K et al (2007) Loss of raf-1 kinase inhibitor protein expression is associated with tumor progression and metastasis in colorectal cancer. Am J Clin Pathol 127:820–827
22. Lee HC, Tian B, Sedivy JM, Wands JR, Kim M (2006) Loss of Raf kinase inhibitor protein promotes cell proliferation and migration of human hepatoma cells. Gastroenterology 131:1208–1217
23. Hagan S, Al-Mulla F, Mallon E et al (2005) Reduction of Raf-1 kinase inhibitor protein expression correlates with breast cancer metastasis. Clin Cancer Res 11:7392–7397
24. Park S, Yeung ML, Beach S, Shields JM, Yeung KC (2005) RKIP downregulates B-Raf kinase activity in melanoma cancer cells. Oncogene 24:3535–3540