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RESEARCH

Expression and Significance of RKIP and E-cadherin in Lung Squamous Cell Carcinoma

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Abstract The purpose of this study was to investigate the expression of Raf kinase inhibitor protein (RKIP) and epithelial cadherin (E-cadherin) in lung squamous cell carcinoma tissue and its correlation with the clinical pathology of lung squamous cell carcinoma. RKIP and E-cadherin mRNA (by RT-PCR) and protein (by western blotting) levels were monitored in carcinoma tissues and surrounding normal tissues from 86 lung squamous cell carcinoma cases, and their positive rates were calculated. The rates of positive RKIP and E-cadherin mRNA expression were significantly lower in lung squamous cell carcinoma than in the surrounding normal tissues ($P<0.05$). The positive expression rates were significantly lower in those with lymph node metastasis than in those without ($P<0.05$). The lower the degree of tumor differentiation, the lower the E-cadherin mRNA positive expression rate ($P<0.05$). The rates of positive RKIP and E-cadherin mRNA expression were significantly lower in patients at advanced (III, IV) stages than in patients at early (I, II) stages ($p<0.05$); this rate, however, was independent of gender, age, and tumor size ($P>0.05$). The protein levels of RKIP and E-cadherin were significantly lower in lung squamous cell carcinoma than in the surrounding normal tissues ($P<0.05$). The levels were significantly lower in patients with lymph node metastasis than in those without it ($P<0.05$). The lower the degree of tumor differentiation, the lower the protein level of E-cadherin ($P<0.05$). Both RKIP and E-cadherin are tumor suppressors, their low expression levels may be associated with initiation, invasion and/or metastasis, as well as with the inhibition of lung squamous cell carcinoma differentiation.

Keywords RKIP · E-cadherin · Lung squamous cell carcinoma · RT-PCR · Western blot

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Introduction

Raf kinase inhibitor protein (RKIP), a recently discovered novel tumor metastasis suppressor, is a member of the phosphatidylethanolamine binding protein (PEBP) family. It is named RKIP because PEBP was found to be able to specifically inhibit the Raf-1-MEK1/2-ERK1/2 signaling pathway [1]. PEBPs family members from different species have similar domain structures composed of a large β-sheet connected with smaller β-sheets on both sides and two α-helixes in the C terminus. In this structure, there is a highly conserved phosphate binding pocket, which is very important for the function of PEBP [2]. The human RKIP gene is located on chromosome 12q24.23, and its mRNA of 1507 bp is produced from four exons. Being abundant in the mammalian brain, heart, liver, lungs and testicles, RKIP is involved in the biosynthesis of the plasma membrane, neurodevelopment, spermatogenesis, apoptosis and other physiological and pathological processes. Recent studies have shown that RKIP can interfere with the Raf-1-MEK1/2-ERK1/2 signaling pathway, and that it can also inhibit the NF-κB and G protein-coupled receptor kinase signal transduction pathways [3, 4], which are closely related to cell growth, proliferation, differentiation, tumorigenesis and several other processes. Since PKIP was found to be able to inhibit metastasis of tumor cells in 2003 by Fu et al. [5], it has become a hot topic in tumor research. Recent research has also shown that the weakening or loss of RKIP

expression is related with to the initiation, progression, invasion and metastasis of a variety of tumors. Its expression relative to normal control groups is reduced in prostatic carcinoma, melanoma, colorectal cancer, hepatocarcinoma, breast cancer and related metastatic carcinomas[5–9], suggesting that RKIP is a novel tumor metastasis suppressor gene.

Epithelial cadherin (E-cadherin), a classic inhibitor of metastasis, is a transmembrane protein isoform belonging to the cadherin molecule family. It is found mainly in the epithelial cells of humans and other animals. It is mostly expressed in focal adhesions, it plays an important role in maintaining the morphological and structural integrity of epithelial cells, primarily by mediating adhesion between cells of same type. Located on chromosome 16 near q22.1, the human E-cadherin gene is composed of 723–748 amino acids, and has a hydrophobic domain located in the transmembrane area. Toward the outside of the cell membrane, its amino-terminus has a calcium binding site with high affinity for Ca^{2+}. Its carboxyl-terminus, which is in the cytoplasm and is composed of α, β, γ subunits (catenins) and some other connecting proteins, is connected with actin and plays important roles in maintaining cell morphology and regulating cell adhesion [10]. Low or absent expression of E-cadherin can cause the loss of contact inhibition of tumor cells, leading to unrestricted cell proliferation and loss of differentiation. This occurs because E-cadherin cannot form an intact protein complex with catenin in the cytoplasm and fails to link to the actin cytoskeleton, causing the connections between cells to be lost. In this case, tumor cells can easily come off of the primary tumor and travel to adjacent tissues, blood and lymphatic vessels, which leads to invasion and metastasis. Studies on malignant tumors including thyroid carcinoma, pancreatic cancer and gastric cancer have shown that the lymph node metastasis rates of tumors with down-regulated E-cadherin expression are significantly higher than those of tumors with normal E-cadherin expression [11–13].

To date, no study of associations between RKIP/E-cadherin and the occurrence and development of lung squamous cell carcinoma has been reported in the literature. China is the country with the largest number of lung cancer patients in the world, and its morbidity and mortality are continuously increasing. In recent years, although there has been great progress in the treatment of lung cancer, the survival rate of lung cancer is still not favorable [14]. The main reason is that the biological characteristics of lung cancer are very complex. It is highly malignant, and Non-small cell lung cancer (NSCLC) accounts for approximately 80 % of all cases of lung cancer. Such patients have reached an advanced stage when they are diagnosed, and most patients have metastases and a very poor prognosis. Therefore, more accurate and efficient molecular markers for the early discovery of tumors must be found. In this study, the expression levels of RKIP and E-cadherin were monitored by RT-PCR and western blotting in lung squamous cell carcinoma and surrounding normal tissues. The relationship of RKIP and E-cadherin with the initiation, progression, invasion, metastasis and differentiation of lung squamous cell carcinoma was assessed in order to provide scientific data for predicting the prognosis of and developing targeted treatments for lung squamous cell carcinoma.

Materials and Methods

Specimens

Fresh surgical resection tissue specimens were collected from 86 patients with lung squamous cell carcinoma in our hospital from June 2006 to June 2009. Tumor tissues and adjacent normal tissues were collected from each case. The samples were preserved in liquid nitrogen immediately and stored for later testing. Including 52 males and 34 females, the patients ages ranged from 27 to 91 with a mean of 56.6; 53 patients were younger than 60, and 33 patients were 60 or older. Lacking any preoperative treatment, the 86 cases were all pathologically diagnosed as squamous cell carcinoma. Regarding the degree of differentiation, 25 cases were well differentiated, 28 cases were moderately differentiated and 33 cases were poorly differentiated. Thirty-nine cases had lymph node metastasis and 47 cases lacked lymph node metastasis. The primary tumor was less than 3 cm in 46 cases and greater than or equal to 3 cm in 40 cases.

Reagents

The reagents included but were not limited to: A Trizol kit (INVITROGEN), an RT-PCR kit (GIBCO-BRL), RIPA lysate buffer [150 mM NaCl, 1 % NP40, 0.5 % sodium deoxycholate, 0.1 % SDS, 50 mM Tris (pH 7.9), 10 mM NaF, PMSF and 1×protease inhibitors (Complete cocktail tablets, Roche)], TBST (10 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 0.1 % Tween-20), RKIP (NM_002567.2) upstream primer: 5′-AAGAATAGACCCACCAGCAT-3′, downstream primer: 5′-CTCGTAAACCAGCCAGACAT-3′; E-cadherin (NM_001796.3) upstream primer: 5′-CTTCACCGACTTACCTACT-3′, downstream primer: 5′-GTGCCATACACTTAATTCTC-3′; GAPDH (NG_007073.2) upstream primer: 5′-AATCCCATCAC CATCTTCC-3′, downstream primer: 5′-AGTCCTTCCAC GATACCAA-3′. All primer sequences were provided by Shanghai Invitrogen Biotechnology Co. Ltd. The primary RKIP antibody was a rabbit anti-human polyclonal antibody (Invitrogen, USA), the secondary antibody was HRP labeled goat anti-rabbit polyclonal antibody (Abcam, UK); the

primary E-cadherin antibody was a mouse anti-human polyclonal antibody (Abcam, UK), the secondary antibody was HRP labeled goat anti-mouse polyclonal antibody (Abbiotec Co., USA); the primary GAPDH antibody was a mouse anti-human monoclonal antibody (Abcam, UK), the secondary antibody was HRP labeled goat anti-mouse polyclonal antibody (Abbiotec Co., USA). ECL chemiluminescence reagents were used for western detection (Pierce Co., USA), and the BCA protein concentration assay kit was a product of the domestic Beyotime Institute of Biotechnology.

RT-PCR

Carcinoma tissues and the surrounding tissues were obtained, with 100 mg of each. For each sample, 1 ml Trizol reagent was added and the sample was homogenized. After transferring to a 1.5 ml centrifuge tube, 0.2 ml chloroform was added, and the preparation was shaken for 20 s and then incubated for 3 min. After centrifuging at 12 000 g for 10 min, the aqueous upper phase was taken; 0.5 ml of isopropanol was added and mixed gently. After incubation at room temperature for 10 min, it was centrifuged at 15,000 r/min for 10 min, and the supernatant was discarded. After adding 1 ml 75 % ethanol, the precipitate was gently washed and centrifuged, and the supernatant was discarded. After air drying, the precipitate was dissolved in 20 μl of DEPC water (65 °C dissolution for 10–15 min). Then, the RT reaction was performed. In a 20 μl reaction volume, 1.0 μg of total RNA was added and incubated at 37 °C for 1 h. The samples were inactivated at 95 °C for 5 min and then immediately incubated on ice. They were stored at −20 °C for later use. Then, primers were designed followed by a PCR reaction. To a 25 μl PCR reaction system, 5 μl of cDNA was added. The amplification conditions were: initial denaturing at 94 °C for 3 min, denaturing at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 30 s; after 30 cycles, final extension at 72 °C for 10 min. The PCR products were detected by 1.5 % agarose gel electrophoresis.

Western Blotting

After 100 mg of tissue sample was preserved in liquid nitrogen, ground and then homogenized with 1 ml of RIPA lysate buffer, the homogenate was transferred to a 1.5 ml centrifuge tube and centrifuged at 16 000 g for 30 min. The supernatant was saved and its protein concentration was determined by the BCA method. Protein samples of lung squamous cell carcinoma were obtained by mixing 10 μg of carcinoma tissue from each patient; protein samples of tissues surrounding the tumor were similarly obtained by mixing 10 μg of surrounding tissue from each patient. The lymph node metastasis positive group was a mixture of 10 μg samples from all the patients with positive lymph node metastasis; the lymph node metastasis negative group was a mixture of 10 μg samples from all the patients with negative lymph node metastasis. The high differentiation group was a mixture of 10 μg samples from all the patients with high differentiation; the moderate differentiation group was a mixture of 10 μg samples from all the individual patients with moderate differentiation; the low differentiation group was a mixture of 10 μg samples from all the individual patients with low differentiation. After a 6 % stacking gel and a 12 % separating gel were cast, 50 μg of total protein was applied to each lane and electrophoretic separation was performed. The protein from the gel was then transferred to a PVDF membrane by semi-dry transfer (Amresco, USA). The PVDF membrane was blocked with TBST solution containing 5 % skim milk at room temperature for 1 h. Then, rabbit anti-human polyclonal RKIP antibody (1:1000 dilution), mouse anti-human polyclonal E-cadherin antibody and mouse anti-human GAPDH monoclonal antibody (1:1000 dilution) were added one by one, and incubation proceeded overnight at 4 °C. Then, HRP labeled goat anti-rabbit IgG (1:2000 dilution) and HRP labeled goat anti-mouse polyclonal antibody (1:2000 dilution) were added and incubation proceeded at 37 °C for 1 h. After TBST washing, the ECL chemiluminescence reagent was used for autoradiography. The relative levels of RKIP and E-cadherin were assessed as grayscale RKIP/GAPDH and E-cadherin/GAPDH ratios. The grayscale values were analyzed with QuantityOne software (Bio-Rad, USA).

Statistical Analysis

Stata 7.0 software was used for the statistical analysis with the χ^2 test and t test statistical methods. Statistical significance was established at $P<0.05$.

Results

Our RT-PCR results showed that the positive rate of RKIP mRNA expression was 47.7 % (41/86) in lung squamous cell carcinoma and 76.7 % (66/86) in the tissues surrounding the tumor, indicating that RKIP mRNA expression in lung squamous cell carcinoma tissues was significantly lower than that in the surrounding tissues ($P<0.001$). The positive rate of RKIP mRNA expression was 30.8 % (12/39) in those with lymph node metastasis and 61.7 % (29/47) in those without lymph node metastasis, and this difference was significant ($P=0.004$). Moreover, the mRNA expression of RKIP was unrelated to gender, age or tumor size ($P>0.05$) (Table 1). The positive rate of E-cadherin mRNA expression was 41.9 % (36/86) in lung squamous cell carcinoma and

81.4 % (70/86) in the surrounding tissues, indicating that E-cadherin mRNA expression in lung squamous cell carcinoma tissues was significantly lower than that in the surrounding tissues (P=0.000). The positive rate of E-cadherin mRNA expression was 23.1 % (9/39) in those with lymph node metastasis and 57.4 % (27/47) in those without lymph node metastasis, and this difference was significant (P= 0.001). The lower the degree of tumor differentiation, the lower was the positive rate of E-cadherin mRNA expression (P=0.010). The rates of positive RKIP and E-cadherin mRNA expression were significantly lower in patients at advanced (III, IV) stages than in patients at early (I, II) stages (P=0.009, 0.007). The mRNA expression of E-cadherin was unrelated to gender, age or tumor size (P>0.05) (Table 1).

Western blotting results showed that the protein levels of RKIP and E-cadherin were also significantly lower in lung squamous cell carcinoma than in the surrounding normal tissue (P<0.05). These levels were significantly lower in those with lymph node metastasis than in those without lymph node metastasis (P<0.05). The lower the degree of tumor differentiation, the lower was the expression of E-cadherin protein (P<0.05). However, the correlation between RKIP expression and the degree of tumor differentiation was not obvious (Figs. 1 and 2).

Discussion

The early diagnosis and treatment of lung cancer has been the focus of many researchers [15–21]. Investigations of the pathogenesis and early warning signs of lung cancer will provide new ideas for the early diagnosis and clinical treatment of lung cancer [16–18]. Currently, research into lung cancer focuses on trying to find tumor markers involved in the regulation of the cell cycle, apoptosis and tumor angiogenesis [16]. It is expected that uncovering their roles in the development of lung cancer will provide the scientific data needed for the early diagnosis and targeted treatment of lung cancer.

RKIP, a recently discovered tumor metastasis suppressor, is widely expressed in the mammalian brain, heart, liver, lungs, testicles and other tissues. It is involved in the biosynthesis of the plasma membrane, neurodevelopment, spermatogenesis, apoptosis and other physiological and

Table 1 Relationships between RKIP and E-cadherin mRNA expression in lung squamous cell carcinoma and clinical pathological parameters

Pathologic parameter	No.	RKIP mRNA positive rate(%)	χ^2	P	E-cadherin mRNA positive rate(%)	χ^2	P
Normal tissue	86	66 (76.7)	15.4565	<0.001	70 (81.4)	28.4208	<0.001
Carcinoma tissue	86	41 (47.7)			36 (41.9)		
Sex							
Male	52	25 (48.1)	0.0085	0.926	21 (40.4)	0.1177	0.732
Female	34	16 (47.1)			15 (44.1)		
Age							
<60	53	27 (50.9)	0.5917	0.442	22 (41.5)	0.0070	0.933
≥60	33	14 (42.4)			14 (42.4)		
Size of primary carcinoma (cm)							
<3	46	21 (45.7)	0.1621	0.687	20 (43.5)	0.1064	0.744
≥3	40	20 (50.0)			16 (40.0)		
Degree of differentiation							
Well differentiated	25	16 (64.0)	5.4524	0.065	16 (64.0)	9.2552	0.010
Moderately differentiated	28	14 (50.0)			12 (42.9)		
Poorly differentiated	33	11 (33.3)			8 (24.2)		
Lymph nodes metastasis							
Negative	47	29 (61.7)	8.1753	0.004	27 (57.4)	1.3453	0.001
Positive	39	12 (30.8)			9 (23.1)		
Staging of tumor							
I–II	55	32 (58.2)	6.7529	0.009	29 (52.7)	7.4033	0.007
III–IV	31	9 (29.0)			7 (22.6)		

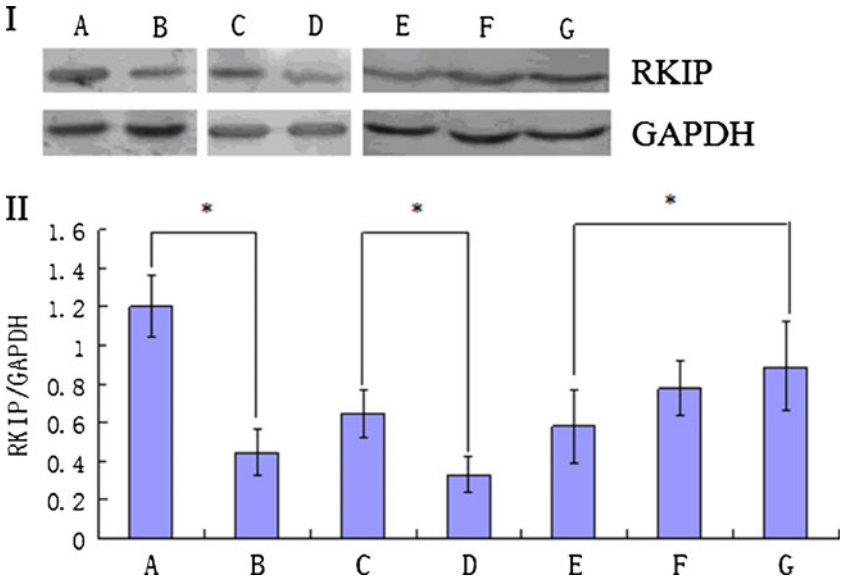

Fig. 1 **I**: The expression of RKIP protein in lung squamous cell carcinoma and in surrounding tissues; **II**: The relative expression of RKIP protein in lung squamous cell carcinoma and in surrounding tissues (A: surrounding tissue, B: lung squamous cell carcinoma, C: lymph node metastasis negative, D: lymph node metastasis positive, E: low differentiation, F: moderate differentiation, G: high differentiation, * indicates $P<0.05$)

pathological processes. Recent studies have shown that RKIP can interfere with the Raf-1-MEK1/2-ERK1/2 signaling pathway, and also that it can inhibit the NF-κB and G protein-coupled receptor kinase signal transduction pathways [3, 4], which are closely related to cell growth, proliferation, differentiation, tumorigenesis and other processes. Because PKIP was found to be able to inhibit tumor cell metastasis in 2003 by Fu et al. [5], it has become a hot topic in tumor research. Lee et al. found that RKIP expression was significantly lower in hepatocarcinoma tissue than in the surrounding normal liver tissue, and that increased expression of RKIP can reduce the proliferation and migration of hepatocarcinoma cells [6, 22]. The expression of RKIP is highest in normal melanocytes, lower in melanoma and the very low or missing in metastatic melanoma. Overexpressed RKIP can inhibit the metastatic potential of highly metastatic melanoma cells [7]. The expression of RKIP in thyroid cancer is lower than that in normal thyroid tissue, and exogenous RKIP can inhibit the growth of thyroid cancer cells [23]. Studies have found that PKIP is highly expressed in primary breast cancer lesions. This expression is unrelated to the tumor type, degree of differentiation, size or ER expression of the breast cancer. However, low or no expression has been detected in metastatic lymph nodes [9, 24]. Recently, by using immunohistochemical RKIP staining of a tissue microarray of non-cancerous prostate tissue and primary and metastatic prostate cancer, Fu et al. [25] found that the expression of RKIP in the non-cancerous prostate tissue, primary and metastatic prostate cancer was reduced by 5 %, 48 % and 89 %, respectively. In this study, the mRNA and protein levels of RKIP were monitored in lung squamous cell carcinoma and the surrounding normal tissues. We found that the rate of

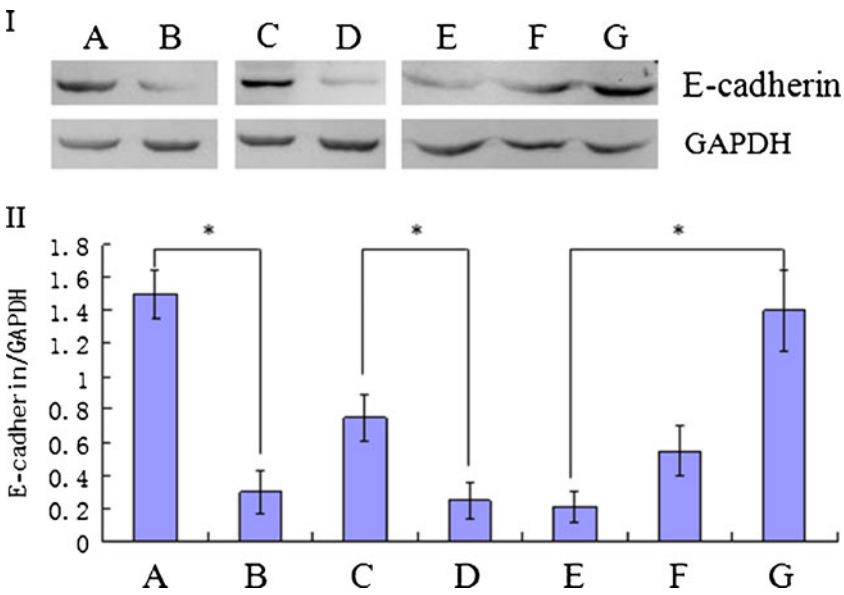

Fig. 2 **I**: The expression of E-cadherin protein in lung squamous cell carcinoma and in surrounding tissues; **II**: The relative expression of E-cadherin protein in lung squamous cell carcinoma and in surrounding tissues (A: surrounding tissue, B: lung squamous cell carcinoma, C: lymph node metastasis negative, D: lymph node metastasis positive, E: low differentiation, F: moderate differentiation, G: high differentiation, * indicates $P<0.05$)

positive RKIP mRNA expression was significantly lower in lung squamous cell carcinoma than in the surrounding normal tissue ($P<0.05$), and it was significantly lower in patients with lymph node metastasis than those without lymph node metastasis ($P<0.05$); moreover, it was independent of gender, age and tumor size ($P>0.05$). The protein expression of RKIP was also significantly lower in lung squamous cell carcinoma than in the surrounding normal tissue ($P<0.05$), and its expression was significantly lower in patients with lymph node metastasis than in those without it ($P<0.05$); The rates of positive RKIP and E-cadherin mRNA expression were significantly lower in patients at advanced (III, IV) stages than in patients at early (I, II) stages ($p<0.05$). These results suggest that the expression of RKIP in lung squamous cell carcinoma is consistent with its expression in other cancers.

In recent years, lung cancer incidence and mortality have risen significantly. The cause of death is usually tumor invasion and metastasis. The important role of E-cadherin in tumor invasion and metastasis has became a research focus. E-cadherin, a transmembrane glycoprotein with a molecular weight of 120 kDa, is a member of the cadherin superfamily. It is an important molecule for the regulation of adhesion between cells and between cells and matrix. Its expression level and its functional activity affect the detachment and reattachment of tumor cells, which plays a key role in the process of tumor invasion and evolution [10]. Researchers have found that the amount of E-cadherin expressed in brain metastatic tumor tissue is also significantly decreased [26]. Showing high expression in surrounding normal liver tissue, E-cadherin expression in hepatoma carcinoma cells has been associated with the degree of differentiation; its expression decreases as the degree of differentiation degreases. It has also been reported that E-cadherin expression is negatively correlated with the lymph node metastasis of liver cancer. Wang et al. [27] found that E-cadherin expression was significantly lower in metastatic lung cancer tissue than in primary lung cancer tissue. The results of this study revealed that the rate of positive E-cadherin mRNA expression in lung squamous cell carcinoma was significantly lower than that in the surrounding normal tissues ($P=0.000$), and this rate was significantly lower in patients with lymph node metastasis than in those without it ($P=0.001$). Moreover, the lower the degree of tumor differentiation, the lower was the E-cadherin mRNA positive expression rate ($P=0.010$). The results regarding E-cadherin protein expression were similar. These results basically agree with those of Sulzer et al. [28]. The loss of adhesion function is the key for tumor cells to achieve dedifferentiation and to become highly invasive [29].

RKIP can disrupt the complex formed between Raf-1 and MEK-1, thereby separating Raf-1 from MEK-1. RKIP can individually bind to Raf-1 or MEK-1. This binding with either one of them is sufficient to inhibit downstream signaling [30]. This study found that the expression of RKIP was significantly reduced or missing in lung squamous cell carcinoma, and this significantly suppressed RKIP's inhibition of the Raf-1-MEK1/2-ERK1/2 signaling pathway. As a result, cell growth and development were promoted, which might be involved in the initiation of squamous cell carcinoma. In addition, RKIP can also interact with the four kinases in the NF-κB activation pathway, including NIK (NF-κB inducing kinase), TAKI (TGF-β activated kinase 1), IKKα (IκB kinase α) and IKKβ (IκB kinase β); thus NF-κB activation was inhibited and the anti-apoptotic properties of the cells were weakened [2]. The expression of RKIP was significantly reduced or missing in lung squamous cell carcinoma. This could promote the activation of the NF-κB pathway and enhance the anti-apoptotic properties of cells, which might be related to the initiation and progression of lung squamous cell carcinoma. Beshir et al. [3] found that RKIP could control the expression of matrix metalloproteinase 1/2 (MMP-1/2), which could promote the invasion and metastasis of tumor cells. Silencing of RKIP was found to enhance the invasive and metastatic abilities of tumor cells and increase the expression of MMP-1/2. Overexpression of RKIP was found to reduce the invasive and metastatic abilities of tumor cells and reduce the expression of MMP-1/2. This study found that the positive rate of RKIP expression was significantly lower in tumor tissues with lymph node metastasis than in those without lymph node metastasis. In this case, the expression of MMP-1/2 might be increased, thus promoting the invasion and metastasis of lung squamous cell carcinoma. The specific mechanism needs to be further verified.

This study also found that the expression of RKIP was positively correlated with the expression of E-cadherin, suggesting that with the decreased expression of RKIP in lung squamous cell carcinoma, E-cadherin expression also decreased, resulting in decreased adhesion between cells and efficient release of tumor cells, thus enhancing the invasion and metastasis of tumor cells in lung squamous cell carcinoma. Research has shown that, after being transfected with the Raf-1 gene, immortalized mouse hepatoma cells show significantly down-regulated E-cadherin protein expression. After the above cell line was treated with inhibitors of MAPK, p38 MAPK and c-Src tyrosine kinase, the expression of proteins related to cell adhesion and tight junctions increased, showing that Raf-1 may be involved in regulating E-cadherin expression by affecting multiple signaling pathways mediated by MAPK, p38 MAPK and c-Src tyrosine kinase [31]. Another study found that TGF-β can reduce the expression of E-cadherin by enhancing the activity of the transcriptional repressors slug and Snail [32]. The above results indicate that Raf-1 and TGF-β are

involved in the regulation of E-cadherin expression. Thus, we speculate that RKIP may regulate the expression of E-cadherin by inhibiting the Raf-1 and TGF-β signaling pathways, among others. The exact regulatory mechanism needs to be further investigated.

The above results suggest that both RKIP and E-cadherin are tumor suppressors. Their low expression may be associated with the initiation, progression, invasion, metastasis and differentiation inhibition of lung squamous cell carcinoma. It is possible that they could serve as targets for the treatment of lung squamous cell carcinoma.

Conflict of Interest None declared.

Disclosure The authors declare no conflicts of interest

References

1. Yeung K, Seitz T, Li S, Janosch P, McFerran B, Kaiser C, Fee F, Katsanakis KD, Rose DW, Mischak H, Sedivy JM, Kolch W (1999) Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. Nature 401:173–7
2. Odabaei G, Chatterjee D, Jazirehi AR, Goodglick L, Yeung K, Bonavida B (2004) Raf-1 kinase inhibitor protein: structure, function, regulation of cell signaling, and pivotal role in apoptosis. Adv Cancer Res 91:169–200
3. Beshir AB, Ren G, Magpusao AN, Barone LM, Yeung KC, Fenteany G (2010) Raf kinase inhibitor protein suppresses nuclear factor-kappaB-dependent cancer cell invasion through negative regulation of matrix metalloproteinase expression. Cancer Lett 299:137–49
4. Zeng L, Imamoto A, Rosner MR (2008) Raf kinase inhibitory protein (RKIP): a physiological regulator and future therapeutic target. Expert Opin Ther Targets 12:1275–87
5. 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 95:878–89
6. 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
7. Lin K, Baritaki S, Militello L, Malaponte G, Bevelacqua Y, Bonavida B (2010) The role of B-RAF mutations in melanoma and the induction of EMT via dysregulation of the NF-kappaB/Snail/RKIP/PTEN circuit. Genes Cancer 1:409–20
8. Al-Mulla F, Hagan S, Al-Ali W, Jacob SP, Behbehani AI, Bitar MS, Dallol A, Kolch W (2008) Raf kinase inhibitor protein: mechanism of loss of expression and association with genomic instability. J Clin Pathol 61:524–9
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 7:832–40
10. van Roy F, Berx G (2008) The cell-cell adhesion molecule E-cadherin. Cell Mol Life Sci 65:3756–88
11. Rachagani S, Senapati S, Chakraborty S, Ponnusamy MP, Kumar S, Smith LM, Jain M, Batra SK (2011) Activated Kras(G12D) is associated with invasion and metastasis of pancreatic cancer cells through inhibition of E-cadherin. Br J Cancer 104:1038–48
12. Gu X, Ma C, Yuan D, Song Y (2012) Circulating soluble intercellular adhesion molecule-1 in lung cancer: a systematic review. Transl Lung Cancer Res 1:36–44
13. Saad AA, Awed NM, Abd Elkerim NN, El-Shennawy D, Alfons MA, Elserafy ME, Darwish YW, Barakat EM, Ezz-Elarab SS (2010) Prognostic significance of E-cadherin expression and peripheral blood micrometastasis in gastric carcinoma patients. Ann Surg Oncol 17:3059–67
14. Metro G, Crinò L (2012) Advances on EGFR mutation for lung cancer. Transl lung Cancer Res 1:5–13
15. Ellis PM, Vandermeer R (2011) Delays in the diagnosis of lung cancer. J Thorac Dis 3:183–8
16. Shao WL, Wang DY, He JX (2010) The role of gene expression profiling in early-stage non-small cell lung cancer. J Thorac Dis 2:89–99
17. Yang HH, Zhang Q, He JX, Lu WJ (2010) Regulation of calcium signaling in lung cancer. J Thorac Dis 2:52–6
18. Xiao DK, He JX (2010) Epithelial mesenchymal transition and lung cancer. J Thorac Dis 2:154–9
19. Korpanty G, Smyth E, Carney DN (2011) Update on anti-angiogenic therapy in non-small cell lung cancer: Are we making progress? J Thorac Dis 3:19–29
20. Shash E, Peccatori FA, Azim HA Jr (2011) Optimizing the use of epidermal growth factor receptor inhibitors in advanced non-small-lung cancer (NSCLC). J Thorac Dis 3:57–64
21. McKeage MJ, Jameson MB, AS1404-201 Study Group Investigators (2010) Comparative outcomes of squamous and non-squamous non-small cell lung cancer (NSCLC) patients in phase II studies of ASA404 (DMXAA)—retrospective analysis of pooled data. J Thorac Dis 2:199–204
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 131:1208–17
23. Akaishi J, Onda M, Asaka S, Okamoto J, Miyamoto S, Nagahama M, Ito K, Kawanami O, Shimizu K (2006) Growth-suppressive function of phosphatidylethanolamine-binding protein in anaplastic thyroid cancer. Anticancer Res 26:4437–42
24. Hagan S, Al-Mulla F, Mallon E, Oien K, Ferrier R, Gusterson B, Garcia JJ, Kolch W (2005) Reduction of Raf-1 kinase inhibitor protein expression correlates with breast cancer metastasis. Clin Cancer Res 11:7392–7
25. Fu Z, Kitagawa Y, Shen R, Shah R, Mehra R, Rhodes D, Keller PJ, Mizokami A, Dunn R, Chinnaiyan AM, Yao Z, Keller ET (2006) Metastasis suppressor gene Raf kinase inhibitor protein (RKIP) is a novel prognostic marker in prostate cancer. Prostate 66:248–56
26. Zeljko M, Pecina-Slaus N, Martic TN, Kusec V, Beros V, Tomas D (2011) Molecular alterations of E-cadherin and beta-catenin in brain metastases. Front Biosci (Elite Ed) 3:616–24
27. Wang G, Dong W, Shen H, Mu X, Li Z, Lin X, Liu Y, Du J (2011) A comparison of Twist and E-cadherin protein expression in primary non-small-cell lung carcinoma and corresponding metastases. Eur J Cardiothorac Surg. Mar 2. [Epub ahead of print]
28. Sulzer MA, Leers MP, van Noord JA, Bollen EC, Theunissen PH (1998) Reduced E-cadherin expression is associated with increased lymph node metastasis and unfavorable prognosis in non-small cell lung cancer. Am J Respir Crit Care Med 157:1319–23
29. Zigler M, Dobroff AS, Bar-Eli M (2010) Cell adhesion: implication in tumor progression. Minerva Med 101:149–62
30. Yeung K, Janosch P, McFerran B, Rose DW, Mischak H, Sedivy JM, Kolch W (2000) Mechanism of suppression of the Raf/MEK/

extracellular signal-regulated kinase pathway by the raf kinase inhibitor protein. Mol Cell Biol 20:3079–85
31. Lan M, Kojima T, Osanai M, Chiba H, Sawada N (2004) Oncogenic Raf-1 regulates epithelial to mesenchymal transition via distinct signal transduction pathways in an immortalized mouse hepatic cell line. Carcinogenesis 25:2385–95
32. Herfs M, Hubert P, Kholod N, Caberg JH, Gilles C, Berx G, Savagner P, Boniver J, Delvenne P (2008) Transforming growth factor-beta1-mediated Slug and Snail transcription factor up-regulation reduces the density of Langerhans cells in epithelial metaplasia by affecting E-cadherin expression. Am J Pathol 172:1391–402