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Original Article

Significance of elevated ERK expression and its positive correlation with EGFR in Kazakh patients with esophageal squamous cell carcinoma

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Abstract: Extracellular signal-regulated kinases (ERKs) are activated by the MAPK pathway. ERKs are downstream effectors of the epidermal growth factor receptor (EGFR), which belongs to the receptor tyrosine kinases family. Studies on the activation of the EGFR-ERK pathway in Kazakh patients with esophageal squamous cell carcinoma (ESCC) have not been reported. Using immunohistochemical staining on tissue microarrays, we investigated the protein expression of EGFR and ERK in 90 ethnic Kazakh patients with ESCC and 48 adjacent normal esophageal tissues (NETs). EGFR and ERK1 expression was localized in the cytoplasm, whereas ERK2 expression was localized in the nucleus. Both were more highly expression in the ESCC tissues than in the NETs, and the difference was considered significant (P = 0.003, 0.002, and 0.005, respectively). ERK1 and EGFR expression was positively correlated with lymph nodes metastasis (P = 0.011 and 0.013, respectively). ERK1 staining was also significantly associated with tumor-node-metastases stage of ESCC (P = 0.044). ERK2 staining was significantly associated with Histological grade (P = 0.012). Furthermore, ERK1 and EGFR expression in the ESCC tissues were positively correlated (r = 0.413, P < 0.001); EGFR was more highly expressed in the ESCC tissues with high ERK1 expression than in the ESCC tissues with low ERK1 expression (4.95 ± 0.57 vs. 3.21 ± 0.35, P = 0.01). This study is thus far the first to demonstrate the correlation between EGFR overexpression and ERK overexpression in Kazakh patients with ESCC. This correlation suggests that the EGFR-ERK signaling pathway participates in ESCC progression and can thus be used as a prognostic marker.

Keywords: Esophageal squamous cell carcinoma, Kazakh, epidermal growth factor receptor, extracellular signal-regulated kinase

Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive malignancies with a very poor outcome in China [1, 2], particularly in the Chinese Kazakh ethnic population residing in Xinjiang, Northwest China [3-5]. The five-year survival rate of ESCC is approximately 42% despite recent advancements in clinical treatment [6]. To improve patient outcome, the molecular mechanisms of ESCC, as well as the biomarkers of tumor growth and development as new prognostic and therapy targets, must be investigated in the Kazakh ethnic population in the Xinjiang.

Epidermal growth factor receptor (EGFR), which is located on the cell surface, is activated by the binding of its specific ligands, such as epidermal growth factor (EGF) and transforming growth factor α (TGFα), resulting in monomer to the dimer [7]. EGFR activation initiates the phosphorylation of tyrosine residues in the EGFR cytoplasmic domain, leading to recruitment and activation of various factors with multiple consequences, such as cell proliferation, cell differentiation and organ morphogenesis. Moreover, EGFR participates in carcinogenesis of tumor metastasis [8, 9]. EGFR overexpression has been found in various solid tumors, including breast cancer [10], colon cancer [11,

Table 1. Antibodies and immunostaining procedures used in this study

Antibody	Source	Dilution	Staining	Restoration	Restoration Condition	Producers
ERK1	Mouse monoclonal	1:100	Immunostainer	/	/	Santa Cruz, CA,USA
ERK2	Mouse monoclonal	1:50	Immunostainer	/	/	Santa Cruz, CA,USA
P-ERK1/2	Mouse monoclonal	1:50	EnVision	Pressure cooker	Citrate	Abcam, USA
EGFR	Mouse monoclonal	1:50	EnVision	Pressure cooker	Citrate	MaixinBio, Fuzhou, China

12] and lung cancer [13, 14]. Several studies have demonstrated that aberrant expression of EGFR exhibits an important function in ESCC [15-17] as an oncogene. Moreover, phosphorylation of the cytoplasmic domains of EGFR can be enhanced by its ligand binding, subsequently activating multiple signaling cascades, such as extracellular signal-regulated kinase (ERK).

ERK is a key component of the classical mitogen-activated protein kinase (MAPK) pathway. Both ERK1 and ERK2 are activated by the MAPK/ERK pathway, which is downstream effectors of the EGFR signal pathway [18]. ERK is activated in human liver and colon cancer [19] and non-small cell lung cancer (NSCLC) [20]. ERK has likewise been reported to be activated in ESCC [21], which is inconsistent with the study by Zheng *et al*, who found that the protein expression of p-ERK1/2 in Kazakh ESCC tissues was lower than that in the adjacent tissues [22]. Numerous studies have reported that the EGFR-ERK pathway exists in various cancers, such as epithelial ovarian cancer tissues [23] and breast cancer [24]. However, no studies on the activation of the EGFR-ERK signal pathway in Kazakh ESCC have been report. Considering that a complex network of genetic and epigenetic aberrations involving various signaling pathways is required in the management of ESCC pathogenesis, we hypothesize that the EGFR-ERK pathway is involved in Kazakh ESCC carcinogenesis.

To address this problem, we assessed the expression of EGFR and ERK-related proteins (ERK1, ERK2, p-ERK1/2) by immunohistochemistry in tissue microarrays (TMAs) to evaluated the possible correlations of the expression with ESCC clinicopathological characteristics. We further explored whether the EGFR-ERK pathway is activated in Kazakh ESCC samples and found that the expression of EGFR, ERK1 and ERK2 is higher in ESCC samples than in normal tissue. ERK1 exhibited a significant positive correlation with EGFR in Kazakh ESCC samples.

Materials and methods

Patients and tumor samples

TMAs were used for immunostaining EGFR and ERK-related proteins in 90 Kazakh patients with ESCC from the First Affiliated Hospital, Shihezi University School of Medicine, Xinjiang Yili Prefecture Friendship Hospital and the People's Hospital of Xinjiang Uyghur Autonomous Region. All patients underwent esophagectomy without prior chemotherapy or radiotherapy. Data on clinicopathologic variables, such as tumor sites, depth of tumor invasion, and lymph node metastasis, were collected. All cases with pathologic diagnoses for tumor-node-metastasis (TNM) stages were evaluated based on Cancer Stage Manual, 7th Edition, issued in 2009 by the American Joint Committee on Cancer. Out of the 90 ESCC specimens, 48 specimens that matched adjacent normal esophageal tissues (NETs) were used as controls. A written informed consent was obtained from each patient, and the study was approved by the hospital ethics committee.

Immunohistochemistry

Immunohistochemistry was performed using the EnVision method. Tumor samples were fixed with 10% formalin in PBS, embedded in paraffin, sectioned into 4 μm slices, and then baked at 60°C for 3 h. Each tissue section was deparaffinized, rehydrated, and incubated with fresh 3% hydrogen peroxide in methanol at room temperature for 15 min (**Table 1**). The sections were then autoclaved in sodium citrate buffer (pH of 6.0) at 100°C for 8 min in a microwave oven and cooled to 30°C for 30 min. Washed thrice with PBS for 5 min each, the section were incubated with primary antibody ERK (Santa Cruz, CA, USA; 1:100 dilution), ERK2 (Santa Cruz, CA, USA; 1:50 dilution),

Table 2. Clinicopathological demographics for the 90 patients with ESCC

Clinical properties	No. of patients (%)
Total	90
Gender	
Male	57 (63.3)
Female	33 (36.7)
Age (years)	
≤ 65	65 (72.2)
> 65	25 (27.8)
Median	58
Range	34-76
Histological grade[1]	
G1+G2	76 (84.4)
G3	14 (15.6)
Invasion depth	
T1-T2	47 (52.2)
T3-T4	43 (47.8)
Lymphatic invasion	
Negative	65 (72.2)
Positive	25 (27.8)
TNM stage[2]	
I/II	67 (74.4)
III/IV	23 (25.6)

[1]Histological grade was with reference to WHO classification; published in 2009. [2]TNM stage was based on the UICC criteria published in 2009.

p-ERK1/2 (1:50; Abcam), and EGFR (MaixinBio, 1:50) at 4°C overnight. Negative controls were prepared by replacing the primary antibodies with PBS. The tissues were washed in PBS for 5 min three thrice being incubated with second antibodies. The sections were then stained for 3 min to 5 min with 3,3'-diaminobenzidine tetrahydrochloride, counterstained with Mayer's hematoxylin, dehydrated, and mounted.

Assessment by immunohistochemistry

Cytoplasmic staining of ERK1 and EGFR in ESCC, as well as nuclear staining of ERK2 in ESCC and p-ERK1/2, was performed. The results were assessment by two investigators. The percentages of positive stained cells were assigned the following scores: 0 (< 5% positive cells), 1 (6% to 25% positive cells), 2 (26% to 50% positive cells), 3 (51% to 75% positive cells), or 4 (> 75% positive cells). The staining intensity was scored on a scale of 0 to 3 as follows: 0, negative; 1, buff; 2, yellow; and 3, brown. The percentages of positive epithelial cells and the staining intensities were then multiplied to generate the immunoreactivity score (IS) for each case. Overall staining scores from 0 to 4 and ≥ 4 were considered low and high expression, respectively.

Statistical analysis

All statistical analyses were performed with SPSS version 13.0 (SPSS Standard version 13.0, SPSS, Chicago, IL, USA). The chi-square test was used to statistical analyze the expression of ERK1, ERK2, p-ERK1/2 and EGFR in normal tissues and ESCC sample, as well as the clinicopathologic factors. The Spearman's rank correlation coefficient was used to evaluate the expression of ERK1, ERK2, p-ERK1/2, and EGFR. All P value were two-sided and considered statistically significant at $P < 0.05$.

Results

Clinical-pathologic variables

We conducted a case series study of 90 ESCC samples. The clinical pathologic variables of the patients are listed in **Table 2**.

Overexpression of ERK1, ERK2 and EGFR in Kazakh ESCC tissues

The expression of EGFR and ERK-related proteins was explored in ESCC tissues and adjacent NETs by immunohistochemistry. As show in **Figure 1A**, the diffuse ISs for ERK1 detected in the cytoplasm of ESCC cells (3.79 ± 0.302) were significantly higher than those in the NETs, with an average IS of 1.78 ± 0.345 ($P < 0.005$, **Figure 1A4**). As shown in **Figure 1B**, high ISs for ERK2 (4.09 ± 0.261) were observed in both nucleus and the cytoplasm of carcinoma cells. These scores for ERK2 in ESCC tissues were significantly higher than those in the NETs (1.958 ± 0.419, $P < 0.001$). However, the protein expression of p-ERK1/2 was stained in the cytoplasm and nucleus (4.616 ± 0.300). Moreover, 47.8% (43/90) of the samples were highly stained in ESCC but 46.4% (13/28) in normal tissues. The expression of p-ERK1/2 was lower in NETs (4.464 ± 0.612); however, this difference was not statistically significant ($P > 0.05$, **Figure 1C**). EGFR expression was observed in the cytoplasm of the ESCC cell (2.522 ± 0.163); 28.9% (26/90) of were highly stained in the samples and none (0/24) in the

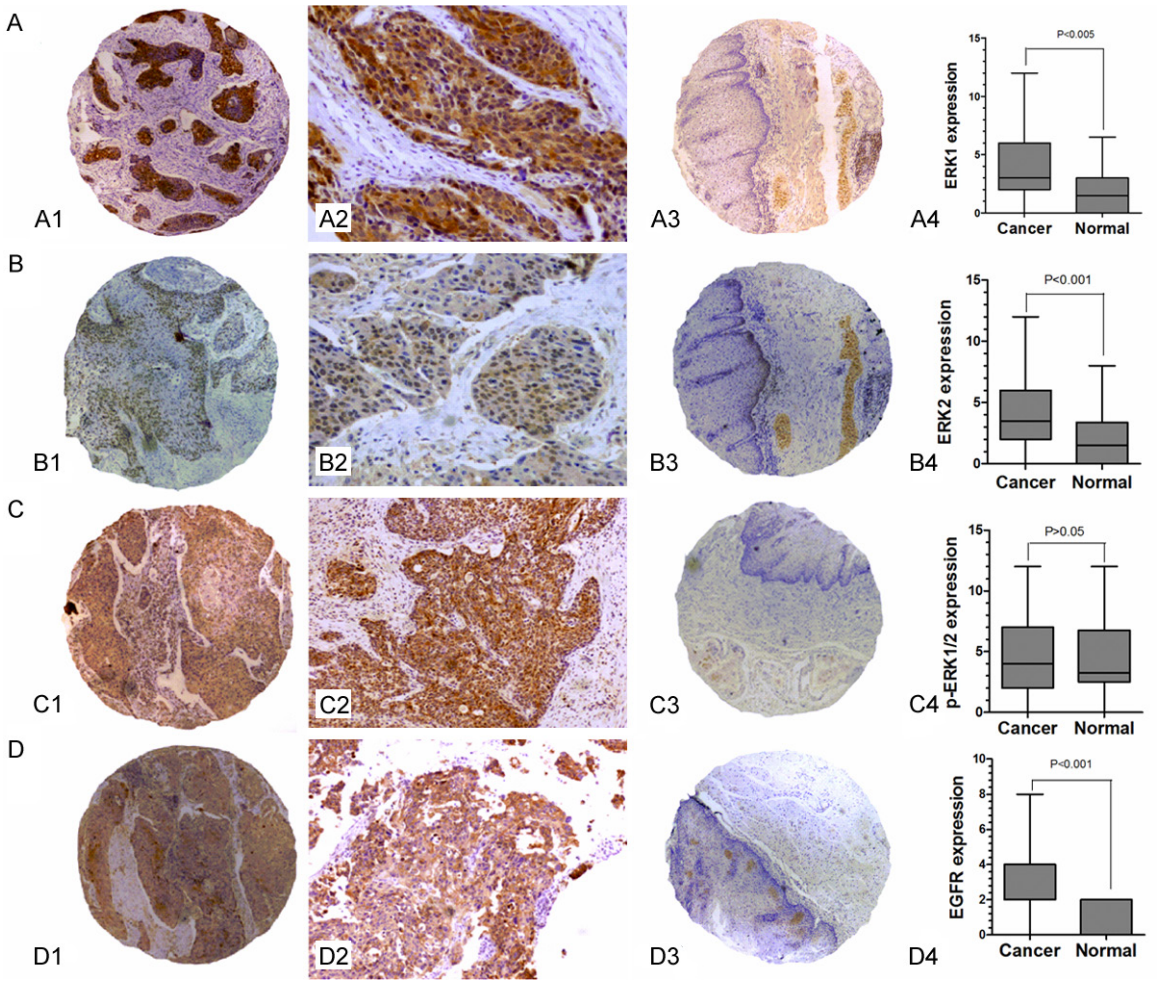

Figure 1. Immunohistochemical staining of the protein expression of ERK1, ERK2, p-ERK1/2, and EGFR in Kazakh esophageal cancer tissues and adjacent normal tissues. High expression of ERK1, ERK2, P-ERK1/2, and EGFR in ESCC (A1, ERK1; B1, ERK2; C1, P-ERK1/2; D1, EGFR. Original magnification ×40). High power view (Original magnification ×200) shows strong staining for ERK1 and EGFR in the cytoplasm of cancer cells (A2, ERK1; A4, EGFR), ERK2 in the nucleus of cancer cells (B2), and P-ERK1/2 in the cytoplasm and the nuclei of cancer cells (C2). Low expression of ERK1, ERK2, P-ERK1/2, and EGFR is detected in NETs (A3, ERK1; B3, ERK2; C3, P-ERK1/2; D3, EGFR. Original magnification ×40). The boxplot shows that the expression of ERK1, ERK2, and EGFR are significantly higher in ESCC tissues than in NETs ($P < 0.005$, $P < 0.001$, and $P < 0.001$, respectively); meanwhile, the results were different for P-ERK1/2 (C4).

adjacent NETs. The expression of EGFR was lower in normal tissues (0.58 ± 0.189) than in the ESCC tissues ($P < 0.001$, **Figure 1D**).

Correlation of ERK and EGFR expression with the clinical features of Kazakh ESCC tissues

The correlation of ERK and EGFR protein expression with several clinicopathologic factors is shown in **Table 3**. Of 25 Kazakh ESCC patients with lymph nodes metastases, 15 cases (60.0%) and 12 cases (48.0%) showed high expression of ERK1 and EGFR, respectively. In comparison, in 65 Kazakh ESCC patients without lymph nodes metastases, 30.7% and 21.5% of which showed overexpression of ERK1 and EGFR, respectively. Therefore, ERK1 expression and EGFR expression in ESCC was significantly correlated with lymph node metastasis ($\chi^2 = 6.492$, $P = 0.011$, $\chi^2 = 6.154$, $P = 0.013$, respectively). Of 23 Kazakh ESCC patients with tumor-node-metastases stage III/IV, 13 cases (56.5%) showed overexpression of ERK1, however, only 22 cases (32.8%) showed overexpression of ERK1 in 67 Kazakh ESCC with tumor-node-metastases stage I/II. Therefore, ERK1 expression in ESCC was significantly correlated with tumor-node-metastases stage

Table 3. Correlations between ERK1, ERK2, EGFR protein expression and clinicopathologic factors

Groups	No. of high expression (%)			
	NO. of patients	ERK1	ERK2	EGFR
Gender				
Male	57	21 (36.8)	28 (49.1)	19 (33.3)
Female	33	14 (42.4)	15 (45.4)	7 (21.2)
Age (years)[1]				
≤ 65	65	26 (40.0)	30 (46.2)	18 (27.7)
> 65	25	9 (36.0)	13 (52.0)	8 (32.0)
Histological grade[2]				
G1+G2	76	28 (36.8)	32 (42.1)	24 (31.6)
G3	14	7 (50.0)	11 (78.5)*	2 (14.3)
Invasion depth				
T1-T2	47	17 (36.1)	24 (51.1)	16 (34.0)
T3-T4	43	18 (41.8)	19 (44.2)	10 (23.3)
Lymphatic invasion				
Negative	65	20 (30.7)	32 (49.2)	14 (21.5)
Positive	25	15 (60.0)*	11 (44.0)	12 (48.0)*
TNM stage[3]				
I/II	67	22 (32.8)*	24 (35.8)	26 (38.8)
III/IV	23	13 (56.5)	11 (47.8)	9 (39.1)

*$P < 0.05$, [1]Mean age. [2]Histological grade was with reference to WHO classification published in 2009. [3]TNM stage was based on the UICC criteria published in 2009.

Table 4. Correlations between ERK1 and EGFR protein expression

	ERK1	ERK2	p-ERK1/2	EGFR
ERK1	1			
ERK2	0.231*	1		
p-ERK1/2	0.04	-0.031	1	
EGFR	0.413***	0.028	0.113	1

*$P < 0.05$, $P < 0.005$, ***$P < 0.001$.

(χ^2 = 7.385, P = 0.007). Of 14 poorly differentiated tissues, 11 cases (78.5%) showed high expression of ERK2, whereas in 76 well and moderately differentiated tissues 42.1% showed overexpression of ERK2. Therefore, ERK2 expression in ESCC was positively correlated with Histological grade (χ^2 = 6.301, P = 0.012).

Relationship between ERK1 and EGFR expression in ESCC

Collectively, our data suggest a significant correlation between ERK1 expression and EGFR expression in the ESCC tissues (Spearman coefficient of 0.413; P = 0.001; **Table 4**, **Figure 2A**). In addition, higher EGFR histoscores were obtained for ERK1-high patients (4.95 ± 0.57), whereas lower EGFR histoscores were found in ERK1-low patients (3.21 ± 0.57, P = 0.01; **Figure 2B**). These results indicate that the up-regulation of EGFR is associated with the up-regulation of ERK1 in Kazakh ESCC.

Discussion

ESCC is a highly malignant tumor of the digestive system; thus, key factors involved in the development and progression of the disease must be investigated. In our previous research, the phospholipase C epsilon 1 (PLCE1) gene was identified as a new susceptibility gene to ESCC [25-27], especially as a potential biomarker for cancer metastasis and aggressiveness in Kazakh ESCC [28-30]. However, multiply factors, such as aberrant activation of many signal pathways, could be involved in ESCC carcinogenesis. Belonging to the subfamily of the MAPK pathway, ERKs contain ERK1 and ERK2. These two kinases are required for physiological processes, such as cell growth, differentiation, apoptosis, and tumorigenesis in various malignancies [31]. However, the MAPK signaling pathway, as a central subject of research on cancer cell signaling, remains undetermined in Kazakh ESCC.

Using immunohistochemistry in TMAs, we found that ERK1and ERK2 is more highly expressed in ESCC tissues than in adjacent NETs. These results are consistent with other findings that ERK1 and ERK2 are more highly expressed in various cancers, such as clear cell renal cell carcinoma, hepatocellular carcinoma and gastric adenocarcinoma [32-34], than in normal tissue. Moreover, p-ERK1/2 was poorly expressed in ESCC; nevertheless, but this difference was not significant. These results contradict the findings by Zheng et al. [22] and Garavello et al. [35], who indicated that p-ERK1/2 is poorly expressed in ESCC or squamous cell carcinoma of the larynx tissues than in adjacent normal tissues. This discrepancy may be attributed to the variation in population

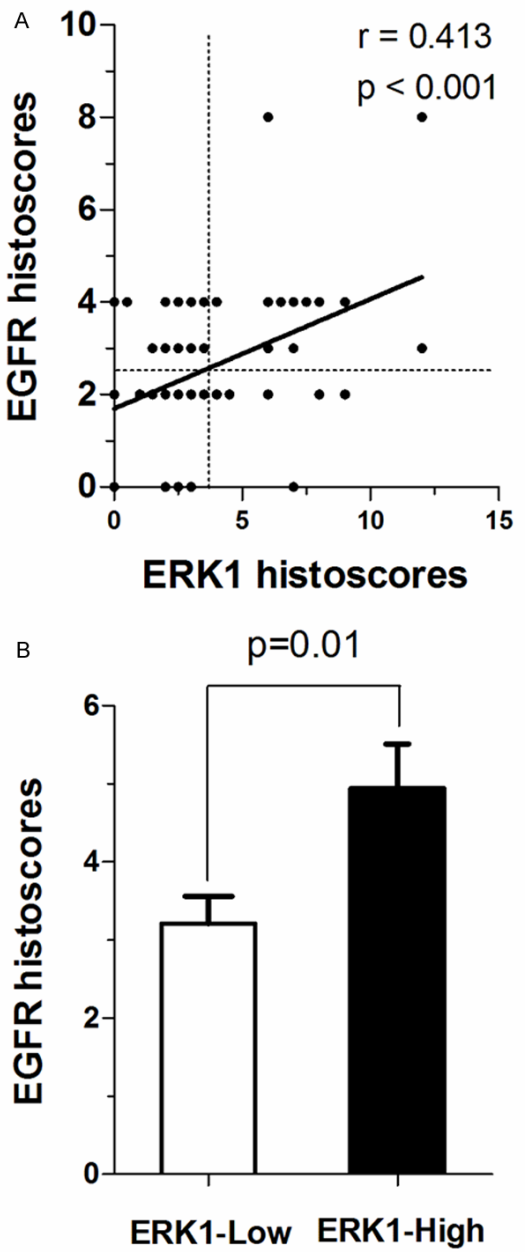

Figure 2. Correlation between ERK1 expression and EGFR expression in Kazakh ESCC. A: ERK1 expression was positively corrected with EGFR expression in ESCC tissues (r = 0.413, *P* < 0.001). The dotted line represents the mean score of protein expression. B: EGFR expression was higher in ESCC tissue with high ERK1 expression (4.95 ± 0.57) than in those tissues with low ERK1 expression (3.21 ± 0.35; *P* = 0.01).

heterogeneity and the influence of p-ERK1/2 in ESCC carcinogenesis between the early and the later stages. Thus, further studies need to be conducted on the same ethnic groups with larger ESCC samples in different stages.

In the present study, we demonstrated that the protein expression of EGFR was higher in Kazakh ESCC tissues than in normal tissues. EGFR overexpression has been reported in breast cancer [10], colon cancer [11, 12], and lung cancer [13, 14]. Silencing of EGFR mRNA in prostate cancer stem-like cells (PCSC) resulted in the self-renewal and robust reduction of propagation in PCSC [36]. Our observation that EGFR expression was upregulated in Kazakh ESCC tissues agree with the study by Itakura et al. Their study indicated that the immunohistochemical overexpression of EGFR in a Japanese population occurred more frequently than did EGFR DNA amplification in ESCC [37]. This result suggests that in several cancers, including Kazakh ESCC, EGFR significantly affects the biological behavior of ESCC and can be used as a biomarker for Kazakh ESCC.

Moreover, we analyzed the correlation of EGFR and ERK expressions with clinicopathological features and found that ERK1 is correlated not only with lymphatic invasion but with the TNM stage as well. ERK2 expression was higher in well and moderately differentiated tissues than in poorly differentiated tissues. Notably, the inhibition of the ERK signal pathway such as Rab25 and dual-specificity phosphatase 6 (DUSP6) can suppress cell migration and ESCC invasion [38, 39]. Protein tyrosine kinase 7 (PTK7) and fibronectin (FN) phosphorylate Raf and further activate ERK, resulting in invasive in ESCC [21, 40]. Similarly, Li et al. suggested that Stomatin-like protein 2 (SLP-2) can be the downstream gene of the ERK pathway and that SLP-2 can promote ESCC invasion through the ERK signaling pathway [41]. In addition, EGF signaling can increase the invasive capability of human prostate cancer cells by the up-regulation of p-ERK and the HH signaling transcriptional factor GLI-1, which may be among the important effectors activated by EGF downstream signaling [42] (**Figure 3**). The present study found that EGFR was correlation with lymphatic invasion. This correlation supports the finding that the higher expression of the EGFR gene in the cytoplasm can significantly affect the progression of invasive in salivary adenoid cystic carcinoma [43]. This finding was derived from the report that EGFR activates mesenchymal to epithelial transition through MAP kinases to enhance NSCLC invasion and brain metastasis [44].

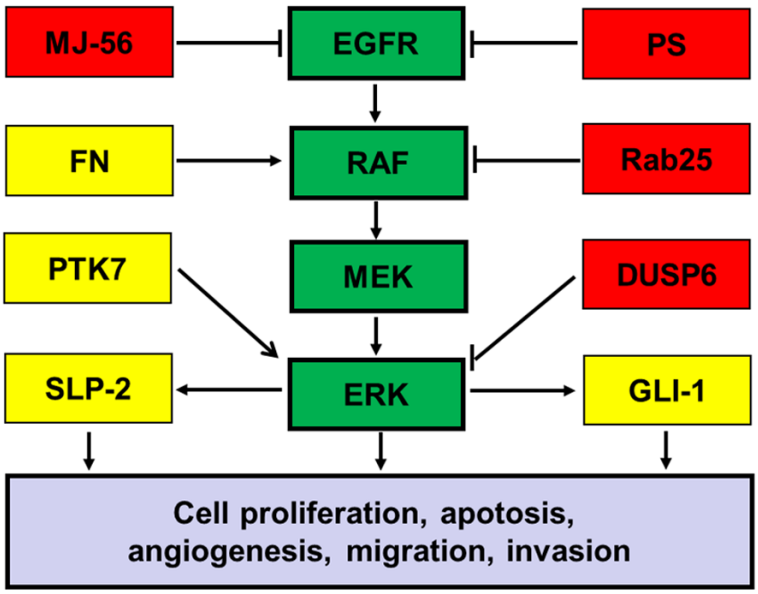

Figure 3. EGFR-ERK pathway is activated by many factors in various tumors. MJ-56 and PS suppress tumorigenesis by reducing the activities of EGFR-Raf/MEK/ERK, Rab25 and DUSP6 inhibit ERK signal pathway, Meanwhile, FN and PTK7 can activate the ERK signal pathway, SLP and GLI-1, which are the downstream genes of the ERK pathway that can increase tumorigenesis.

We assessed the relationship between EGFR expression and ERK1 expression in ESCC and found that ERK1 expression in ESCC tissues is positively corrected with EGFR expression and that EGFR was more highly expression in ESCC tissue with high ERK1 expression than in those with low ERK1 expression, suggesting that the activation of the EGFR-ERK signaling pathway occurs frequently in Kazakh ESCC. This activation is associated with tumorigenesis and progression in several other cancers. The EGFR-ERK signaling pathway activated by the insulin receptor tyrosine kinase substrate (IRTKS) may promote the proliferation of hepatocellular carcinoma cells [45]. In addition, MJ-56 [6-pyrrolidinyl-2-(3-bromostyryl) quinazolin-4-one] inhibited the cell migration and invasion of HT29 human colorectal cancer cells by reducing the activities of EGFR and the downstream ERK-mediated MAPK signaling pathways [46]. Therefore, the involvement of the increased activation of the EGFR-ERK signaling pathway that was determined by those molecules in the development of Kazakh ESCC by the same mechanism needs further investigation.

In conclusion, the current study showed the significant overexpression of EGFR and ERK at the early stage of ESCC and determined a positive correlation between ERK and EGFR in Kazakh patients with ESCC. To the best of our knowledge, this study is the first to report on the involvement of the EGFR-ERK signaling pathway in ESCC carcinogenesis in Kazakh patients. Further studies are necessary to examine the therapeutic potential of the EGFR-ERK inhibitor in anticancer therapy, particularly in ESCC.

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Disclosure of conflict of interest

None.

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References

[1] Parkin DM, Bray F, Ferlay J and Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74-108.

[2] Huang L, Lu Q, Han Y, Li Z, Zhang Z, Li X. ABCG2/V-ATPase was associated with the drug resistance and tumor metastasis of esophageal squamous cancer cells. Diagn Pathol 2012; 7: 1746-1596.

[3] Wang GQ, Abnet CC, Shen Q, Lewin KJ, Sun XD, Roth MJ, Qiao YL, Mark SD, Dong ZW, Taylor PR and Dawsey SM. Histological precursors of oesophageal squamous cell carcinoma: results from a 13 year prospective follow up study in a high risk population. Gut 2005; 54: 187-192.

[4] Cui X, Zhao Z, Liu D, Guo T, Li S, Hu J, Liu C, Yang L, Cao Y, Jiang J, Liang W, Liu W, Li S, Wang L, Wang L, Gu W, Wu C, Chen Y, Li F. Inactivation of miR-34a by aberrant CpG methylation in Kazakh patients with esophageal carcinoma. J Exp Clin Cancer Res 2014; 33: 20.

[5] Cui X, Chen Y, Liu L, Li L, Hu J, Yang L, Liang W, Li F. Heterozygote of PLCE1 rs2274223 increases susceptibility to human papillomavirus infection in patients with esophageal carcinoma among the Kazakh populations. J Med Virol 2014; 86: 608-617.

[6] Rice TW, Rusch VW, Apperson-Hansen C, Allen MS, Chen LQ, Hunter JG, Kesler KA, Law S, Lerut TE, Reed CE, Salo JA, Scott WJ, Swisher SG, Watson TJ and Blackstone EH. Worldwide esophageal cancer collaboration. Dis Esophagus 2009; 22: 1-8.

[7] Yarden Y and Schlessinger J. Epidermal growth factor induces rapid, reversible aggregation of the purified epidermal growth factor receptor. Biochemistry 1987; 26: 1443-1451.

[8] Holbro T, Civenni G and Hynes NE. The ErbB receptors and their role in cancer progression. Exp Cell Res 2003; 284: 99-110.

[9] Pawson T and Nash P. Protein-protein interactions define specificity in signal transduction. Genes Dev 2000; 14: 1027-1047.

[10] Elisa G, Fabio A, Turano C and Silvia C. The protein ERp57 contributes to EGF receptor signaling and internalization in MDA-MB-468 breast cancer cells. J Cell Biochem 2013; 114: 2461-70.

[11] Najar AG, Pashaei-Asl R, Omidi Y, Farajnia S and Nourazarian AR. EGFR antisense oligonucleotides encapsulated with nanoparticles decrease EGFR, MAPK1 and STAT5 expression in a human colon cancer cell line. Asian Pac J Cancer Prev 2013; 14: 495-498.

[12] Hong L, Han Y, Zhang H, Zhao Q, Yang J and Ahuja N. High expression of epidermal growth factor receptor might predict poor survival in patients with colon cancer: a meta-analysis. Genet Test Mol Biomarkers 2013; 17: 348-351.

[13] Zhang YW, Staal B, Essenburg C, Lewis S, Kaufman D and Vande Woude GF. Strengthening Context-Dependent Anti-Cancer Effects on Non-Small Cell Lung Carcinoma by Inhibition of Both MET and EGFR. Mol Cancer Ther 2013; 12: 1429-41.

[14] Zhang M, Mu XY, Jiang SJ, Liu QL and Li DW. Relationship between epidermal growth factor receptor gene expression and radiosensitivity of non-small-cell lung cancer cells. Zhonghua Zhong Liu Za Zhi 2013; 35: 94-97.

[15] Abedi-Ardekani B, Dar NA, Mir MM, Zargar SA, Lone MM, Martel-Planche G, Villar S, Mounawar M, Saidi F, Malekzadeh R and Hainaut P. Epidermal growth factor receptor (EGFR) mutations and expression in squamous cell carcinoma of the esophagus in central Asia. BMC Cancer 2012; 12: 602.

[16] Yang YL, Xu KL, Zhou Y, Gao X and Chen LR. Correlation of epidermal growth factor receptor overexpression with increased epidermal growth factor receptor gene copy number in esophageal squamous cell carcinomas. Chin Med J (Engl) 2012; 125: 450-454.

[17] Okines A, Cunningham D and Chau I. Targeting the human EGFR family in esophagogastric cancer. Nat Rev Clin Oncol 2011; 8: 492-503.

[18] Oberst MD, Beberman SJ, Zhao L, Yin JJ, Ward Y and Kelly K. TDAG51 is an ERK signaling target that opposes ERK-mediated HME16C mammary epithelial cell transformation. BMC Cancer 2008; 8: 189.

[19] Peng H, Dara L, Li TW, Zheng Y, Yang H, Tomasi ML, Tomasi I, Giordano P, Mato JM and Lu SC. MAT2B-GIT1 interplay activates MEK1/ERK 1 and 2 to induce growth in human liver and colon cancer. Hepatology 2013; 57: 2299-2313.

[20] Dong QZ, Wang Y, Tang ZP, Fu L, Li QC, Wang ED and Wang EH. Derlin-1 is overexpressed in non-small cell lung cancer and promotes cancer cell invasion via EGFR-ERK-mediated up-regulation of MMP-2 and MMP-9. Am J Pathol 2013; 182: 954-964.

[21] Zhang J, Zhi H, Zhou C, Ding F, Luo A, Zhang X, Sun Y, Wang X, Wu M and Liu Z. Up-regulation of fibronectin in oesophageal squamous cell carcinoma is associated with activation of the Erk pathway. J Pathol 2005; 207: 402-409.

[22] Zheng ST, Liu T, Middottuersun A, Huo Q, Liu Q, Huang CG, Feng JG, Lu GD, Wang X, Lin RY, Sheyhidin I and Lu XM. Expression of ERK1/2 MAPK signaling transduction pathway in esophageal cancers in Kazakh patients. Zhonghua Zhong Liu Za Zhi 2011; 33: 421-425.

[23] Pengnan Z, Zhang P, Min Z, Hua J, Haiyan Z, Bizhi S, Xiaorong P, Huiping G, Hong S and Zonghai L. Exon 4-Deletion Variant of Epidermal Growth Factor Receptor Enhances Invasiveness and Cisplatin Resistance in Epithelial Ovarian Cancer. Carcinogenesis 2013; 34: 2639-46.

[24] Zhang X, Diaz MR and Yee D. Fulvestrant regulates epidermal growth factor (EGF) family ligands to activate EGF receptor (EGFR) signaling in breast cancer cells. Breast Cancer Res Treat 2013; 139: 351-360.

[25] Wu C, Hu Z, He Z, Jia W, Wang F, Zhou Y, Liu Z, Zhan Q, Liu Y, Yu D, Zhai K, Chang J, Qiao Y, Jin G, Shen Y, Guo C, Fu J, Miao X, Tan W, Shen H, Ke Y, Zeng Y, Wu T and Lin D. Genome-wide association study identifies three new susceptibility loci for esophageal squamous-cell carcinoma in Chinese populations. Nat Genet 2011; 43: 679-684.

[26] Wang LD, Zhou FY, Li XM, Sun LD, Song X, Jin Y, Li JM, Kong GQ, Qi H, Cui J, Zhang LQ, Yang JZ, Li JL, Li XC, Ren JL, Liu ZC, Gao WJ, Yuan L, Wei W, Zhang YR, Wang WP, Sheyhidin I, Li F, Chen BP, Ren SW, Liu B, Li D, Ku JW, Fan ZM, Zhou SL, Guo ZG, Zhao XK, Liu N, Ai YH, Shen FF, Cui WY, Song S, Guo T, Huang J, Yuan C, Wu Y, Yue WB, Feng CW, Li HL, Wang Y, Tian JY, Lu Y, Yuan Y, Zhu WL, Liu M, Fu WJ, Yang X, Wang HJ, Han SL, Chen J, Han M, Wang HY, Zhang P, Dong JC, Xing GL, Wang R, Guo M, Chang ZW, Liu HL, Guo L, Yuan ZQ, Liu H, Lu Q, Yang LQ, Zhu FG, Yang XF, Feng XS, Wang Z, Li Y, Gao SG, Qige Q, Bai LT, Yang WJ, Lei GY, Shen ZY, Chen LQ, Li EM, Xu LY, Wu ZY, Cao WK, Wang JP, Bao ZQ, Chen JL, Ding GC, Zhuang X, Zhou YF, Zheng HF, Zhang Z, Zuo XB, Dong ZM, Fan DM, He X, Wang J, Zhou Q, Zhang QX, Jiao XY, Lian SY, Ji AF, Lu XM, Wang JS, Chang FB, Lu CD, Chen ZG, Miao JJ, Fan ZL, Lin RB, Liu TJ, Wei JC, Kong QP, Lan Y, Fan YJ, Gao FS, Wang TY, Xie D, Chen SQ, Yang WC, Hong JY, Wang L, Qiu SL, Cai ZM and Zhang XJ. Genome-wide association study of esophageal squamous cell carcinoma in Chinese subjects identifies susceptibility loci at PLCE1 and C20orf54. Nat Genet 2010; 42: 759-763.

[27] Abnet CC, Freedman ND, Hu N, Wang Z, Yu K, Shu XO, Yuan JM, Zheng W, Dawsey SM, Dong LM, Lee MP, Ding T, Qiao YL, Gao YT, Koh WP, Xiang YB, Tang ZZ, Fan JH, Wang C, Wheeler W, Gail MH, Yeager M, Yuenger J, Hutchinson A, Jacobs KB, Giffen CA, Burdett L, Fraumeni JF Jr, Tucker MA, Chow WH, Goldstein AM, Chanock SJ and Taylor PR. A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. Nat Genet 2010; 42: 764-767.

[28] Chen YZ, Cui XB, Hu JM, Zhang WJ, Li SG, Yang L, Shen XH, Liu CX, Pan QF, Yu SY, Yuan XL, Gu WY, Chen JZ, Wang LD and Li F. Overexpression of PLCE1 in Kazakh esophageal squamous cell carcinoma: implications in cancer metastasis and aggressiveness. APMIS 2013; 121: 908-18.

[29] Cui XB, Chen YZ, Pang XL, Liu W, Hu JM, Li SG, Yang L, Zhang WJ, Liu CX, Cao YW, Jiang JF, Gu WY, Pang J, Yang L, Yuan XL, Yu SY, Li F. Multiple polymorphisms within the PLCE1 are associated with esophageal cancer via promoting the gene expression in a Chinese Kazakh population. Gene 2013; 530: 315-322.

[30] Cui XB, Pang XL, Li S, Jin J, Hu JM, Yang L, Liu CX, Li L, Wen SJ, Liang WH, Chen YZ, Li F. Elevated expression patterns and tight correlation of the PLCE1 and NF-kappaB signaling in Kazakh patients with esophageal carcinoma. Med Oncol 2014; 31: 791.

[31] Balmanno K and Cook SJ. Tumour cell survival signalling by the ERK1/2 pathway. Cell Death Differ 2009; 16: 368-377.

[32] Lee HJ, Kim DI, Kang GH, Kwak C, Ku JH and Moon KC. Phosphorylation of ERK1/2 and prognosis of clear cell renal cell carcinoma. Urology 2009; 73: 394-399.

[33] Huynh H, Nguyen TT, Chow KH, Tan PH, Soo KC and Tran E. Over-expression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: its role in tumor progression and apoptosis. BMC Gastroenterol 2003; 3: 19.

[34] Bang YJ, Kwon JH, Kang SH, Kim JW and Yang YC. Increased MAPK activity and MKP-1 overexpression in human gastric adenocarcinoma. Biochem Biophys Res Commun 1998; 250: 43-47.

[35] Garavello W, Nicolini G, Aguzzi A, Maggioni D, Leone BE, Vigano P, Gaini RM and Tredici G. Selective reduction of extracellular signal-regulated protein kinase (ERK). Oncol Rep 2006; 16: 479-484.

[36] Tang DG, Rybak AP, Ingram AJ and Tang D. Propagation of Human Prostate Cancer Stem-Like Cells Occurs through EGFR-Mediated ERK Activation. PLoS One 2013; 8: e61716.

[37] Itakura Y, Sasano H, Shiga C, Furukawa Y, Shiga K, Mori S and Nagura H. Epidermal growth factor receptor overexpression in esophageal carcinoma. An immunohistochemical study correlated with clinicopathologic findings and DNA amplification. Cancer 1994; 74: 795-804.

[38] Tong M, Chan KW, Bao JY, Wong KY, Chen JN, Kwan PS, Tang KH, Fu L, Qin YR, Lok S, Guan XY and Ma S. Rab25 is a tumor suppressor gene with antiangiogenic and anti-invasive activities in esophageal squamous cell carcinoma. Cancer Res 2012; 72: 6024-6035.

[39] Wong VC, Chen H, Ko JM, Chan KW, Chan YP, Law S, Chua D, Kwong DL, Lung HL, Srivastava G, Tang JC, Tsao SW, Zabarovsky ER, Stanbridge EJ and Lung ML. Tumor suppressor dual-specificity phosphatase 6 (DUSP6) impairs cell invasion and epithelial-mesenchymal transition (EMT)-associated phenotype. Int J Cancer 2012; 130: 83-95.

[40] Shin WS, Kwon J, Lee HW, Kang MC, Na HW, Lee ST and Park JH. Oncogenic role of protein tyrosine kinase 7 in esophageal squamous cell carcinoma. Cancer Sci 2013; 104: 1120-6.

[41] Cao W, Zhang B, Ding F, Zhang W, Sun B and Liu Z. Expression of SLP-2 Was Associated with

Invasion of Esophageal Squamous Cell Carcinoma. PLoS One 2013; 8: e63890.
[42] Zhu G, Zhou J, Song W, Wu D, Dang Q, Zhang L, Li L, Wang X and He D. Role of GLI-1 in epidermal growth factor-induced invasiveness of AR-CaPE prostate cancer cells. Oncol Rep 2013; 30: 904-10.
[43] Ge MH, Ling ZQ, Tan Z, Chen C, Xu JJ and Yu JL. Expression of epidermal growth factor receptor in salivary adenoid cystic carcinoma and its role in cancer invasion. Zhonghua Zhong Liu Za Zhi 2012; 34: 278-280.
[44] Breindel JL, Haskins JW, Cowell EP, Zhao M, Nguyen DX and Stern DF. EGF Receptor activates MET through MAP kinases to enhance non-small cell lung carcinoma invasion and brain metastasis. Cancer Res 2013; 73: 5053-65.
[45] Wang YP, Huang LY, Sun WM, Zhang ZZ, Fang JZ, Wei BF, Wu BH and Han ZG. Insulin receptor tyrosine kinase substrate activates EGFR/ERK signalling pathway and promotes cell proliferation of hepatocellular carcinoma. Cancer Lett 2013; 337: 96-106.
[46] Chen HJ, Jiang YL, Lin CM, Tsai SC, Peng SF, Fushiya S, Hour MJ and Yang JS. Dual inhibition of EGFR and c-Met kinase activation by MJ-56 reduces metastasis of HT29 human colorectal cancer cells. Int J Oncol 2013; 43: 141-150.