Expression and clinical significance of NF-κB and VEGF-C in esophageal squamous cell carcinoma

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Abstract AIM: To detect the expression of Nuclear transcription factor-kB(NF-κB) and vascular endothelial growth factor-C(VEGF-C) protein in esophageal squamous cell carcinoma (ESCC) and explore their relationship with the prognosis of ESCC. **Methods:** SP immunohistochemical method was used to detect the expression of NF-κB and VEGF-C protein in 124 cases of ESCC and 62 cases of normal esophageal mucosa. **Results:** The positive expression rates of NF-κB and VEGF-C protein in specimens of ESCC were 53.2% and 69.4%, in normal esophageal mucosa were 17.7% and 3.2%, respectively. Both of them had the significant differences between ESCC and normal mucosa ($P < 0.05$). The positive expression of NF-κB and VEGF-C protein were closely correlated with the infiltration and lymph node metastasis of ESCC ($P < 0.05$), but were not correlated with the tumor grade, age or gender of the patients ($P > 0.05$). Moreover, the expression of NF-κB protein was positively correlated with VEGF-C ($P<0.05$). **Conclusion:** The expression of NF-κB and VEGF-C protein is closely correlated with the clinic pathological characteristics of ESCC. The genes of NF-κB and VEGF-C may play important roles in the infiltration and metastasis ESCC. United detection of them may be used as important prognostic predictors in ESCC.
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Key words: esophageal squamous cell carcinoma; nuclear transcription factor-κB; vascular endothelial growth factor-C; invasion; metastasis; prognosis

Introduction

Esophageal cancer is a significant health problem worldwide due to the aggressive nature of invasion and metastasis of this disease; these patients undergo systemic and local recurrences even after radical surgery, and the survival rate remains dismal.Nuclear transcription factor-kB (NF- kB) is a kind of significant pleiotropic nuclear transcription factor, which can regulate the transcription of a variety of cancer-related genes . Vascular endothelial growth factor C (VEGF-C) is a critical activator of tumor lymphangiogenesis that recently has been strongly implicated in the tumor metastasis proces. Expression of VEGF-C is correlated with progression in a number of different cancers. The present study tends to explore the expression of NF-κB and VEGF-C protein in ESCC and normal mucosa tissues, and determine the correlation between these data with clinicopathologic and prognostic features.

Material and Methods

General material

All the samples were taken from the stored paraffin blocks of esophageal specimens by surgical resection in the Third Teaching Hospital of Xinxiang Medical University. Preoperatively, all the cases had no chemotherapy, radiotherapy and immunotherapy history. The age of 124 patients with esophageal carcinoma, 66 males and 58 females, varied from 38 to 75 (average age 60.6±9.5). The HE staining had confirmed that all the cases belongs to squamous cell carcinoma, with 30 cases in level Ⅰ, 50 cases in Level Ⅱ and 44 cases in level Ⅲ.Lymphatic metastasis was found in 84 cases, but was not found in other 40 cases. Low infiltration was found in 18 cases, which lied in or under mucous layer. Deep infiltration was found in 106 cases, which was in muscular layer or theca externa. Besides, 62 cases of normal mucosa of oesophagus were taken in this study for the control group.

Main Agent and Method

Immunohistochemistry SP was used in the research. Monoclonal mouse anti-human NF-κB antibody was the product of American Santa Cruz, purchased from Beijing Zhong Shan Jin Qiao Biological Company Limited, with working concentration of 1:50; Rabbit anti-human VEGF-C polyclonal antibody fluid was purchased from Wu Han Bo Shi De Biological Company, at a 1:100 dilution. The experiment was conducted according to the kit introduction. Diaminlbenzidin (DAB) was used for colour development, and PBS was used to replace monoclonal antibody as negative contrast.

Result Judgment Standard

NF-κB protein showed positive expression when the nucleus or endochylema was yellowish brown or brown. VEGF-C positive staining was mainly in endochylema. Each slice was randomly observed in 10 visual fields and counted the positive

cells. It was grouped as positive cases when the number of positive cells ≥10% and negative ones when the number of positive cells < 10%.

Statistical Analysis

Statistical analysis was performed with SPSS software (version 10.0). The comparation of rate used $\chi 2$ test. Associations for NF-κB and VEGF-C expression were assessed using Spearman's rank correlation test. Differences were considered significant at $P < 0.05$.

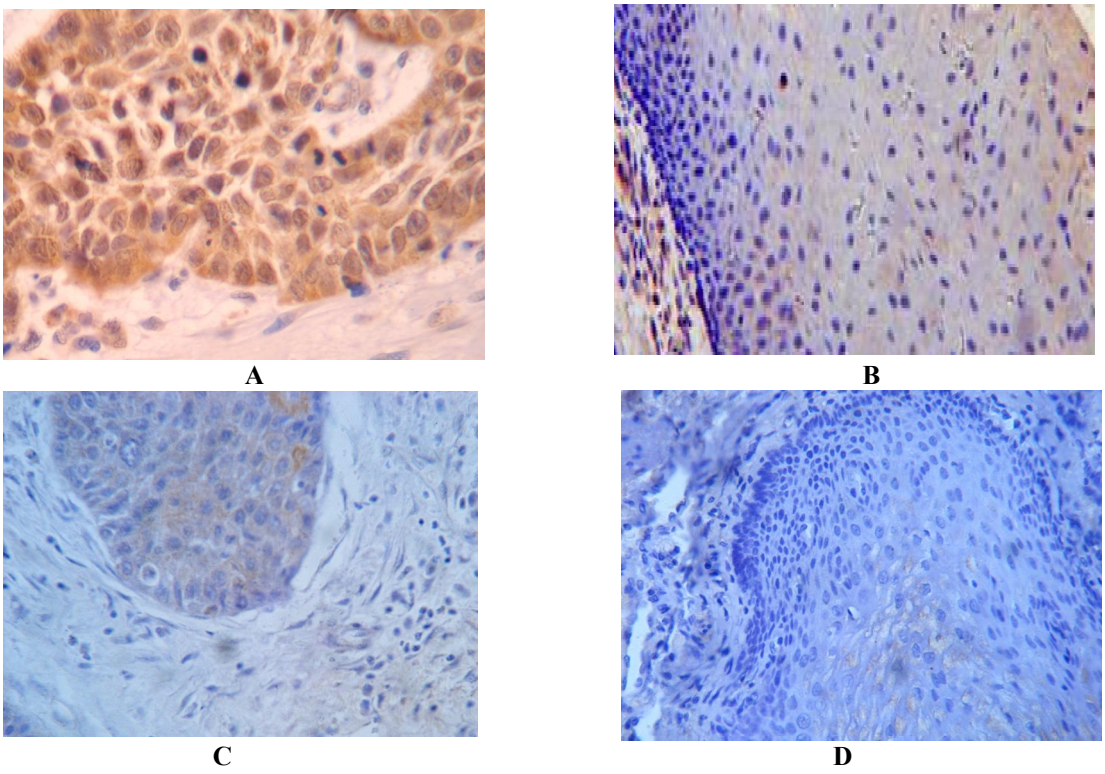
Results

Expression of NF-κB and VEGF-C in esophageal ECC and normal mucosa

The positive expression of NF-κB showed yellowish brown or brown colouring in nucleus or endochylema（fig. A, fig. B）. The positive expression of VEGF-C protein mainly demonstrates strong colouring in endochylema (fig.C, fig.D). And the positive expression rates of NF-κB and VEGF-C increased in ESCC than in normal esophageal mucosa (table 1).

Table 1. Expression of NF-κB and VEGF-C in esophageal ECC and normal mucosa

Tissue types		NF-κB			VEGF-C		
		Positive number（%）	$\chi 2$	*P*	Positive number（%）	$\chi 2$	*P*
ECC	124	66（53.2）	21.452	0.000	86（69.4）	72.511	0.000
Normal	62	11（17.7）			2（3.2）		



A **B** **C** **D**

Figure 1. Immunohistochemical staining of NF-κB and VEGF-C. Expression of NF-κB in **A** esophageal squamous cell carcinoma (ESCC) (400×) and **B** normal mucoma (400×); Expression of VEGF-C in **C** ESCC (400×) and **D** normal mucoma (400×)

Expression of NF-κB and VEGF-C in esophageal squamous cell carcinoma and its relationship with clinicopathologic features

The positive expression of *NF-κB and VEGF-C* protein in ESCC was closely correlated with the infiltration and lymph metastasis (P<0.05), but had no correlation with the differentiation degree and patients' age or sex (p>0.05) (table 2).

Table 2. Relationship between the expression of NF-κB, VEGF-C and the clinicopathologic features of esophageal SCC

Clinicopathologi-cal features		Expression of NF-κB			Expression of VEGF-C		
		Positive number	$\chi 2$	*P*	Positive number	$\chi 2$	*P*
Gender							
Male	66	36	0.099	0.753	46	0.008	0.930
Female	58	30			40		
Age							
≥60	72	40	0.374	0.541	50	0.001	0.980
＜60	52	26			36		
Differentiation degree							
Ⅰ	30	14	2.982	0.225	22	5.406	0.067
Ⅱ	50	24			29		
Ⅲ	44	28			35		
Infiltration depth							
Superficial layer	18	4	8.130	0.004	5	17.126	0.000
Deep layer	106	62			81		
Lymph node metastasis							
Positive	84	50	4.149	0.042	64	5.725	0.017
Negative	40	16			22		

Correlation between NF-κB and VEGF-C in esophageal squamous cell carcinoma

According to the spearman analysis. The expression of *NF-κB and VEGF-C* shows positive correlation in ESCC ($p<0.01$) (table 3).

Table 3. Relationship between between NF-κB and VEGF-C in tumor tissues

Immunohistochemical index		VEGF-C		*R*	*P*
		+	—		
NF-κB	+	54	12	0.288	0.001
	—	32	26		

Discussion

Tumor progression and metastasis rely mainly on both vascular and lymphatic systems by which cancer cells can spread widely into regional or distant tissues. The high tumor metastasis is strongly associated with short disease-free survival periods and poor prognosis in cancer patients. It is important to identify the activity of the tumor proteins to better understand the complexity of invasion and metastasis. In the present study, we investigated the expression of NF-κB and VEGF-C proteins in ESCC and esophageal normal mucosa.

NF-κB is an important type of nuclear transcription factor. The main form of NF-κB is a dimmer composed of p50 and p65 protein, the former is the part where NF-κB is linked with DNA, and the latter takes part in the primary regulation of gene's transcription and enhances the linkage between p50 and DNA. So, this study mainly detected the expression p65 to represent the activity of NF-κB. Recent studies found that NF-κB can be expressed in many tumors and was closely linked with the invasion and metastasis of various tumors [9, 10].

VEGF-C possesses two different functions: stimulate the proliferation of vascular endothelial cell and to promote angiogenesis; increase in vascular permeability to provide a favorable local environment for tumor cell invasion and metastasis [11, 12]. In the past decades, many subsets of molecules have been reported to be critically involved in regulating the blood microvessel formation in tumor development [13]. Little is known about how cancer cells can migrate to regional lymph nodes or promote the proliferation of lymphatic vessel. Recent evidence showed that VEGF1-C and VEGF-D, two members of the VEGF family, are the ligands for VEGF receptor(R)-3, which can stimulate the lymphatic vessel growth (lymphangiogenesis) and also enhance lymphatic metastasis in animal model [14]. The former study had shown that knockdown of VEGF-C expression suppressed ESCC viability in vitro and tumor formation and growth in nude mouse xenografts [15]. Other impressive studies showed that VEGF-C expression was associated with lymph node metastasis and poor prognosis in patients with resected esophageal cancer [16, 17].

Our study showed that NK-κB and VEGF-C proteins were highly expressed in ESCC than in esophageal normal mucous ($P<0.05$) and associated with the infiltration depth and lymph node

metastasis of ESCC (P<0.05). The study also showed there was positive correlation between NF-κB and VEGF-C. It indicates that over expression of NF-κB can up regulate the expression of VEGF-C which is coincided with the previous finding [18]. The results suggested that NK-κB and VEGF-C may be useful biomarkers for the prognosis prediction in ESCC.

In conclusion, we demonstrated in this study that the expression of NF-κB and VEGF-C was significantly associated with the clinicopathologic characteristics of ESCC patients. That is to say patients with high expression of NF-κB and VEGF-C may have a poorer prognosis.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer [J].Cell, 2000, 100:57–70.
2. Morales CP, Souza RF, Spechler SJ. Hallmarks of cancer progression in Barrett's oesophagus[J]. Lancet, 2002, 360:1587–1589.
3. A.C. Berger, J. Farma, W.J. Scott, et a1.Complete response to neoadjuvant chemoradiotherapy in esophageal carcinoma is associated with significantly improved survival [J]. J. Clin. Oncol. 2005, 23(7):4330–4337.
4. Sethi G, Sung B, Aggarwal BB. Nuclear factor-kappaB activation: from bench to bedside [J]. Exp Biol Med, 2008, 233(1):21-31.
5. Jenny B, Harrison JA, Baetens D, et a1. Expression and localization of VEGF-C and VEGFR-3 in glioblastomas and haemangioblastomas[J]. J Pathol 2006, 209(1):34–43.
6. Li R, Younes M, Wheeler TM, Scardino P, et a1.Expression of vascular endothelial growth factor receptor-3 (VEGFR-3)in human prostate[J]. Prostate, 2004, 58(2):193–199.
7. Juttner S, Wissmann C, Jons T, et a1.Vascular endothelial growth factor-D and its receptor VEGFR-3: two novel independent prognostic markers in gastric adenocarcinoma[J]. J Clin Oncol, 2006, 24(2):228–240.
8. Van Trappen PO, Steele D, Lowe DG, et a1. Expression of vascular endothelial growth factor (VEGF)-C and VEGF-D, and their receptor VEGFR-3, during different stages of cervical carcinogenesis[J]. J Pathol, 2003, 201(4):544–554.
9. Liu C, Zhou S, Ke CS, et al. Activation and prognostic significance of AKT, NF-kappaB and STAT3 in breast cancer with lymph node metastasis and estrogen receptor expression [J]. Ai Zheng, 2007, 26(9): 929-936.
10. Sethi G, Sung B, Aggarwal BB. Nuclear factor-kappaB activation: from bench to bedside [J]. Exp Biol Med (Maywood), 2008, 233(1):21-31.
11. Wang F, Wei L, Chen L.The relationship between vascular endothelial growth factor, microvascular density, lymph node metastasis and prognosis of breast carcinoma[J]. Zhonghua Binglixue Zazhi, 2000, 29(3):172–175.
12. Adams J, Carder PJ, Downey S, et a1.Vascular endothelial growth factor (VEGF) in breast cancer: comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen[J]. Cancer Res, 2000, 60（11): 2898–2905.
13. Wang F, Wei L, Chen L. The relationship between vascular endothelial growth factor, microvascular density, lymph node metastasis and prognosis of breast carcinoma [J]. Zhonghua Binglixue Zazhi 2000, 29(3):172–175.
14. Adams J, Carder PJ, Downey S, Forbes MA, et a1.Vascular endothelial growth factor (VEGF) in breast cancer: comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen[J]. Cancer Res 2000, 60(11): 2898–2905.
15. Zhang H, Yin Y, Zhang L, et a1.The effects of vascular r endothelial growth factor C knockdown in esophageal squamous cell carcinoma[J]. J Cancer Res Clin Oncol, 2012, 138(1):133–139.
16. Kozlowski M, Naumnik W, Niklinski J, et a1.Vascular endothelial growth factor C and D expression correlates with lymph node metastasis and poor prognosis in patients with resected esophageal cancer[J]. Neoplasma, 2011, 58(4):311-319.
17. Tanaka T, Ishiguro H, Kuwabara Y, et a1.Vascular endothelial growth factor C (VEGF-C) in esophageal cancer correlates with lymph node metastasis and poor patient prognosis[J]. J Exp Clin Cancer Res, 2010, 29:83.
18. Yang MH, Lee KT, Yang S, et a1. Guggulsterone enhances antitumor activity of gemcitabine in gallbladder cancer cells through suppression of NF-κB[J]. J Cancer Res Clin Oncol, 2012, 138(10):1743-1751.

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