

# Changes of COX-2 and VEGF expressions in esophageal precancerous and cancerous lesions from the patients at high incidence area in Henan province<sup>☆</sup>

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## Abstract

**Objective.** To determine the changes and clinical significance of cyclooxygenase-2 (COX-2) and vascular endothelial growth factor (VEGF) in esophageal precancerous and cancerous lesions from the patients at Linzhou, Henan, a well-known region for its high prevalence of esophageal cancer. **Methods.** The expressions of COX-2 and VEGF in esophagi with normal epithelium ( $n = 34$ ), basal cell hyperplasia (BCH,  $n = 36$ ), mild dysplasia ( $n = 15$ ), severe dysplasia ( $n = 18$ ) and esophageal squamous cell carcinoma (SCC,  $n = 32$ ) was examined by immunohistochemistry. **Results.** Immunoreactivity of COX-2 and VEGF in severe esophageal dysplasia and SCC was significantly higher than in other tissues ( $P < 0.05$ ). The correlation between COX-2 and VEGF was statistically significant ( $\chi^2 = 41119, P < 0.01$ ). **Conclusion.** COX-2 and VEGF expressions are up-regulated in severe dysplasia and SCC, suggesting that COX-2 and VEGF may play important roles in esophageal carcinogenesis and may be potential molecular targets for high risk subject screening, and early detection. [Life Science Journal. 2007; 4(2): 11 – 14] (ISSN: 1097 – 8135).

**Keywords:** esophageal cancer; precancerous lesion; COX-2; VEGF

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## 1 Introduction

Esophageal carcinoma is one of the six most common malignant diseases worldwide. The prognosis of the esophageal carcinoma is very poor, with a five-year survival rate as low as 10%, due to lack of specific and sensitive early detection biomarkers and reliable biological prevention and treatment methods<sup>[1]</sup>. Cyclooxygenases (COXs), including two isoforms of COX-1 and COX-2, are important rate-limiting enzymes in the metabolism of arachidonic acid. COX-1, which is often constitutionally

expressed in most mammalian tissues, serves as a house-keeping gene; COX-2, which is not detectable in normal tissues, is highly inducible when tissues are exposed to inflammation or mitogens<sup>[2,3]</sup>. Recent studies have revealed that the expression of COX-2 is closely correlated to the development, progression and metastasis of tumors. Vascular endothelial growth factor (VEGF) plays an important role in stimulating the growth of blood vessels in tumors and is correlated closely to tumor growth, infiltration and metastasis. In this study, to determine the role of COX-2 and VEGF in the pathogenesis of esophageal carcinoma, we have examined the expression of COX-2 and VEGF in the normal esophageal epithelia, esophageal precancerous and cancerous lesions.

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## 2 Materials and Methods

### 2.1 Tissue samples

All the human esophageal specimens were taken from paraffin blocks archived in the Department of Pathology, Yaocun Esophageal Cancer Hospital, Linzhou, Henan, China and the Department of Pathology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China. The age of the 135 subjects was  $53 \pm 9$  years old, ranging from 38 to 70 years old. Among the patients, 71 were male and 64 were female. All these patients had not been treated by either radiotherapy or chemotherapy before surgical treatment. All the specimens were fixed in 5% formaldehyde, routinely dehydrated, embedded in paraffin and serially sectioned at 5  $\mu\text{m}$ -thick for histopathological diagnosis and immunohistochemistry.

### 2.2 Histopathological analysis

Histopathological diagnosis for esophageal epithelia was made based on the changes in cell morphology and tissue architecture using previously established criteria<sup>[4]</sup>. In brief, the normal esophageal epithelium contained one to three proliferating basal cell layers; the papillae were confined to the lower half of the whole epithelium thickness. In basal cell hyperplasia (BCH), the proliferating basal cells surpassed 15% of the total epithelial thickness. Dysplasia (DYS) was characterized by nuclear atypia (enlargement, pleomorphism, and hyperchromasia), loss of normal cell polarity, and abnormal tissue maturation. Squamous cell carcinoma (SCC) was characterized by confluent and invasive sheets of cohesive, polymorphous cells with hyperchromatic nuclei.

### 2.3 Immunohistochemistry detection

**2.3.1 Reagents:** Avidin-biotin peroxides complex (ABC) kit, DAB substrate kit, as well as COX-2 and VEGF mouse monoclonal antibodies were purchased from Zhongshan Biochemical Ltd, Beijing, China. The working concentration was 1:200. Bovine serum albumin (BSA) was purchased from Sigma Chemical Inc, USA.

**2.3.2 Laboratory procedure:** The ABC method was used for immunohistochemical staining<sup>[5]</sup>. In brief, after sectioning, dewaxation and dehydration, incubate the specimens with 1% hydrogen peroxide for 20 minutes. Incubate with normal horse serum (1:50) for 20 minutes and then incubate with 1:400 COX-2 or VEGF antibodies containing 2% BSA for overnight. Dilute the biotin-labeled secondary antibody with 2% BSA, 1:200, and incubate for 45 minutes and then with freshly prepared ABC for 60 minutes. Mix DAB and 0.3%  $\text{H}_2\text{O}_2$  and incubate together for 2 to 5 minutes. Reaction was observed under a microscope and all the procedure was carried out at room temperature.

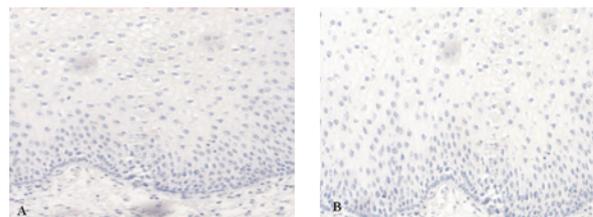
**2.3.3 Semi-quantification of immunostaining results:** The immunostaining was semi-quantified based on the following criteria: (i) Score A: score 0 stands for no increased staining compared to negative control; score 1 means that faint yellow particles were observed in cell membrane or cytoplasm, which was obviously higher than the negative control. Other scores: score 2 was given to those with some pale brown particles; score 3 was given to those with large number of deep pale brown particles; (ii) Score B: In each section, 5 high-power fields were observed randomly, count the number of positive cells out of 500 cells observed. Groups were separated based on the percentage of the positive cells: scores of 0, 1, 2 and 3 represent positive cell percentage <5%, 5% – 25%, 25% – 75% and >75%, respectively; (iii) The final comprehensive scores were represented by the cross products of the two scores A and B. The severity of the staining was as follows: Scores 0 – 3 stand for (–), scores 4 – 6 stands (+), (++) and (+++), respectively.

### 2.4 Statistical analysis

SPSS 10.0 statistical package was used throughout the study. Statistical significance is evaluated by chi-square test and spearman rank correlation test.  $P < 0.05$  was considered statistically significant.

## 3 Results

The expression of COX-2 in different kinds of esophageal epithelium: the expression of COX-2 was not observed in normal epithelia (NOR). The positive expression rates of COX-2 in BCH, mild dysplasia, severe dysplasia and SCC were 23% (8/36), 38% (6/15), 69% (12/18), and 79% (25/32), respectively. Immunoreactivity of COX-2 in severe dysplasia and SCC was significantly higher than in other groups ( $\chi^2 = 41.119$ ,  $P < 0.05$ ) (Figures 1A, 2A, 3A) The expression of VEGF in different kinds of esophageal epithelium: The expression rate of VEGF in normal esophagus epithelia, BCH, mild dysplasia, severe dysplasia and SCC were 16% (5/34), 21% (8/36), 39% (6/15), 69% (10/18), and 78% (25/32), respectively (Figures 1B, 2B, 3B).



**Figure 1.** A: Negative expression of COX-2 in NOR; B: Negative expression of VEGF in NOR.

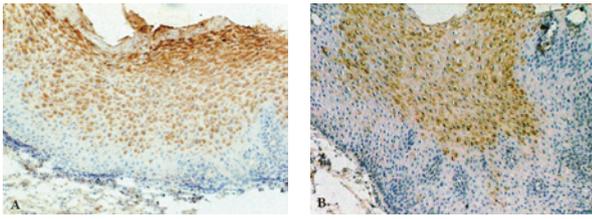


Figure 2. A: Expression of COX-2 in DHS; B: Expression of VEGF in DHS.

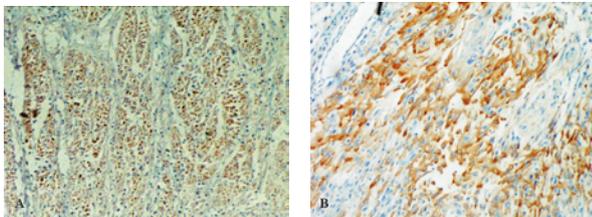


Figure 3. A: Expression of COX-2 in SCC; B: Expression of VEGF in SCC.

The correlation between COX-2 and VEGF: All the tissues with a positive COX-2 also had positive staining for VEGF; in the tissues where COX-2 expression was negative, only 25% (21/84) had a positive staining for VEGF (Figure 4). The correlation between COX-2 and VEGF was statistically significant ( $r = 2.11, P < 0.01$ ).

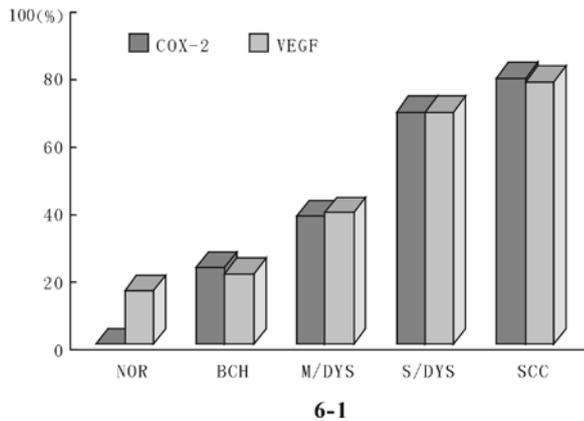


Figure 4. The expression of COX-2 and VEGF in different kinds of esophageal epithelium.

## 4 Discussion

The present results demonstrate that the expression rates of COX-2 and VEGF increase with the progression of the esophageal epithelial lesions from normal to BCH to mild and severe dysplasia and SCC, indicating that COX-2 and VEGF may participate in the pathogenesis and progression of esophageal carcinogenesis. In addition, the expression of

COX-2 and VEGF is significantly correlated. One explanation is that the high expression level of COX-2 may lead to the expression of VEGF to induce blood vessel growth, thus provide more nutrients to support the growth and metastasis of the tumor.

COXs are the key enzymes in catalyzing arachidonic acid into prostaglandin. They are membrane-binding proteins that reside in nuclear and microsomal membranes. COXs include two types of isoforms, COX-1 and COX-2, which share 60% homology and 70% of their core sequences are the same. Especially the amino acid sites with catalytic activity in COX-1 and COX-2 are highly conservative. However, these two COXs have significant differences in inducibility and tissue location: COX-1 is considered a house-keeping gene and resides in many tissues, including stomach, kidney, and platelets. In contrast, COX-2 is considered an immediately early gene, which is undetectable in physiological conditions in most tissues, except brain, kidney and anaphase fetation placenta. However, when cells are stimulated by inflammation, COX-2 is induced rapidly to participate in many kinds of pathophysiologic processes, such as inflammation, fever, pain and so on. Recent studies have shown that high expression levels of COX-2 are found in many malignant tumors and precancerous tissues. COX-2 may help the growth and development of tumor through the following mechanisms: (1) stimulating cell growth; (2) suppressing apoptosis; (3) stimulating neovascularization; (4) participating in tumor invasion and metastasis; (5) affecting the proliferation of cells by catalyzing arachidonic acid metabolism to promote the growth of tumor and inhibit local immune function. Accumulated data indicate the expression of COX-2 increases in various tumor tissues, such as squamous carcinoma of hypistoma and oral cavity, uterine cervix cancer, colorectal cancer, gastric cancer, non-small-cell lung cancer, nasopharyngeal carcinoma, breast cancer and so on. This research shows that COX-2 is also upregulated in esophageal tumor and may be one of important factors in the progression of esophageal carcinogenesis. Thus, selective COX-2 inhibitors might inhibit the growth and progression of esophageal carcinoma.

VEGF is a secretory glycoprotein with a molecular weight of 40 – 45 kD. There are 5 different polypeptides of VEGF due to different mRNA splicing<sup>[6]</sup>. Many studies have revealed that VEGF is a major factor in stimulating angiogenesis in tumors. VEGF secreted by tumor cells promotes neovascularization and is related to the genesis, infiltration and metastasis of tumors. In normal conditions, the expression of VEGF is low. However, under inflammatory and cancerous conditions, over-expression of VEGF is a common finding. VEGF plays a key role in the formation of new tumor blood vessels and is related to infiltration and metastasis of tumors<sup>[7]</sup>. Our previous reports have shown that the life span of esophageal carcinoma patients with

high levels of VEGF expression tended to be shorter<sup>[8]</sup>. This study shows that normal esophageal epithelium expresses little VEGF, while in pathological esophageal epithelium the expression of VEGF increases and is positively related to the malignancy of the diseases, indicating that the expression of VEGF may reflect the pathological processes of esophageal epithelium and provide a reliable molecular marker for high risk subject screening and early detection of SCC in high-risk populations. It may also be a new target for clinical intervention and treatment.

The correlation analysis of COX-2 and VEGF expression shows that COX-2 and VEGF correlate closely and have some concordance in esophageal carcinoma and precancerous lesions. COX-2 may promote the formation of tumor blood vessels through inducing VEGF. The overexpression of COX-2 and VEGF in severe atypical hyperplasia and esophageal carcinoma tissues suggest its importance in detecting COX-2 and VEGF for early diagnosis. Selective COX-2 and VEGF inhibitors should be observed in animal studies and clinical trials to examine their potential in prevention and treatment of esophageal carcinoma.

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