

## Alteration of protein profiles in human esophageal multistage carcinogenesis: highlight on promising biomarker and challenges for high-risk subject screening and early diagnosis<sup>☆</sup>

Lidong Wang<sup>\*</sup>, Aiqun Wu, Yupei Qin, Xiaoshan Feng, Mei Zhang, Baochi Liu, Guolan Xing

Henan Key Laboratory for Esophageal Cancer; Basic Medical College, Zhengzhou University, Zhengzhou, Henan 450052, China

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### Abstract

Human esophageal carcinogenesis has been well-recognized as a multistage progressive process. The early indicator for the subject predisposed to esophageal cancer (EC) is the esophageal epithelial cell hyperproliferation, morphologically manifested as basal cell hyperplasia (BCH) dysphasia (DYS) and carcinoma *in situ* (CIS), which could be considered as esophageal precancerous lesions. Follow-up studies on the subjects at high incidence area for EC have demonstrated that these precancerous lesions are unstable, i. e. these lesions could develop to cancer, or remain at the same stage for long time, and even return to normal. The molecular mechanism underlying is largely unknown. It has been demonstrated that multiple proteins with aberrant expression are involved in esophageal carcinogenesis. In this review, evidences for protein profiles in human esophageal precancerous and cancerous lesions were summarized to highlight the promising biomarkers and challenges for high-risk subject screening and early diagnosis for EC. [Life Science Journal. 2007;4(1):1-5] (ISSN: 1097-8135).

**Keywords:** esophageal carcinoma; carcinogenesis; protein profile; biomarker

### 1 Introduction

Esophageal carcinoma (EC) is one of the sixth most common malignant tumors worldwide. Linzhou (formerly Linxian) and nearby counties have been well-documented as the highest incidence area for EC, and EC remains the leading cause of cancer related death in these areas. Moreover, EC in late stage has a very poor prognosis, with a five year survival rate of less than 10%. However, the 5-year survival rate for EC in the early stage could be as high as 90%. Obviously, early diagnosis is the crucial factor in reducing mortality. But, more than 80% of the EC patients are diagnosed at the advanced stage clinically for the first time at present. One leading cause for this poor diagnosis is lack of specific biomarkers for the early EC patients who have not obvious special symptom in early stage and for large-scale high-risk subject screening. So far, endoscopic biopsy and histopathological examination in mass survey and follow-up at high incidence area remain the most effective method to the identify early cancer and precancerous lesions. It is rather difficult to apply these methods for high-risk subject screening and early diagnosis in large-

scale mass survey for symptom-free subjects from high incidence area. Thus, it becomes critically important to identify promising biomarker for high-risk subject screening and early diagnosis through charactering the morphological and molecular changes in multistage carcinogenesis of EC<sup>[1]</sup>.

Esophageal carcinogenesis has been well-recognized as a multistage and progressive process. The early indicator for the subject predisposed at EC is the aberrant hyper-proliferation of epithelial cells, morphologically manifested as basal cell hyperplasia (BCH), dysphasia (DYS) and carcinoma *in situ* (CIS), which could be regarded as esophageal precancerous lesions. High risk subject screening and follow-up studies in high-incidence area for EC have indicated that about the natural history for esophageal carcinogenesis from these precancerous lesions to cancer could be 5-10 years<sup>[1-4]</sup>. But, the molecular mechanism underlying is still largely unknown. In this review, the progress for aberrant protein expression in human esophageal multistage carcinogenesis and the challenges in this area were summarized to highlight the promising biomarker for high-risk subject screening and early diagnosis.

### 2 Challenges in Studying the Mechanisms of Human Esophageal Multistage Carcinogenesis

The obvious clinical characteristic of human esophageal precancerous lesions is its instability, i. e. , it

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<sup>\*</sup>Corresponding author. Tel and Fax: 86-371-6665-8335; Email: ldwang@zzu.edu.cn

could constantly develop to the direction of cancer or remain at the same stage for many years, or even return to normal. It is difficult to interpret the phenomenon only based on morphological changes. The underlying molecular changes may be of importance in elucidating the mechanism of human esophageal multistage carcinogenesis and establishing the promising biomarkers for high-risk subject screening and early diagnosis. The challenges in these areas include: (1) To establish a large scale follow-up design on symptom-free subjects from high-risk area with repeated esophageal biopsies. This case-control study is crucial in identifying the promising biomarkers for high-risk subject screening and early diagnosis; (2) Through large scale mass survey with the promising biomarkers and esophageal biopsies to confirm the consistence in diagnosis of precancerous and cancerous lesions; (3) To establish "one drop of blood test" method for large scale high-risk subject screening and early diagnosis. Recent studies have indicated that through one drop of blood to test the autoantibodies against tumor suppressor and monogenic proteins could predict the subjects with esophageal precancerous and cancerous lesions in a small group<sup>[5]</sup>. Obviously, this "one drop of blood test" would be more easier, economic and acceptable for the large scale mass survey. It could narrow down the number of subjects for endoscopic examination. The key scientific questions to be addressed in the mechanisms of human esophageal multistage carcinogenesis include: What are the key molecular events occurred in multistage carcinogenesis? Which of these molecular events are key factor to drive the mild esophageal precancerous lesions to severe or cancer? Would the subjects with these molecular changes during the follow-up develop to esophageal cancer earlier or more quickly than those without these molecular changes? Based on these studies, could the promising biomarkers be identified for high-risk subject screening and early diagnosis? Apparently, to answer these questions, it is very important to establish the follow-up subjects in high-risk area with repeated esophageal biopsies.

### 3 Nomenclature and Protein Profiles for Human Esophageal Multistage Carcinogenesis

#### 3.1 Nomenclature of human esophageal multistage carcinogenesis

The concept of esophageal precancerous lesions comes from the histopathological observation and follow-up studies on the large-scale mass survey in high-risk area, on surgical specimen adjacent to carcinoma and on animal experiment model. Morphologically, the precancerous lesions of the esophageal epithelium are quite similar in symptom-free subjects, tissues adjacent to EC and the rat EC models induced by nitrosamine<sup>[6,7]</sup>. It is

noteworthy that the patterns of molecular changes are not the same in morphologically similar precancerous lesions. The typical sample is that, the positive p53 immunostaining rate in human esophageal precancerous lesions is much higher than in rat model induced by nitroamine. In contrast, ras mutations are frequently observed in rat model induced by nitrosamine, not in human esophageal precancerous and cancerous lesions<sup>[8]</sup>. Even in the same subject with similar morphological type of precancerous lesions at the different parts of the esophagus, p53 mutation pattern is different<sup>[9]</sup>. These results indicate the discordance of "tissue phenotype" and "genetic phenotype" in esophageal multistage carcinogenesis. Thus, it becomes important to nominate these morphological changes based on molecular events, which may predict the development of these lesions either to cancer or not.

#### 3.2 Histogenesis model of human esophageal multistage carcinogenesis<sup>[10]</sup>

Based on the literature and recent works of our lab, the histogenesis model for the human esophageal multistage carcinogenesis is summarized as in Figure 1.

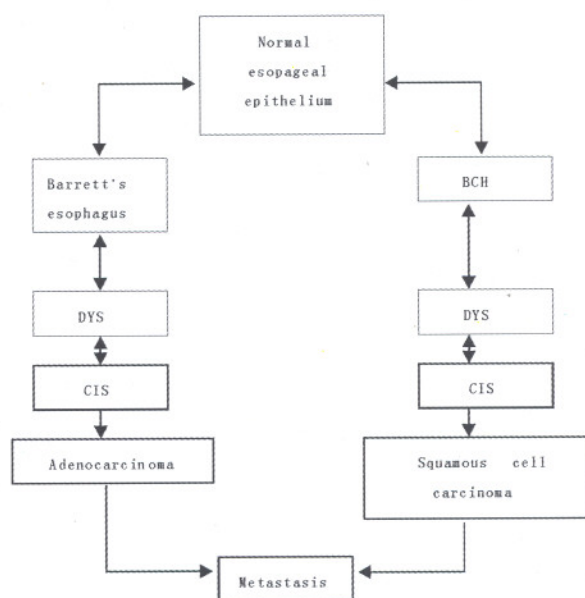


Figure 1. Histogenesis model for human esophageal multistage carcinogenesis

What needs to be emphasized is that the histological pattern of EC is quite different in western countries and in China. Primary esophageal adenocarcinoma almost takes up 50% in western countries. The histogenesis model from reflux esophagitis (gastroesophageal reflux disease, GERD) to Barrett's esophagus to *DYS* to *CIS* to esophageal adenocarcinoma is the most common type of esophageal carcinogenesis in the western countries.

However, squamous cell carcinoma almost takes up 95% in China, the incidence for primary esophageal adenocarcinoma is very low. The incidence of the reflux esophagitis in high-risk area in China is about 6% (14%-16% in the western countries). Barrett's esophagus occurrence is also very low in China (0.5% - 2%). The mechanisms for these differences are not clear. The histogenesis model from normal to BCH to DYS to CIS is the most common type of esophageal carcinogenesis in China.

### 3.3 Protein profiles for human esophageal multistage carcinogenesis

Figure 2 and Table 1 summarized the alterations of 57 proteins aberrant expression in human esophageal normal, precancerous and cancerous lesions<sup>[11-58]</sup>. All these subjects were from Linzhou, Henan province, the highest incidence area for EC. Most of the precancerous and normal tissues were from high-risk subject screening in this area with endoscopic examination. These primary results demonstrated that multiple proteins changed in the multistage carcinogenesis of EC with a different degree of severities. It is noteworthy that with the lesions progressed from normal to mild and severe stage, most of proteins from p53-Rb pathway (including p53, Rb, p16, p15, p14, CyclinD1, waf1-p21, PCNA, etc.), shows apparent aberrant expression, especially the p53, and PCNA proteins. These data suggest that these molecular changes will be one of mechanisms to drive the mild lesions to severe and cancer. Further characterization is needed to verify the significance of these biomarkers in high-risk subject screening and early diagnosis.

## 4 Perspectives

### 4.1 Establish the sample and information bank for human esophageal multistage carcinogenesis and identi-

### fication of the key genetic biomarkers for high-risk subject screening and early diagnosis.

Although the accumulated data have indicated that esophageal carcinogenesis is a multistage and progressive process involved by multiple genetic changes, the key genetic changes to drive the mild lesions to severe and cancer in EC is largely unknown. To establish the informative sample bank is crucial in illustrating the mechanism of human esophageal multistage carcinogenesis and identifying the biomarkers for high-risk subject screening and early diagnosis. Actually, these genetic changes at the same time could provide important clues in designing new target for treatment and prevention. It could not be overemphasized to perform systemic studies on pedigree of EC to identify the key genetic changes.

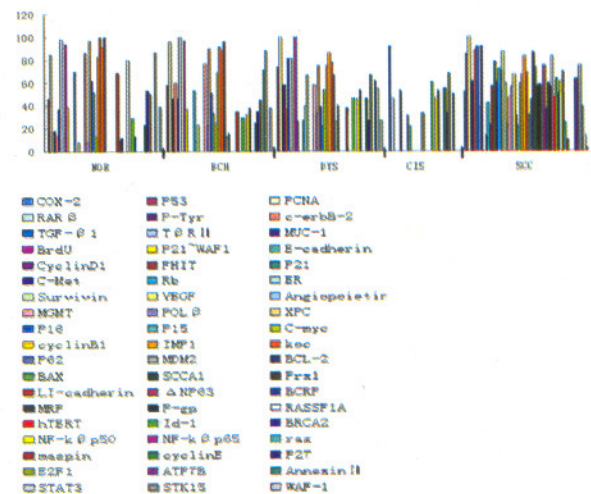


Figure 2. Alterations of 57 proteins in human esophageal multistage carcinogenesis

Table 1. Frequency of 57 proteins expression in human esophageal multistage carcinogenesis

Histological types	Positive immunostaining rate (%)		
	<50%	50% - 80%	>80%
Normal	TGF-β2, ER, POL-β, Id-1, P53, NF-κBp50, MUC1, IMP1	PCNA, COX-2, LI-cadherin	TGF-β1, E-cadherin, MGMT, cyclin-B1, P21 - WAF1
BCH	POL-β, Id-1, TGF-β2, ER, MUC1, IMP1, NF-κBp65	MGMT, P53, NF-κBp50, PCNA, COX-2	TGF-β1, E-cadherin, cyclin-B1, P21 - WAF1
DYS	ER, POL-β, Id-1, MUC1, LI-cadherin	E-cadherin, MGMT, P53, NF-κB P50, PCNA, NF-κBp65	TGF-β2, TGF-β1, COX-2, P21 - WAF1, P21 - WAF1
CIS	E-cadherin	ER, Id-1, PCNA, NF-κBp65	TGF-β2, P53
SCC	TGF-β2, ER, P16, MGMT, LI-cadherin, P21 - WAF1, Annexin II, cyclin-D1, P62, MET, GSTM1, FHIT	E-cadherin, BRCA2, POL-β, Id-1, NF-κBp50, COX-2, P21 - WAF1, NF-κBp65, VEGF, MDM2, NF-κBp49, BAX, KOC, ΔNp63, RARβ, Rb, c-myc, SCCA1, c-erb-2, c-met	TGF-β1, P53, MUC1, PCNA, IMP1, Bcl-2, Prx1, Survivin

## 4.2 Basic-clinic translation

"One drop of blood test" for high-risk subject screening and early diagnosis has been dreamed by generations of esophageal cancer researchers. Although the key molecular events involved in esophageal multistage carcinogenesis is not clear, the present accumulated data have showed that multiple autoantibody assay could predict the high-risk subjects and even identify the early EC patient. Basic-clinic translation should be emphasized to narrow down the scale for high-risk subject screening with endoscopic examination.

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