• ESOPHAGEAL CANCER •

Clinicopathologic analysis of esophageal and cardiac cancers and survey of molecular expression on tissue arrays in Chaoshan littoral of China

Min Su, Xiao-Yun Li, Dong-Ping Tian, Ming-Yao Wu, Xian-Ying Wu, Shan-Ming Lu, Hai-Hua Huang, De-Rui Li, Zhi-Chao Zheng, Xiao-Hu Xu

Min Su, Xiao-Yun Li, Dong-Ping Tian, Ming-Yao Wu, Xian-Ying Wu, Xiao-Hu Xu, Department of Pathology, Shantou University Medical College, Shantou 515031, Guangdong Province, China
De-Rui Li, Tumor Hospital of Shantou University Medical College, Shantou 515031, Guangdong Province, China
Shan-Ming Lu, First Affiliated Hospital of Shantou University Medical College, Shantou 515031, Guangdong Province, China
Hai-Hua Huang, Zhi-Chao Zheng, Second Affiliated Hospital of Shantou University Medical College, Shantou 515031, Guangdong Province, China
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Correspondence to: Professor Min Su, Department of Pathology, Shantou University Medical College, 22 Xinling Road, Shantou 515031, Guangdong Province, China. minsu@stu.edu.cn
Telephone: +86-754-8900429   **Fax:** +86-754-8900429
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Abstract

AIM: To investigate clinical and pathologic data of esophageal carcinoma (EC) and cardiac carcinoma (CC) among residents in Chaoshan region of China.

METHODS: Clinical and pathologic data of 9 650 patients with EC and 4 173 patients with CC in the Chaoshan population were collected and analyzed. Moreover, Chaoshan esophageal carcinoma tissue arrays were made for high-throughput study.

RESULTS: Male to female ratio was 3:1 in patients with EC and 4.75:1 in CC. The average age of the occurrence of EC was 54.6 years, and of CC was 58.1 years. For both EC and CC, age at diagnosis was a little younger in Chaoshan region than in most other areas. The most commonly affected site of esophageal carcinoma was the middle third of esophagus (72.0%); the second was the lower third (15.3%). The main gross type of esophageal carcinoma was ulcerative type (41.50%); the medullary type was the second (39.6%). Squamous cell carcinoma accounted for the overwhelming majority of esophageal cancer (96.4%); adenocarcinoma accounted for the overwhelming majority of cardiac carcinoma (94.5%). Chaoshan esophageal carcinoma tissue arrays were easily for high-throughput study, and tissue cores with a diameter of 1.5 mm could better keep more structure for molecular expression study.

CONCLUSION: Both EC and CC are common in males. The average occurrence age of EC and CC is younger in Chaoshan than in most other regions of China. The most commonly affected site of esophageal carcinoma was the middle third of esophagus (72.0%). Squamous cell carcinoma accounted for the overwhelming majority of esophageal cancer; adenocarcinoma accounted for the overwhelming majority of cardiac carcinoma. Tissue arrays technology is applicable for rapid molecular profiling of large numbers of cancers in a single experiment.

INTRODUCTION

Esophageal cancer (EC) ranks among the 10 most common cancers in the world, and is almost uniformly fatal. Chaoshan area is a unique littoral high-risk area of EC in China, within which Nanao island has the highest risk, the second being Jieyang county. According to the report from the Department of Public Health, Guangdong Province in 1993, the mortalities of EC in Nanao island were: 108.68±7.88/100 000 in standardized Chinese population, 145.44±10.49/100 000 in standardized world population, 261.16±25.01/100 000 in standardized world population between the age of 35-64. The annual average incidence rates in males and females were 132.19/100 000 and 69.20/100 000 in Nanao island from 1987 to 1992[1].

The predominant inhabitants of Chaoshan are offsprings of immigrants who hundreds or thousands of years ago came from the Central Plains of China, now a world well-known high risk region for EC. Chaoshan residents who have a high risk of EC and cardiac carcinoma (CC) are a relatively isolated population who have kept the old Chinese language (Chaoshan dialect) and customs. It is important to see if there is any evidence for the reducing incidence and mortality of EC and CC in Nanao island so far[2] as the incidences of EC and CC present a downward trend in most other high risk regions. This unique society provides us an unparalleled base for the genetic and also environmental study of esophageal carcinoma. In the current study, we explored the clinical and pathologic features of EC and CC.

In addition, scholars have discovered that many genes and signaling pathways are involved in EC and CC development[3-7]. However, genetic tumor markers have not gained in EC and CC diagnostics and prognosis prediction. Identification and evaluation of new molecular parameters are of utmost importance in cancer research. Here we present a high-throughput approach to rapidly identify relevant molecular expression changes in Chaoshan EC tissue arrays.

MATERIALS AND METHODS

Clinical data

Data about age, gender, and X-ray or pathological diagnoses of 13 823 patients with carcinoma of esophagus (9 650 cases) or

cardia (4 173 cases) were collected from the Tumor Hospital (1978-1998), First Affiliated Hospital (1989-1998), and Second Affiliated Hospital (1983-1998) of Shantou University Medical College, the Central Hospital of Shantou and the Hospital of Jieyang.

Sample collecting and EC tissues arrays construction

Seventy esophageal squamous carcinoma tissue specimens were selected from Department of Pathology, Shantou University Medical College in 2002. The specimens were fixed in 40 g/L neutrally buffered formaldehyde, embedded in paraffin. Sections of 5 µm stained with hematoxylin and eosin were obtained to confirm the diagnosis and to identify different viable, representative areas of the specimen. From these defined areas core biopsies were taken with a precision instrument. Sixty-eight EC and para-cancerous tissue cores with a diameter of 1.5 mm from each specimen were punched and arrayed in 8×9 on a recipient paraffin block. Five-µm sections of the EC tissue array block was cut and placed on adhesive coated slides (cooperated with Cybrdi, Dr. Li Jun).

Immunohistochemistry

The expression of Erk1/Erk2 MAPK signaling protein was analyzed using Erk1/Erk2 Mouse derived anti-activated MAP kinase monoclonal antibody (1:400, Sigma), Histostain™-SP kit and DAB visualization methods according to the manufacture's instruction (China Beijing Zhongshan Biological Technology CO., LTD.) And the expression of epidermal growth factor receptors were primarily analyzed using phosphor-EGFR (Try845) rabbit polyclonal antibodies and HRP-linked anti-rabbit IgG (Cell Signaling Technology, Inc. #2231, #7074). Four conventional normal esophageal epithelium tissues from autopsy were used as normal tissue controls. The human breast cancer tissue was used as positive control. Negative control was designed using phosphate-buffered saline (PBS) instead of primary antiserum. The detailed immunohistochemical process was carried out according to the manufacture's instructions.

Assessment of staining

The positive immunohistochemical staining of Erk1/Erk2 proteins was shown as brown signals in the nuclei; and the immunohistochemical signals of phosphor-EGFR (Tyr845) was in membrane and cytoplasm. The percentage of positive stained cells was evaluated for each tissue sample by counting all cells at 5 high power fields of micrometric rule (5 mm×5 mm). The cases having positive cancer cells or epithelium accounting for more than 75% of all cancer cells or epithelium on the slide were defined as a score of ++++, 50-74% were defined as a score of +++, 25-49% were defined as a score of ++, 6-24% were defined as a score of +, 1-5% were defined as a score of ±, less than 1% were defined as a score of -.

Statistical analysis

Data were stored in a computer data base (FoxPro, version 2.5 b) and analyzed using a computer (Pentium 4) spread sheet (Microsoft Excel 97) and professional statistical computer software (SPSS, version 11.0 and SAS, version 6.08). $P<=0.05$ was taken as significant. Immunoreactivity was classified as continuous data (undetectable levels or 0% to homogeneous staining or 100%) for all markers.

RESULTS

Gender and age

Genders of the patients were recorded in 9 635 cases. The 8 665 EC cases had age records, the youngest and the oldest were 17 years and 91 years respectively, and 3714 CC cases had age records. The male to female ratio and average age of morbidity for EC and CC in Chaoshan region are shown in Table 1. Constituent ratio of EC, CC in every age group is shown in the histogram (Figure 1).
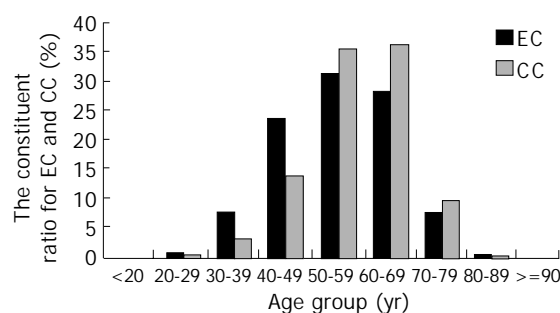
Figure 1 The histogram of the constituent ratio for EC and CC in Chaoshan region.
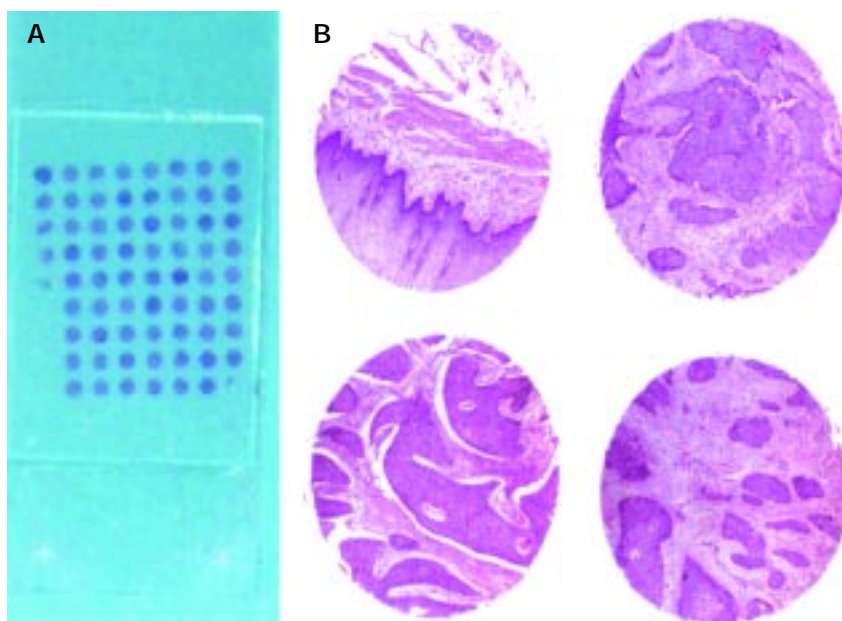
Figure 2 A: Overview of Chaoshan esophageal carcinoma tissue array (tissue cores with a diameter of 1.5 mm with 68 samples); B: Esophageal para-cancerous tissue and squamous carcinoma tissue (HE staining, original magnification: ×40).

Table 1 Sex ratio, average age of morbidity for EC and CC in Chaoshan littoral region (mean±SD)

|	n	Sex (male:female)	Age(yr)	F	P
EC	9 635	7 228:2 407(3.0:1)	54.61±10.73	103.24	<0.001
CC	4 167	3 442:725(4.75:1)	58.14±9.45		

EC:CC=2.31:1.

Pathology

The overwhelming majority of ECs were squamous cell carcinoma (96.4%); whereas CCs were composed mainly of adenocarcinomas (94.5%), and squamous cell carcinoma ranked second (4.4%). The detailed information of pathology is shown in Table 2.

Table 2 Pathology for EC and CC in Chaoshan littoral region (%)

	Site			Gross type				Histological type			
	U	M	L	UT	MT	ST	FT	SCA	ACA	UCA	O
EC	12.7	72.0	15.3	41.5	39.6	9.6	9.4	96.4	2.7	0.6	0.3
CC								4.4	94.5	0.9	0.2

Site (EC, *n*=6 384); Gross type (EC, *n*=1 193); Histological type (EC, *n*=7 272; CC, *n*=3 086); U: Upper third; M: Middle third; L: Lower third; UT: Ulcerative type; MT: Medullary type; ST: Scirrhous type; FT: Fungating type; SCA: Squamous cell carcinoma; ACA: Adenocarcinoma; UCA: Undifferentiated carcinoma; O: others.
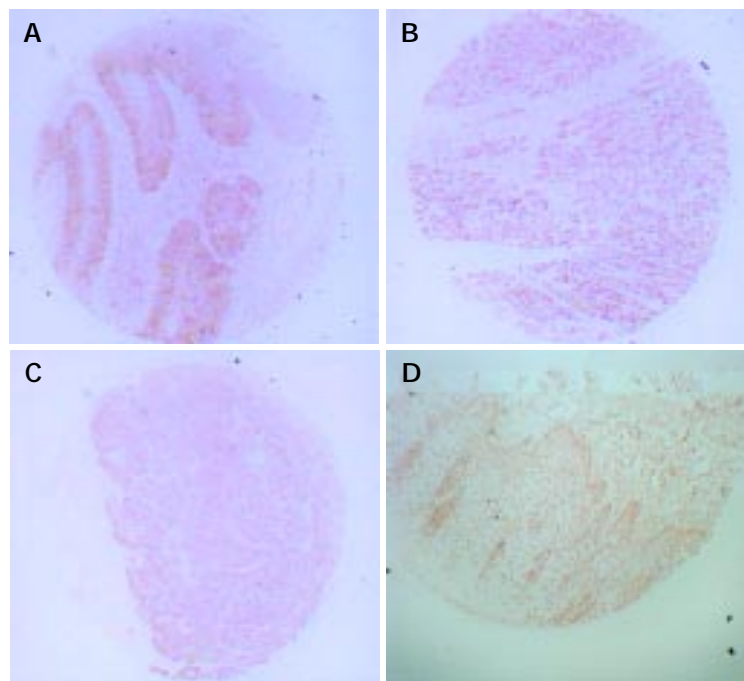
Figure 3 Phosphor-EGFR (Tyr845) expression on esophageal squamous carcinoma tissue (A, B, C) and esophageal para-cancerous tissue (D) (Cell Signaling Technology kit, original magnification: ×40).
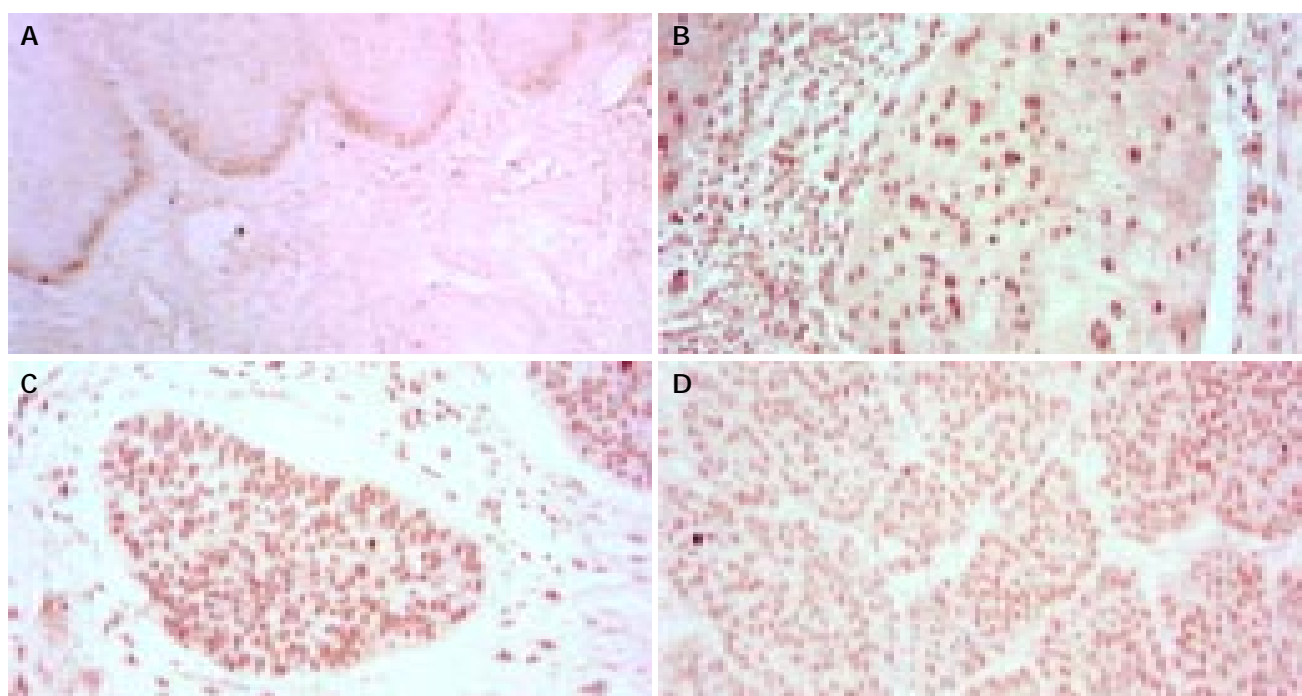
Figure 4 Erk1/Erk2 expression on normal esophageal squamous tissue (A, conventional tissue section, SP×100) and esophageal squamous carcinoma tissue (B, C, D tissue arrays) (SP×200).

Table 3 Expression of Erk1/Erk2 in esophageal squamous carcinoma tissue and para-cancerous tissue (%, mean±SD)

	Normal esophageal squamous cell (n=5)	Para-cancerous tissue (n=5)	Grade I (n=19)	Grade II (n=34)	Grade III (n=9)	F	P
Activated ERK1/ERK2	5.10±1.44	76.80±0.14	76.80±0.09	75.80±0.09	69.30±0.14	2.60	0.04

There was marked difference between normal esophageal epithelium and cancer tissues or para- cancerous tissue. But there was no marked difference among para–cancerous tissues and esophageal squamous carcinoma tissues (F=1.463, P=0.236).

Chaoshan EC tissues arrays and immunohistochemistry

Sections from tissue arrays were kept structure well for pathologic and immunohistochemical research (Figure 2). The expression of phosphor-EGFR(Tyr845) in esophageal squamous carcinoma tissue are relative diversity from ± to ++++, but no distinct differences among esophageal squamous carcinoma tissues according to grading (Figure 3). The expression of activated ERK in esophageal squamous carcinoma tissue and para-cancerous tissue are shown in Table 3 (Figure 4).

DISCUSSION

In data from Yangquan city of Shanxi Province (502 cases)[8], the median age of EC patients was 59.17 years, 4.56 years older than that of Chaoshan EC patients. And also data from Linxian of Henan Province (2 601 cases)[9], the proportion of EC patients from 20 to 30 years was 0.35%, from 30 to 40 was 5.19%; whereas both of the two proportions were lower than those from our data (0.70% and 7.70% respectively). In our data from Chaoshan region, the ulcerative type (41.5%) was the most common gross type of EC, which suggests that most EC patients were in the terminal stages when they arrived at hospitals.

Both preceding evidences indicated the average age of EC occurrence was younger in Chaoshan population than those in central plains of China. Synthetically analysis of two reports from Henan Province (1 045 cases and 1 332 cases respectively)[9,10] showed the proportion of CC patients less than 50 years old was 15.56%, which was slightly lower than that from Chaoshan CC data (17.60%). The similar phenomena were seen in other papers[11,12]. It might indicate that the age of incidence of cardiac cancer is also younger in Chaoshan region than in most other areas. Pathological analysis of 1 572 EC patients from Henan Province indicated histological type for the overwhelming majority of EC was squamous cell carcinoma (SCC), and the proportion of SCC (95.1%) was similar to that in our data (96.44%)[13]. In comparison of the constituent ratios of histological types for cardiac carcinoma with data respectively from the Chinese Academy of Medical Science[14], Erlangen - Nurmberg University in Germany[15] and our data, it homoplastically shows that adenocarcinoma accounts for the overwhelming majority of cardiac carcinoma, while the proportions of other histological types are relatively low.

Both data from Yangquan and Linxian[16] also showed the middle third of esophagus was the most commonly affected site analogously, followed by the lower third and then the upper third, similar to our results.

Tissue array technology is applicable for rapid molecular profiling of large numbers of cancers in a single experiment. But a possible limitation of the tissue array technology is that the minute tissue samples acquired from the original tissues may not always be representative of the entire tumor, in light of the intratumor heterogeneity characteristic to most cancers. The comparisons between similarly acquired specimens from different stages of tumor progression placed on the same tissue microarray should be less problematic. If tumor arrays are used to investigate prevalence or prognostic significance of molecular changes, the critical issue is the extent to which minute tissue samples are representative of their donor tumors. The findings of this study suggest that significant results

can be obtained on tumor arrays issue cores with a diameter of 1.5 mm. Well and truly, one should consider the tumor tissue array technology as a rapid, high-throughput survey method to pinpoint the biologically most prevalent or clinically most promising genes and molecular markers for detailed studies combined with conventional tissue specimens.

The epidermal growth factor (EGF) peptide induces cellular proliferation through the epidermal growth factor receptor (EGFR), a M_r 170 000 single-pass transmembrane tyrosine kinase, which is believed to play important roles in the control of cell growth and differentiation. The EGFR activates ras and the MAP kinase pathway, ultimately causing phosphorylation of transcription factors such as c-Fos to create AP-1 and ELK-1 that contribute to proliferation. Gene amplification and overexpression of EGFR have been reported in various human tumors, including head and neck/oral cancer[17-22].

Mitogen-activated protein kinase (MAPK) cascades have been shown to play a key role in transduction extracellular signals to cellular responses. Extracellular signal-regulated kinase (ERK) has been the best characterized MAPK and the Raf-MEK-ERK pathway represents one of the best characterized MAPK signaling pathway. The activated ERKs translocate to the nucleus and transactivate transcription factors, changing gene expression to promote growth, differentiation or mitosis[23].

Our primary immunohistochemical study showed that phosphor-EGFR (Tyr845) and activated ERK were expressed in both para- cancerous and esophageal cancerous cells. The intensity of the expression was much higher in cancer than in normal tissue, suggesting that EGFR, Erk1/Erk2 MAPK signaling pathway might play an important role in the regulation of proliferation of esophageal cancer[24,25].

In summary, Both EC and CC are common in males. The average age of occurrence is younger in Chaoshan than in most other regions of China. It is suggested that either genetic factors might play an important role in the pathogenesis of esophageal and cardiac cancers in Chaoshan or Chaoshan residents exposed themselves to some high risk environmental factors. Tissue microarrays technology is applicable for rapid molecular profiling of many tissue samples in a single experiment.

ACKNOWLEDGMENTS

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