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

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 9650 patients with EC and 4173 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 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; 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.

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 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[2,3]. 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...
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 database (FoxPro, version 2.5 b) and analyzed using a computer (Pentium 4) spreadsheet (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 9635 cases. The 8665 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).

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 manufacturer'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 antisemur. The detailed immunohistochemical process was carried out according to the manufacturer'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 (Try845) 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 -.

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

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Sex (male:female)</th>
<th>Age(yr)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>9 635</td>
<td>7 228:2 407(3.0:1)</td>
<td>54.61±10.73</td>
<td>103.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CC</td>
<td>4 167</td>
<td>3 442:725(4.75:1)</td>
<td>58.14±9.45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 (%)

<table>
<thead>
<tr>
<th>Site</th>
<th>Gross type</th>
<th>Histological type</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>M</td>
<td>L</td>
</tr>
<tr>
<td>EC</td>
<td>12.7</td>
<td>72.0</td>
</tr>
<tr>
<td>CC</td>
<td>4.4</td>
<td>94.5</td>
</tr>
</tbody>
</table>

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: Mediullary 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)

<table>
<thead>
<tr>
<th>Normal esophageal squamous cell (n=5)</th>
<th>Para-cancerous tissue (n=5)</th>
<th>Grade I (n=19)</th>
<th>Grade II (n=34)</th>
<th>Grade III (n=9)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated ERK1/ERK2</td>
<td>5.10±1.44</td>
<td>76.80±0.14</td>
<td>76.80±0.09</td>
<td>75.80±0.09</td>
<td>69.30±0.14</td>
<td>2.60</td>
</tr>
</tbody>
</table>

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)[9], 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 (2601 cases), 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 (1045 cases) and our data, it homoplastically indicated the average age of occurrence is younger in Chaoshan than in most other regions of China. 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 1572 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%)[12]. In comparison of the constituent ratios of histological types for cardiac carcinoma with data respectively from the Chinese Academy of Medical Science[14], Erlangen-Nurnberg 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 tissue 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, 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[11-22].

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 pathways. The activated ERKs translocate to the nucleus and transactivate transcription factors, changing gene expression to promote growth, differentiation or mitosis[23].

In summary, both EC and CC patients in the central plains of China have much younger average age of occurrence than those in other regions of China. 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 both genetic factors might play an important role in the pathogenesis of esophageal and cardiac cancers in Chaoshan. The authors acknowledge the participation of the following members of the undergraduate scientific research group in Shantou University Medical College of China led by Professor Min Su: SM Ying, Y Ni, YS Gao, J Lin, JK Sun, BC Yuan, YF Li, XL Chen, JS X, YF Chen, who helped us collect the case data of esophageal carcinoma and cardiac cancer. We express our special thanks to Professor Bruce AJ Ponder, Hutchison MRC Research Centre, MRC Cancer Cell Unit and University of Cambridge, for his invaluable suggestions, Dr. John KL, for his kind assistance in the preparation of the grammatical structure of this paper.
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