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Long telomere length predicts poor clinical outcome in esophageal cancer patients

Yanyan Lv[a,b], Yong Zhang[c], Xinru Li[a], Xiaojuan Ren[a], Meichen Wang[a], Sijia Tian[a], Peng Hou[a,d], Bingyin Shi[a,d], Qi Yang[a,d,*]

[a] Department of Endocrinology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, People's Republic of China
[b] Department of Rheumatology, Xi'an No. 5 Hospital, Xi'an 710082, People's Republic of China
[c] Department of Thoracic Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, People's Republic of China
[d] Key Laboratory for Tumor Precision Medicine of Shaanxi Province, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, People's Republic of China

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ABSTRACT

Background: Abnormal telomere length is widely reported in various human cancers, and it is considered to be an important hallmark of cancer. However, there is remarkably little consensus on the value of telomere length in the prognostic evaluation of esophageal cancers. Here, we attempted to determine the association of variable telomere length with clinical outcome of esophageal cancer patients.
Materials and methods: Using real-time quantitative PCR, we examined relative telomere lengths (RTL) in a cohort of esophageal cancer and normal esophageal tissues, and statistically investigated the association between RTL and clinical outcomes of esophageal cancer patients.
Results: The majority of esophageal cancers in this study had longer RTLs as compared to adjacent non-tumor tissues. Enhanced tumor RTL was associated with smoking habit, poor differentiation, advanced tumor stage, lymph node metastasis and cancer related death. In particular, a close relationship between longer RTL and poor survival was fully demonstrated by using cox regression and Kaplan-Maier survival curves.
Conclusions: We found frequent telomere elongation in esophageal cancer tissues, and demonstrated longer RTL may be an independent poor prognostic factor for esophageal cancer patients.

1. Introduction

Esophageal cancer leads the eighth most common cancer and the sixth cancer related death worldwide [1]. Squamous cell carcinoma (ESCC) constitutes the major (90%) classification of esophageal cancers and prevails in developing countries [2,3]. Esophageal cancers can be successfully treated if diagnosed early [4]. However, over decades, the tumors are still usually diagnosed at an advanced stage and the prognosis is poor [5,6], reflecting limited advances in our understanding of the pathogenesis of this cancer. Thus, there is pressing need to find the biomarkers for diagnosis, prevention, treatment and/or prognosis evaluation of esophageal cancer.

Telomere is a special nucleotide structure that caps the distal ends of chromosomes in eukaryote cells and consists of tandem TTAGGG repeats [7], and plays critical roles in safe guarding chromosome integrity and genomic stability through prevention of DNA decay and chromosomal ends fusion [8,9]. Usually, several factors such as ageing and oxidative stress can drive cells into senescence and apoptosis by inducing telomeric repeats erosion during mitosis [10,11]. On the other hand, there is a great deal of evidence to suggest that telomeres are continuously elongated by telomerase in cancer cells [12–14]. Increased telomerase activity is considered to be one of the hallmarks of human cancers including esophageal cancer, overcoming telomere shortening and contributing to unrestricted growth of tumor cells [14]. This abnormality is even identified during early epithelial carcinogenesis and may be an initiating event in many human epithelial cancers [15].

In recent years, a number of studies attempted to explore the relationship between telomere length and cancer prognosis. For

* Corresponding author at: Department of Endocrinology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710061, People's Republic of China.
E-mail addresses: lyywjplxj@163.com (Y. Lv), zhangyongyang05@163.com (Y. Zhang), 245228632@qq.com (X. Li), xiaojuanren0803@163.com (X. Ren), wmc_2010@stu.xjtu.edu.cn (M. Wang), tiansijia5020@163.com (S. Tian), phou@mail.xjtu.edu.cn (P. Hou), shibingy@126.com (B. Shi), yangqi2015@mail.xjtu.edu.cn (Q. Yang).

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example, a previous study showed that shortened telomere length was closely associated with poor outcome of non-small cell lung cancer [16]. However, meta analysis involving 956 colorectal cancers demonstrated poor prognosis was significantly correlated with long telomere instead of the shortened [17]. In breast cancer, telomere length was not found to be associated with clinicopathological characteristics or clinical outcomes [18]. In esophageal cancer (including Barrett's esophageal adenocarcinoma) and gastric cancer, shortened telomere and elevated telomerase activity were considered as tumorigenesis risks [19,20]. However, in an esophageal balloon cytology study among 89 asymptomatic cases of esophageal squamous dysplasia and 92 matched normal controls, it failed to reveal any difference of telomere length for precursory cancer lesions [21]. A recent study demonstrated short telomere only involves in chronic inflammation and intestinal metaplasia of gastric mucosa. No relationship was found between telomere length and clinicopathological characteristics and outcomes of gastric cancer [22].

Altogether, the prognostic implication of variable telomere length in esophageal cancer remains largely unknown. In this study, we attempted to investigate relative telomere length (RTL) in a cohort of the patients with well-characterized esophageal cancers, and explore its association with clinical outcomes of these patients.

2. Materials and methods

2.1. Patients and tissue samples

A total of 161 paraffin-embedded esophageal cancer tissues were obtained from the First Affiliated Hospital of Xi'an Jiaotong University between October 2003 and May 2008. Moreover, 38 paraffin-embedded cancer adjacent esophageal tissues were used as controls. None of these patients received any chemotherapy and radiotherapy before surgery, and informed consent was obtained from each patient before the surgery. All samples were histologically examined by a senior pathologist at the Department of Pathology of the hospital according to the World Health Organization (WHO) criteria for esophageal diseases. Clinicopathological characteristics of these patients were summarized in Table 1. This study was approved by our institutional review board and human ethics committee.

Table 1
Clinicopathological characteristics of esophageal cancer patients.

Characteristics	No. of patients(%)
Gender	
Male	117 (72.7)
Female	44 (27.3)
Age, years	
Mean	59.0
SD	8.55
Smoking	
BI = 0	83 (51.6)
0 < BI ≤ 300	22 (13.7)
300 < BI ≤ 600	37 (23.0)
BI > 600	19 (11.8)
Tumor localization	
esophagus upper	34 (21.1)
esophagus middle	94 (58.4)
esophagus lower	33 (20.5)
Differentiation	
well/moderate	127 (78.9)
poor/undifferentiation	34 (21.1)
Tumor stage	
I	45 (27.9)
II	80 (49.7)
III	36 (22.4)
IV	0 (0)
Lymph node metastasis	
Yes	66 (41.0)
No	95 (59.0)
Survival	
Dead	65 (40.4)
Alive	96 (59.6)

2.2. DNA extraction

Serial sections were cut from paraffin embedded tumor and control tissues. One of sections from each samples was stained using hematoxylin and eosin (H&E) staining, and a tumor representative tissue was marked by an expert surgical pathologist for esophageal cancer. Tumor tissues were then isolated by manual microdissection under an inverted light microscope according to the marked H&E section. After microdissection, tissues were deparaffinized in xylene at the room temperature for 12 h, and digested with 1% sodium dodecyl sulfate (SDS)/proteinase K at 48 °C for 48 to 72 h. Genomic DNA was subsequently extracted from these tissues by a standard phenol-chloroform extraction and ethanol precipitation protocol. DNA was stored at −80 °C until use.

2.3. Relative telomere length (RTL) measurement

Relative telomere length (RTL) was measured by using a quantitative PCR (qPCR) method as previously described [23–25]. Briefly, each sample underwent two separate qPCR reactions to determine the cycle threshold (Ct) values for the amplification of the telomere and single-copy control gene. The primer sequences were shown in Table 2. The reaction were carried out in a toal of 20 μL containing 1 × Kapa SYBR Fast qPCR Mix (Kapa biosystems, Woburn, MA), 900 nM of each forward and reverse primers and 8 ng genomic DNA. The amplification was run in CFX96^{TM} real-time PCR detection system (Bio-Rad Laboratories, Inc., CA) as follows: denaturation at 95 °C for 10 min; followed by 40 cycles of 95 °C for 15 s and 54 °C for 2 min to amplify telomere; or 32 cycles of 95 °C for 15 s and 62 °C for 30 s to amplify globulin gene. The standard curve was established using serial dilution of normal leukocyte DNA with a quantity range of 0.59–48 ng. The copy number ratio of the telomere repeat (T) and the single copy human globulin gene (S) was determined for each sample using this standard curve. The derived telomere/single-copy-gene (T/S) ratio was defined as RTL.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 16.0 package for Windows (SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov tests were used to examine the normality of distribution and data were expressed as mean ± standard deviation (SD). Non-normal distribution data were expressed as median (range). The Mann–Whitney *U* test was used to compare RTLs between esophageal cancer and adjacent normal esophageal tissues. Patient smoking habit was quantified using Brinkman Index (BI: the number of cigarettes smoked per day multiplied by the number of years of smoking). Multivariate models were developed to adjust for the most important covariates, including age, tumor size, differentiation and lymph node metastasis. Survival time was defined as the length of time from the day of primary tumor surgery to the day of death or the last clinical follow-up examination. Kaplan-Meier survival curves were used for prognosis evaluation. Differences between curves were analyzed using the log-rank test. Multivariate Cox regression analysis was used to evaluate the effect of RTL on survival, independent of the number of lymph node metastasis, tumor stage and differentiation. $P < 0.05$ was considered statistically significant.