Telomeric associations of chromosomes in patients with esophageal squamous cell carcinomas

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Abstract
AIM To investigate the role of telomeric association in the development of esophageal cancer.
METHODS Using chromosome R banding technique, telomeric association of chromosome in peripheral blood lymphocytes from 16 untreated patients with esophageal squamous cell carcinoma were observed and 16 healthy adults served as controls.
RESULTS The telomeric association frequencies of cell and chromosomes were significantly higher than those of controls ($\chi^2=9.56, P<0.01$), but its distribution on the chromosome showed no significant difference ($\chi^2=1.01, P>0.05$) between the two groups.
CONCLUSION Chromosomal instability can be initiated by telomeric associations, and sequential chromosome analysis can aid the understanding of the tumor occurrence and progression.

INTRODUCTION
Telomeres are genetic elements located at the end of all eukaryotic chromosomes and are essential for normal cell viability. One or their basic functions is to protect themselves. DNA at the terminal end of all chromosomes has a special structure to avoid binding to the end of DNA from other chromosomes, thus preventing end-to-end fusion or telomeric association (TAs). Telomeric DNA consists of terminal repeat arrays in the 3' strand (TTAGGG)$_n$ and has been isolated from telomeres in human. Recent studies have suggested that abnormal telomeric behavior plays a key role in cancer development. Fitzgeral[1] reported a case of B-cell leukemia and found chromosomal translocation resulting from TAs. The phenomenon has also been observed in tumor cells from a malignant histiocytoma[2] and a case of pre-T-cell acute lymphoblastic leukemia[3], three cases of cardiac myxoma[4], and two cases of renal tumor[5]. In the present report, we observed the telomeres in the peripheral blood lymphocytes from 16 untreated patients with esophageal cancer and 16 controls. So as to explore the relationship between abnormal telomeric behavior and chromosomal instability.

MATERIALS AND METHODS
Samples
Sixteen patients (12 men, 4 women) with esophageal squamous cell carcinoma admitted to the Institute of Oncology, Yanling County, Sichuan Province, aged 42 to 60 years were selected before the start of any kind of therapy. Sixteen healthy adults (12 men and 4 women) with no history of cancer, genetic diseases aged 35-65 years and radiation exposure served as controls.

Methods
Whole blood lymphocytes from all individuals were cultured in 199 medium (pH 7.5) containing 5% fetal calf serum, phytohemagglutinin and antibiotics. The cells were grown in the dark at 37°C for 96 hours. At 94 hours, colcemid was added to a final concentration of 0.1ng/L. After hypotonic treatment with KCl and fixation in methanol: acetic (3:1), the slides were prepared by air drying. R banding of chromosomes were obtained according to the technique of GAO Chung-
Sheng\(^{[7]}\) with slight modification. For each individual, 50 metaphases were studied under a same microscope to record the frequency of TAs and the distribution on chromosomes.

**RESULTS**

Data on the frequency of TAs per cell in esophageal cancer patients and normal control are shown in Table 1.

| Chromosomal groups | Patients | Controls |
|--------------------|----------|----------|
| No of TAs | Frequencies(%) | No of TAs | Frequencies(%) |
| A     | 4800 | 115 | 14.38 |
| B     | 3200 | 75 | 9.38 |

The patients had a TAs frequency of 14.38%, which was significantly higher than that observed in normal controls (9.38%) (\(\chi^2=9.56, P<0.01\)).

Comparison of distribution of TAs on chromosomes between the patients and controls is shown in Table 2. The frequency of TAs per chromosome was increased as compared with the controls (\(P<0.01\)). Statistical analysis showed that the distribution of TAs in two groups was nonrandom. In the patients, there was a high frequency in groups E and B, and a low frequency in groups D and G+Y (\(P<0.01\)).

Proportion analysis of telomeric associations indicated that TAs distribution on chromosome was not different between the two groups (\(P>0.05\)).

| Chromosomal groups | Patients | Control |
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**DISCUSSION**

Recently, the study on the relationship between abnormal telomeric behavior and mechanism of canceration has become a ‘hot spot’ in the field of molecular genetics. TAs of human chromosome is a rare phenomenon, which has been observed mostly in metephase cell of a pathologic nature. Since the 1990s, it has been found that telomeric lengths are evidently shortened in human colorectal carcinoma, Wilms’ tumor; giant cell tumor of bone, breast cancer, lung cancer, etc. while loss of telomeric sequences would lead to chromosomal instability. Because telomere integrity is critical for the normal replication of chromosomes in mitosis, telomeric reduction may lead to chromosomal dysfunction and manifest cyogenetically as TAs. Sawyer\(^{[8]}\) reported TAs evolving to ring chromosomes in a Pleomorphic xanthoastrocytoma. TAs between chromosomes 15pter and 20qter, and between chromosome 1q and 22qter, evolved in a stepwise fasion to ring chromosome 20 and 22. Thus, TAs is one of the mechanisms that can initiate chromosomal instability by generating subclones with unstable chromosome intermediates and result in ring chromosomes and subsequent chromosome loss. Adamson\(^{[9]}\) postulated that absent telomere sequence causes chromosome loss and instability, and that it may cause bridge break-fusion cycles, leading to partial chromosome deletion/duplication. In the early tumorgenesis, the telomere repeat sequence (TTAGGG)\(_n\) is often shortened in tumor cells, which may trigger the activation of telomerase to elongate telomere sequence. The shortened telomeric sequence was often shown to be TAs. TAs not only causes nondisjunction but also trigger further structural changes, which may contribute to the complexity of karyotypes of solid tumors and could be one of mechanisms of oncogene activation and/or tumor-suppressor gene disruption.

We had reported genetic instability in patients with esophageal cancer. However, it is necessary to prove whether there is a relationship between chromosomal instability and TAs. In our experiment cellular TAs rate was 14.38% in patients and 9.3% in controls, compared with the report in literature\(^{[10]}\), the frequency of TAs was decreased slightly, which may be the difference of susceptibility of tumor and chromosome banding techniques.

To our knowledge, this is the first report on TAs in esophageal cancer. It is not known whether the biologic changes resulting in TAs have any causal role in the carcinogenic process, or are of any importance in tumor cell progression. Molecular studies should increase our understanding of mechanisms involved in telomeric rearrangements and the relationship between chromosome change and pathogenesis and disease course in esophageal cancer.

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