論　文　内　容　要　旨

白血球由来テロメア G-Tail 長を指標とした新規食道がんバイオマーカーの開発

主指導教員：田原　栄俊教授
（医系科学研究科　細胞分子生物学）
副指導教員：高野　幹久教授
（医系科学研究科　医療薬剤学）
副指導教員：高橋　陵宇准教授
（医系科学研究科　細胞分子生物学）

韓　　佳延
（医歯薬保健学研究科　医歯薬学専攻）

Introduction

Esophageal cancer (ESC) is the eighth most common cancer worldwide and the sixth most common cause of cancer related deaths. Due to the lack of early clinical symptoms, ESC is commonly diagnosed at an advanced stage. This is one of the main reasons for the poor 5-year survival rates (approximately 10%) of patients with ESC. Patients with advanced-stage cancer are unable to undergo surgery, leading to a poor prognosis. Despite improvements in therapeutic approaches such as surgery, chemotherapy, radiotherapy, and chemoradiotherapy (CRT), the prognosis remains poor, with the 5-year post-esophagectomy survival rate ranging from 15–40%. The disadvantages of conventional methods for diagnosis are expensive and invasive to patients. Therefore, it is critical to develop the biomarkers for ESC that enable the rapid and non-invasive diagnosis at the early stage.

Telomeres, which contain tandem TTAGGG repeats, are nucleoprotein complexes located at the ends of eukaryotic chromosomes. Their main function is to maintain genomic stability. Telomeres form large loops (called telomeres or t-loops) by the invasion of the G-rich single-stranded overhang (G-tail) into double-stranded telomeric DNA to prevent the chromosome ends from being recognized as a DNA break. The telomeric G-tail is vital for the formation of the telomeric loop structure. Telomeric sequences are lost during successive cell divisions, and DNA damage accelerates telomere shortening, which eventually results in genomic destabilization. We previously established a high-sensitivity and high-throughput method, known as the G-tail telomere hybridization protection assay (Gt-telomere HPA), to measure telomeric G-tail length in various types of cells and clinical samples.

Using Gt-telomere HPA, we previously reported that G-tail length was shorter in breast cancer patients at early stage compared with cancer-free individuals, suggesting G-tail length is a promising blood-based biomarker for cancer diagnosis. Considering our previous findings, in the current study, we investigated the possibility whether the shortening of G-tail and total telomere is also correlated with status of ESC patients.

Materials & Methods

A total of 147 ESC patients aged 45-88 (median age 69.0 years, interquartile range (IQR) = 63.0 - 74.0 years; 122 men and 25 women) were registered at Hiroshima University Hospital between May 2018 and April 2022. To enroll control subjects, blood samples were obtained from volunteers. From these, we selected control subjects who were matched with our study participants for age and gender, and finally assessed 170 healthy participants aged 40-80 (median age 62.0 years, IQR = 58.0 - 68.3 years; 88 men and 82 women) as control subjects. This study approved by the Institutional Research and Ethics Committee at Hiroshima University Hospital, Hiroshima, Japan. All participants provided informed consent to participate. Peripheral blood samples (10 mL) were collected in each participant. Total telomere length was measured in genomic DNA of peripheral blood leukocytes by telomere hybridization protection assay (HPA) methods, and telomeric G-tail length was measured by a telomere G-tail HPA. The average coefficient of variances (CVs) in all samples was 5.7% for telomeric G-tail length and 4.9% for total telomere length, while the inter-assay CVs was 5.6% for telomeric G-tail length and 5.5% for total telomere length.

Results & Discussion

3.1. Telomeric G-tail length is Associated with Cancer Status

We compared LTL and G-tail lengths in controls and patients with ESC. Our data showed that the telomeric G-tail length in patients with ESC was shorter than that in the controls ($p = 0.02$), while there was no significant difference in LTL between controls and patients with ESC ($p = 0.07$).

To investigate the determinants of these results, we compared LTL and G-tail length in patients with ESC according to the disease stage. A significant difference was observed only in the G-tail length between controls and patients with stage II ESC ($p = 0.01$). In contrast, there was no significant difference in LTL between controls and patients with ESC at any stage.

3.2. Correlation between Aging and Telomeric G-tail Length

Next, we investigated the correlations between aging, LTL, and G-tail length. Our data showed that LTL was negatively correlated with age in both controls ($r = -0.312$, $p < 0.0001$) and patients with ESC ($r = -0.287$, $p = 0.0004$). G-tail length also correlated with age in controls ($r = -0.178$, $p = 0.020$) and patients with ESC ($r = -0.258$, $p = 0.002$). The trend of LTL shortening with age among patients with ESC was similar to that among the controls. However, we observed that the trend of G-tail length shortening among patients with ESC over 50 years of age was more rapid than that among the controls, suggesting that G-tail shortening was mainly caused by ESC incidence.

3.3 Telomeric G-tail Length Shortening Correlates with Recurrence

Next, we compared LTL and G-tail length in controls and preoperative patients with recurrent or non-recurrent ESC following treatment to explore the association between G-tail length and the possibility of recurrence. Our data revealed that there were significant differences in G-tail length between controls and preoperative patients with recurrent ESC ($p = 0.002$), and between preoperative patients with recurrent ESC and those without recurrent ($p = 0.02$), while no significant difference was observed in LTL in the same compared groups ($p = 0.30$; $p = 0.99$).

Conclusion

In this study, we found a significant reduction of telomeric G-tail length in ESC patients compared to healthy participants using 147 ESC patients and 170 age-matched healthy participants. Our study also revealed that Shorter G-tail length was associated with an increased risk of recurrence of ESC. Therefore, these results provide the evidence that G-tail length reflects the physiological status of ESC patients.

Our current study suggests that the evaluation of G-tail length is a promising approach for diagnosis of ESC. As circulating tumor cells, DNAs, and RNAs are considered to reflect the status of cancer patients, a combination of G-tail evaluation and these circulating tumor cells might lead to the development of more accurate and sensitive markers.