Human Pathology (2008) **39**, 1647–1655

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Luminal and cancer cells in the breast show more rapid telomere shortening than myoepithelial cells and fibroblasts☆

Rie Kurabayashi MD[a,b,*], Kaiyo Takubo MD[b], Junko Aida DDS, PhD[b], Naoko Honma MD[b], Steven S.S. Poon PhD[c], Makoto Kammori MD[b,d], Naotaka Izumiyama-Shimomura PhD[b], Ken-ichi Nakamura PhD[b], Ei-ichi Tsuji MD[a], Masaaki Matsuura PhD[e], Toshihisa Ogawa MD[a], Michio Kaminishi MD[a]

[a]*Division of Metabolic Care and Endocrine Surgery, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan*
[b]*Research Team for Geriatric Diseases, Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0015, Japan*
[c]*Terry Fox Laboratory, BC Cancer Research Center, Vancouver, BC, Canada V5Z 1L3*
[d]*Department of Surgery, Nihon University School of Medicine, Tokyo 173-8610, Japan*
[e]*Department of Cancer Genomics, The Cancer Institute, The Japanese Foundation for Cancer Research, Tokyo 135-8550, Japan*

Received 24 January 2008; revised 3 April 2008; accepted 4 April 2008

Keywords:
Tissue Q-FISH;
Telomere;
Breast;
Fibroblast;
Centromere

Summary Critically shortened, dysfunctional telomeres may play a role in the genetic instabilities commonly found in cancer. We analyzed 30 surgical specimens of invasive breast carcinoma from women aged 34 to 91 years and estimated telomere lengths as telomere-to-centromere ratio values in the 5 different cell types comprising breast tissue in order to clarify telomere length variations within and between individuals using our tissue quantitative fluorescence in situ hybridization method. We obtained 3 novel findings. (1) In corresponding normal tissues, telomere length decreased in the order myoepithelial cells > normal-appearing fibroblasts > luminal epithelial cells, and telomere lengths were characteristic in these 3 cell types within each individual. (2) As expected, cancer cells had significantly shorter telomeres than myoepithelial cells ($P < .0001$) and normal-appearing fibroblasts ($P = .0161$), but there was no significant difference in telomere length between luminal cells and cancer cells ($P = .6270$). (3) Fibroblasts adjacent to cancer had longer telomeres than normal-appearing fibroblasts distant from cancer ($P < .0001$). This study, which represents the first reported assessment of telomere length variations in the 5 cell types comprising breast tissue within and between individuals, revealed that normal luminal epithelial cells and cancer cells had the shortest telomeres. Our new findings indicate that telomeres of background luminal cells are as short as those

☆ This work was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (nos. 18659364, 18591432, 17390108, 18659104).

* Corresponding author. Division of Metabolic Care and Endocrine Surgery, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan.
E-mail address: kurabar-tky@umin.ac.jp (R. Kurabayashi).

doi:10.1016/j.humpath.2008.04.005

of cancer cells. Tissue quantitative fluorescence in situ hybridization, applicable to analysis of individual cells in tissue sections, is considered to be a powerful technique with considerable promise for studies in oncology.

1. Introduction

Telomeres in humans, located at the ends of chromosomes, are composed of a tandemly repeated DNA sequence and specific binding proteins [1]. These structures protect chromosome ends and prevent them from being recognized as DNA double-strand breaks [2]. The telomere repeat sequences of all cells can be shortened by each cell division, DNA damage due to oxidative stress, or changes in telomere-associated proteins [3,4]. Conversely, telomeres may be elongated through the activity of the telomeric DNA-synthesizing enzyme telomerase, which is expressed at high levels in germline cells and stem cells and also in immortal cells such as cancer cells [5]. Critically shortened, dysfunctional telomeres can result in chromosome fusion and breakage and may play a role in the genetic instabilities commonly found in cancer [6,7]. Cancers and their precursor lesions have been reported to show telomere shortening [8-12].

Prior studies of telomere length in breast cancers using Southern blotting or slot blot analysis have revealed telomere shortening in breast cancer tissues, but the data regarding correlation with clinical factors such as histologic grade and prognosis have not always been concordant [13-15]. Although Odagiri et al [13] reported that grade 3 tumors had shorter telomeres and that telomere length was not correlated with prognosis, Rogalla et al [14] found no correlation between telomere length and histologic grade. On the other hand, Fordyce et al reported that telomere length was correlated with both stage and prognosis [15]. These disagreements may be partly explained by the limitations of Southern blotting and slot blot analysis, which are unable to estimate the telomere lengths of individual cells and provide only information on telomere lengths for a mixed cell population including inflammatory cells and other stromal cells.

Recently, quantitative fluorescence in situ hybridization (Q-FISH) using tissue sections from paraffin-embedded blocks has allowed the estimation of telomere length of individual cells in each slice [11,12]. Two previous studies of breast tissue using tissue Q-FISH have reported telomere shortening in invasive ductal carcinomas, ductal carcinoma in situ, and normal luminal epithelial cells [16,17]. In these studies, only a small numbers of cells were analyzed (10-20 cells [16] or a minimum of 20 cells [17]), and there was no quantitative comparison of telomere lengths among various cell types within individuals. Furthermore, although stromal cells are sometimes used as an internal control in tissue Q-FISH [11], the definition of "stromal cells" is ambiguous, and few studies have investigated telomere length in stromal cells per se. The fibroblast is one of several component cell types in breast stroma.

In our method, the centromere signals act as an internal control to calibrate for telomere measurement in nuclei that are incomplete due to sectioning and to minimize hybridization- and preparation-dependent deviations. As a result, we obtained the telomere-to-centromere ratio (TCR) as an estimate of the telomere length in each cell. We have

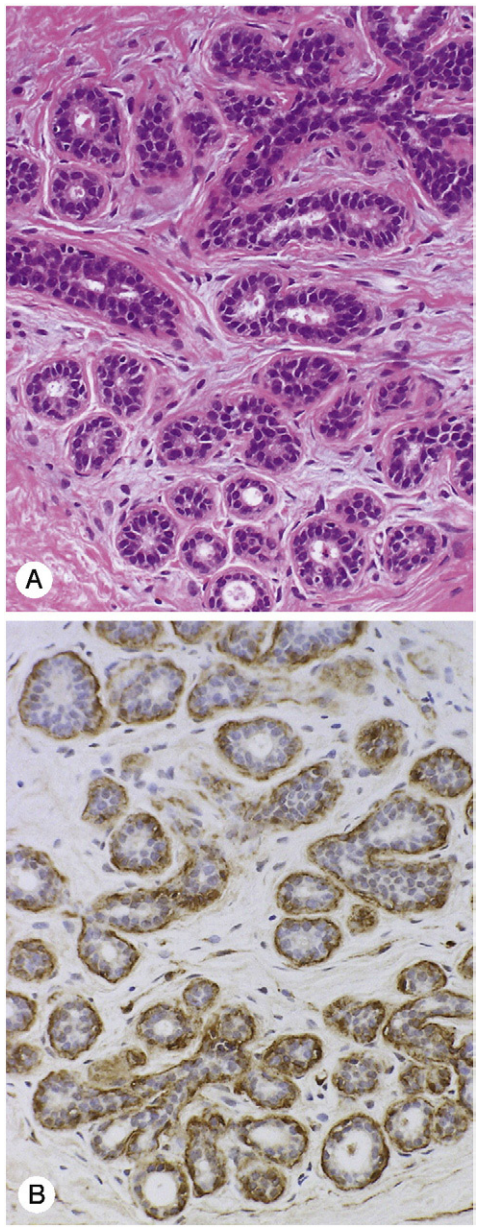

Fig. 1 Normal breast TDLU (original magnification ×200) A, Normal breast TDLU, hematoxylin-eosin, case 5. B, Immunohistochemical staining for smooth muscle actin, case 5. Outer myoepithelial cells are positive for smooth muscle actin.