Human Pathology (2014) **45**, 473–480

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Quantitative fluorescence in situ hybridization measurement of telomere length in skin with/without sun exposure or actinic keratosis☆,☆☆

Hiroyuki Ikeda MD[a,b], Junko Aida DDS, PhD[a,c,*], Atsushi Hatamochi MD[b], Yoichiro Hamasaki MD[b], Naotaka Izumiyama-Shimomura MD[a], Ken-ichi Nakamura PhD[a], Naoshi Ishikawa MD[a], Steven S. Poon PhD[a], Mutsunori Fujiwara MD[d], Ken-ichiro Tomita MT[d], Naoki Hiraishi MT[e], Mie Kuroiwa PhD[f], Masaaki Matsuura PhD[g], Yukihiro Sanada MD[h], Youichi Kawano MD[i], Tomio Arai MD[a,c], Kaiyo Takubo MD[a,c,*]

[a]*Research Team for Geriatric Pathology, Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0015, Japan*
[b]*Department of Dermatology, Dokkyo Medical University, School of Medicine, Mibu, Tochigi 321-0293, Japan*
[c]*Department of Pathology, Tokyo Metropolitan Geriatric Hospital, Tokyo 173-0015, Japan*
[d]*Department of Pathology and Laboratory Medicine, Japanese Red Cross Medical Center, Tokyo 150-8935, Japan*
[e]*Department of Laboratory Medicine, Hadano Red Cross Hospital, Hadano, Kanagawa 257-0017, Japan*
[f]*Department of Analytical Chemistry, Yokohama College of Pharmacy, Yokohama 245-0066, Japan*
[g]*Department of Cancer Genomics, The Cancer Institute, The Japanese Foundation for Cancer Research, Tokyo 135-8550, Japan*
[h]*Department of Transplant Surgery, Jichi Medical University, Yakushiji, Shimotsuke City, Tochigi 329-0498, Japan*
[i]*Department of Surgery, Nippon Medical School, Tokyo 113-8603, Japan*

Received 23 August 2013; revised 2 October 2013; accepted 4 October 2013

Keywords:
Actinic keratosis;
Telomere;
Aging;
Photoaging;
Epidermis;
Q-FISH;
Dermis

Summary Chromosomal and genomic instability due to telomere dysfunction is known to play an important role in carcinogenesis. To study telomere shortening in the epidermis surrounding actinic keratosis, we measured telomere lengths of basal, parabasal, and suprabasal cells in epidermis with actinic keratosis (actinic keratosis group, n = 18) and without actinic keratosis (sun-protected, n = 15, and sun-exposed, n = 13 groups) and in actinic keratosis itself as well as in dermal fibroblasts in the 3 groups, using quantitative fluorescence in situ hybridization. Among the 3 cell types, telomeres of basal cells were not always the longest, suggesting that tissue stem cells are not necessarily located among basal cells. Telomeres of basal cells in the sun-exposed group were shorter than those in the sun-protected group. Telomeres in the background of actinic keratosis and in actinic keratosis itself and those of fibroblasts in actinic keratosis were significantly shorter than those in the controls. Our findings

☆ Funding: This study was supported by JSPS KAKENHI 21390109, 22590327, 22590343, 23659193, 23590440, 24590468, and 25460447.
☆☆ The present study was performed by the Research Team for Geriatric Pathology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan.
* Corresponding authors. Research Team for Geriatric Pathology, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi-ku, Tokyo 173-0015, Japan.
E-mail addresses: aidaj@tmig.or.jp (J. Aida), takubo@tmig.or.jp (K. Takubo).

http://dx.doi.org/10.1016/j.humpath.2013.10.009

demonstrate that sun exposure induces telomere shortening and that actinic keratosis arises from epidermis with shorter telomeres despite the absence of any histologic atypia.

1. Introduction

Actinic keratosis is a premalignant condition of the skin and carries up to a 20% risk of progression to squamous cell carcinoma [1]. Actinic keratosis is associated with frequent exposure to the sun and is usually accompanied by solar damage [2]. Cumulative and intermittent exposure to solar radiation is implicated in the development of actinic keratosis [3]. The lesion may appear on any sun-exposed area, such as the face, ears, lateral neck, balding scalp, backs of the hands, forearms, or lips and in middle-aged and elderly persons [4].

On the other hand, aging itself appears to play an important role in carcinogenesis generally, as elderly individuals tend to have a much higher incidence of carcinoma and also multiple carcinomas than the young, and carcinoma occurrence in the elderly is related to telomere dysfunction, epigenetic gene silencing, and other factors [5]. In the epidermis, aging or photoaging may be an important factor related to tumorigenesis [6]. Telomeres are repetitive G-rich DNA sequences and associated binding proteins found at the ends of linear eukaryotic chromosomes and appear to play a key role in preventing genomic instability [7,8]. Using Southern blotting, we have previously analyzed telomere lengths in almost all human organs and tissues including the epidermis and confirmed that telomeres shorten with age in the epidermis [9]. We have also reported that the estimated annual reduction rate of telomere length in the epidermis is 36 base pairs [10], and similarly, telomere shortening with age in the skin has been reported elsewhere [11]. The progression of telomere shortening with age could lead to genomic instability during the initial stage of tumorigenesis [5].

We have also confirmed the telomere length distributions of different cell types in many tissues using quantitative fluorescence in situ hybridization (Q-FISH) and our original software, Tissue Telo [9]. We demonstrated that squamous cell carcinomas in situ of the esophagus [12] and oral mucosa [13] arise from epithelium with short telomeres and chromosomal instability. On the other hand, orthokeratotic dysplasia, a type of oral leukoplakia, is a premalignant condition associated with short telomeres and also arises from oral epithelium with chromosomal instability [14]. In a survey of PubMed, as of August 1, 2013, we were unable to find any reported studies of actinic keratosis and telomeres of epidermis, although the relationship between telomere lengths of peripheral blood cells was positively associated or inversely associated with skin neoplasms [15,16].

In the present study, we postulated that actinic keratosis is also likely to occur in epidermis with excessively shortened telomeres, as we had demonstrated previously for the esophageal and lingual mucosae. Therefore, we measured and analyzed telomere lengths in histologically normal epidermis with or without actinic keratosis and in actinic keratosis itself, in comparison with controls.

2. Materials and methods

2.1. Subjects

For this study, we surveyed patient files at the Department of Dermatology, Dokkyo Medical University. We established 3 groups of patients: 15 consecutive patients (6 men and 9 women, aged 62-77 years; mean, 69.3 years) with benign tumors, including epidermal cyst (n = 12) and nevocytic nevus (n = 3) of the back (n = 11), chest (n = 2), abdomen (n = 1), and thigh (n = 1) (sun-protected control group); 13 consecutive patients (8 men and 5 women, aged 61-96 years; mean, 72.1 years) with benign tumors, including epidermal cyst (n = 7) and nevocytic nevus (n = 6) of the face (n = 10) and neck (n = 4) (sun-exposed control group); and 18 consecutive patients (7 men and 11 women, aged 54-94 years; mean, 78.0 years) with actinic keratosis of the face (n = 17) and back of the hand (n = 1) (actinic keratosis group), dating from 2011.

Concerning the subjects' occupations, the sun-protected group (n = 15) included 11 subjects (73.3%) who had worked indoors, for example, office workers, and 4 subjects (26.7%) who had worked outdoors, for example, farmers or drivers. The sun-exposed group (n = 13) included 9 subjects (69.2%) who had worked indoors and 4 (30.8%) who had worked outdoors. The actinic keratosis group (n = 18) included 3 subjects (16.7%) who had worked indoors and 15 (83.3%) who had worked outdoors.

Samples of uninvolved epidermis with epidermal cyst and nevocytic nevus were defined as controls. Samples of uninvolved epidermis with actinic keratosis were defined as the background of actinic keratosis. We measured telomere lengths in the epidermis uninvolved with epidermal cyst, nevocytic nevus, or actinic keratosis and that in actinic keratosis itself. We also measured telomere lengths of fibroblasts under the epidermis without atypia.

We used archival paraffin blocks from these 46 cases (28 controls and 18 cases of actinic keratosis). In our pathology laboratories, all specimens removed at surgery were fixed for 5 hours in 10% buffered formalin. Both sets of samples were then subjected to standard tissue processing and paraffin embedding. From the archival blocks, tissues were sliced