ELSEVIER

Urologic Oncology: Seminars and Original Investigations 32 (2014) 135–145

UROLOGIC ONCOLOGY

Original article

Short telomeres and chromosome instability prior to histologic malignant progression and cytogenetic aneuploidy in papillary urothelial neoplasms

Naotaka Izumiyama-Shimomura, M.D.[a], Ken-ichi Nakamura, Ph.D.[a], Junko Aida, D.D.S., Ph.D.[a,i], Naoshi Ishikawa, M.D.[a], Mie Kuroiwa, M.D.[b], Naoki Hiraishi[c], Mutsunori Fujiwara, M.D.[d], Yuichi Ishikawa, M.D.[e], Naoko Inoshita, M.D.[e], Junji Yonese, M.D.[f], Masaaki Matsuura, Ph.D.[g], Steven S.S. Poon, Ph.D.[h], Tomio Arai, M.D.[i], Kaiyo Takubo, M.D.[a,i,*]

[a] *Research Team for Geriatric Pathology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*
[b] *Department of Pathophysiology, Yokohama College of Pharmacy, Yokohama, Japan*
[c] *Department of Laboratory Medicine, Hadano Red Cross Hospital, Hadano, Kanagawa-ken, Japan*
[d] *Department of Pathology and Laboratory Medicine, Japanese Red Cross Medical Center, Tokyo, Japan*
[e] *Division of Pathology, The Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan*
[f] *Department of Urology, The Cancer Institute Hospital, Tokyo, Japan*
[g] *Bioinformatics Group, Genome Center and Department of Cancer Genomics, The Cancer Institute, The Japanese Foundation for Cancer Research, Tokyo, Japan*
[h] *Terry Fox Laboratory, British Columbia Cancer Research Centre, Vancouver, BC, Canada*
[i] *Department of Pathology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan*

Received 1 November 2012; received in revised form 11 December 2012; accepted 31 December 2012

Abstract

Purpose: Evaluation of the relationships existing among 3 histologic types of urothelial tumors, chromosomal instability, and telomere length.

Patients and methods: We examined 37 consecutive cases of papillary urothelial neoplasm, from which 26 (70.3%) were suitable for karyotype analysis, comprising 7 papillary urothelial neoplasms of low malignant potential (PUNLMPs), 10 low-grade papillary urothelial carcinomas (PUCs), and 9 high-grade PUCs. We performed karyotype and anaphase bridge analyses, and measured telomere lengths by quantitative fluorescence in situ hybridization.

Results: PUNLMPs were always diploid and had anaphase bridges. Low-grade PUCs showed diploidy ($n = 2$), hypoploidy ($n = 4$) and polyploidy ($n = 4$), and high-grade PUCs showed diploidy ($n = 1$) and polyploidy ($n = 8$); both had anaphase bridges. The incidence of anaphase bridges did not differ significantly between PUNLMPs and high-grade PUCs ($P = 0.105$). The telomere lengths of PUNLMP, low-grade PUC, and high-grade PUC, expressed as mean telomere fluorescence units (TFU) ± SD, were 7906 ± 3197, 4893 ± 1567, and 3299 ± 1406, respectively. The differences among the 3 groups were significant. However, 42.9% of the PUNLMPs had shorter telomeres than the mean value for low-grade PUCs, and 30.0% of the low-grade PUCs had shorter telomeres than those for high-grade PUCs. There was an inverse correlation between telomere length and the incidence of anaphase bridges.

Conclusions: PUNLMP appears to progress to low-grade PUC and high-grade PUC in association with telomere shortening and chromosomal instability. Our data suggest that critically shortened telomeres cause chromosomal instability during progression of papillary urothelial neoplasms.

Keywords: Telomere; PUNLMP; Urothelial carcinoma; Q-FISH; Chromosomal instability; Anaphase bridge; Karyotype analysis

All of the present authors directly participated in planning, execution, and analysis of the present study.

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (KT, Nos. B17390108, B21390109; KIN: No. C18590354).

* Corresponding author. Tel.: +81-3-3964-3241; fax: +81-3-3579-4776.
E-mail address: takubo@tmig.or.jp (K. Takubo).

1078-1439/$ – see front matter
http://dx.doi.org/10.1016/j.urolonc.2012.12.005

1. Introduction

There are 2 independent pathways for the development of urothelial carcinoma, although occasional overlap between them can occur [1–5]. One pathway is represented by low-grade papillary urothelial neoplasms, including papillary urothelial neoplasm of low malignant potential (PUNLMP) and low-grade papillary urothelial carcinoma (PUC), which is characterized by frequent local recurrence without distant metastasis, and which only occasionally progresses to high-grade PUC or urothelial carcinoma with invasion to the muscular layer [1,6]. PUNLMP has been defined as a papillary urothelial tumor that resembles exophytic urothelial papilloma, but shows increased cellular proliferation exceeding the thickness of the normal urothelium [7]. The second pathway is represented by carcinoma in situ and muscle-invasive urothelial carcinoma [2].

Telomeres play critical roles in the maintenance of chromosomal stability, as well as in limiting the ultimate replication capacity of cells [8], and it has been suggested that telomere shortening is an important biological factor associated with cell senescence [9], carcinogenesis, and aging [8–10]. McGrath et al. [11] considered that there was a need to investigate the associations between telomere length and histologic grade, but no such study has yet been reported. Therefore, to clarify the relationships existing among 3 different histologic types of bladder neoplasm, chromosomal instability, and telomere length, we performed karyotype and anaphase bridge analyses [12,13], and measured telomere lengths using quantitative fluorescence in situ hybridization (Q-FISH) [14,15].

2. Subjects and Methods

2.1. Subjects

The tumor samples studied were obtained, with informed consent, from 37 consecutive patients with papillary urothelial neoplasms of the urinary bladder by transurethral resection (TUR) performed by a single urologist (JY) at the Cancer Institute Hospital, Tokyo. These neoplasms were histologically divided into 3 groups—PUNLMP, low-grade PUCs, and high-grade PUCs—by 2 pathologists (YI, NI), based on the description by Epstein et al. [16]. From 26 (70.3%) of the 37 neoplasms, we were able to acquire metaphase spreads that were suitable for karyotype analysis. Among the 26 patients, 7 (4 men and 3 women, aged 53–82 y, mean 71.0 y) were diagnosed as having PUNLMP, 10 (10 men, aged 48–74 y, mean 64.0 y) as having low-grade PUC, and 9 (8 men and 1 woman, aged 50–79 y, mean 63.0 y) as having high-grade PUC. Approval for this study was obtained from the ethics committee of Tokyo Metropolitan Institute of Gerontology.

2.2. Estimation of chromosomal instability

2.2.1. The Q-band technique and chromosome number in FISH samples

Fresh tumor samples were examined as described elsewhere [17]. Chromosomes were analyzed using the Q-band technique. Chromosome identification and karyotype designations were performed in accordance with the International System for Human Cytogenetic Nomenclature [18]. Two of the authors (MF and KIN) tried to examine 10 metaphase spreads, if possible, for each case using the Q-band technique. However, in practice, the number of metaphase cells analyzed ranged from 2 to 10. For FISH samples, one of the authors (KIN) recorded the number of chromosomes in 1 metaphase spread. All of the metaphase spreads obtained for FISH samples were analyzed, and we were able to obtain 3 to 18 metaphase spreads for each of these cases (Table 1).

2.2.2. Anaphase bridge analysis

The presence of anaphase bridges as a possible morphologic indicator of chromosomal instability [12,13] was examined by 3 of the authors (KIN, NIS, and NI). The carcinoma cells in primary culture were fixed without colcemid treatment, and then cell smears were prepared and analyzed after staining with Giemsa for 30 seconds. At least 1000 carcinoma cells were evaluated to estimate the rate of occurrence of chromatin bridges in each case. An anaphase bridge was defined as a filamentous connection linking 2 well-separated nuclei [15].

2.3. Telomere measurements by Q-FISH

2.3.1. Probes

The metaphase chromosomes were hybridized using the peptide nucleic acid (PNA)-FISH preparation method [14,19]. A Cy3-labeled $(CCCTAA)_3$ peptide nucleic acid probe (catalog number F1002; Fasmac, Atsugi, Japan) was used to label the telomeres, and a FITC-labeled CTTCGTTGGAAACGGGGT peptide nucleic acid probe (a nonspecific centromere probe, custom-made; Fasmac) was used for the centromere. The chromosomes were counterstained with 4′, 6-diamidino-2-phenylindole(DAPI, Molecular Probes, Eugene, OR, USA).

2.3.2. Q-FISH and image analyses of telomeres

Q-FISH and image analyses were performed as described previously [14,15]. A total of 3 to 18 metaphase spreads for each of the 26 primary-cultured cells were analyzed. Telomere intensities of individual arms in the metaphase spreads were measured and expressed as telomere fluorescence units (TFUs). Digital images were recorded with a CCD camera, AxioCam MRm (Zeiss, Oberkochen, Germany) mounted on an Axio Imager MAT (Zeiss) epifluorescence microscope equipped with a triple band-pass filter for Cy3/FITC/DAPI (61010 Chroma