SPANDIDOS PUBLICATIONS

Specific subtelomere loss on chromosome der(11)t(3;11)(q23;q23)x2 in anaplastic thyroid cancer cell line OCUT-1

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Abstract. One of the most aggressive human malignancies, anaplastic thyroid carcinoma (ATC), has an extremely poor prognosis that may be explained by its genomic instability. We hypothesized that the very rapid cell turnover observed in ATC might accelerate telomere shortening and chromosomal instability associated with tumor cell malignancy. To compare and measure chromosomal aberrations and telomere shortening in the anaplastic thyroid cancer cell line OCUT-1, we applied quantitative fluorescence *in situ* hybridization (Q-FISH) techniques. In all 15 metaphases studied, telomere length estimates from Q-FISH of chromosomes in ATC were shorter than those of a fibroblast cell line derived from the stroma adjacent to the carcinoma. OCUT-1 cells display several chromosomal abnormalities, but have a near-normal chromosome complement of 46, XX, making it easy to analyze the karyotype. The karyotype showed 50, XX, +7, +11, der(11)t(3;11)(q23;q23)x2, del(12)(p11.2p12), +20, +1mar. We analyzed carefully the abnormalities in karyotype of OCUT-1 associated with telomere shortening on each chromosome and expression of subtelomeres. Telomere lengths in the q-arms of the abnormal chromosome del(12)(p11.2p12) were shorter than the average length in the q-arms of the normal chromosome 12 in OCUT-1. Subtelomeres on the abnormal chromosome der(11)t(3;11)(q23;q23)x2 also showed loss of signals on 11p, but there was no loss of signals in the cytogenetically normal trisomies 7 and 20 or the abnormal chromosome del(12)(p11.2p12). Subtelomeres of 3q had eight signals, one pair remaining in place on 3q and another pair on the abnormal 11p. Our findings suggest that telomere shortening and subtelomere loss are correlated with genetic instability in this anaplastic thyroid carcinoma cell line.

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Introduction

Telomeres are nucleoprotein complexes located at the ends of eukaryotic chromosomes, playing important roles in the maintenance of genomic integrity. Because conventional DNA replication cannot completely replicate the ends of linear chromosomes, normal somatic cells lose telomeric repeats with each cell division, both *in vivo* and *in vitro*. The enzyme telomerase, with associated proteins (1) and other telomerase-independent mechanisms (2,3), counteracts the progressive shortening of telomeres in highly proliferative cell types such as immortal cell lines, cancer cells, and stem cells. Although telomerase is also expressed in hematopoietic stem cells and lymphocytes, the level of telomerase activity in these cells appears to be insufficient to prevent gradual telomerase shortening with age (4-6).

Human telomeres play critical roles in the maintenance of chromosomal stability as well as in limiting the ultimate replication capacity of cells. Telomere shortening has been suggested as an important biological factor in carcinogenesis, cell senescence, cell replication, cell immortality, and aging (7-11). It is therefore important to clarify telomere metabolism in individual chromosomes using cancer cell lines.

In recent years, strategies have been developed to investigate the possible role of chromosomal subtelomere regions in genetic disorders. Familial subtelomeric abnormalities have been documented among individuals with developmental delay, idiopathic mental retardation, or non-specific congenital abnormalities (12). However, little is known about the association between subtelomeres and cancer. It is therefore

of interest to analyze the subtelomeres on chromosomes of cancer cell lines.

Differentiated thyroid carcinoma (DTC) is known to be one of the least aggressive cancers; the overall survival rate of patients 10 years after surgery is >90% (13,14). Even patients with recurrent disease can survive for some time after relapse. In contrast, anaplastic thyroid carcinoma (ATC) is one of the most aggressive human malignancies. Extensive combination therapy involving irradiation, chemotherapy, and surgery is usually attempted, but the prognosis remains extremely poor (13,15). A number of studies have described the characteristics of ATC and analyzed its biologic background in order to explain its extremely aggressive nature. Several differences between DTC and ATC have been clarified using molecular biological methods. We have established and characterized the ATC cell line OCUT-1 (16), derived from a surgical specimen obtained from a 74-year-old Japanese woman with advanced ATC. The cell line has already been maintained for >100 passages and stably cultured for more than a year. We have also established and characterized a fibroblast cell line derived from stroma adjacent to the carcinoma in the same patient. These cells have a normal 46, XX karyotype.

Using Southern blot analysis, we have determined that telomere shortening occurs after 50 years of age in normal thyroid tissue and that human thyroid tissue does not seem to show rapid reduction of telomere length in early life (17,18). On the other hand, it is well known that thyroid cancer can show a change in malignant potential in individuals >45 years old and an increase in anaplasia, changing from DTC to ATC, in individuals >50 years of age.

In this study, we compared our results for telomere length measurements of quantitative fluorescence *in situ* hybridization (Q-FISH) samples derived from our established OUCT-1 cell line with those from the fibroblast cell line derived from the same individual, and with the analysis of OCUT-1 cell subtelomeres by fluorescence *in situ* hybridization (FISH). We also studied the expression of telomerase activity in OCUT-1, both quantitatively and qualitatively. On the basis of our findings, we discuss the genomic instability of OCUT-1.

Materials and methods

Cell culture. The established human ATC cell line OCUT-1, and the cell line established from normal fibroblasts in the stroma adjacent to the original carcinoma from which OCUT-1 was derived, were obtained from Osaka City University. Cells were grown in 25-cm^2 plastic tissue-culture flasks (Falcon/Becton Dickinson Co., Franklin Lakes, NJ) in a humidified incubator (37˚C, 84% humidity, 5% CO_2/ambient air atmosphere) in DMEM supplemented with 5% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 mg/ml streptomycin. After centrifugation at 1,000 rpm for 3 min, the cell pellet was re-suspended in the medium and cultured at 37˚C in a 5% CO_2 atmosphere under humidified conditions. Cells were passaged weekly at 75% confluence using 0.25% trypsin/0.02% EDTA (Life Technologies, Inc., Gaithersburg, MD) for detachment, followed by inactivation with serum-containing medium (normal or charcoal-stripped as appropriate) and seeding in new flasks using a ratio of 1:10. All cell lines were tested using a polymerase chain reaction-based (PCR-based) mycoplasma detection kit obtained from the American Type Culture Collection (ATCC; catalog no. 90-1001K) and found to be free of mycoplasma contamination. The additional cell lines used were also obtained from the ATCC. Media and passaging procedures followed the accompanying ATCC recommendations. Cells were trypsinized, plated at near-confluent cell numbers, and incubated with colcemid for 12 h before cell harvest. The two cell types were cultured in the presence of phytohaemagglutinin for 3 days before addition of colcemid for 1 h. All cells were treated with hypotonic KCl buffer for 30-60 min at 37˚C after colcemid incubation. They were then fixed in methanol-acetic acid and dropped on clean slides and/or stored at -20˚C in fixative.

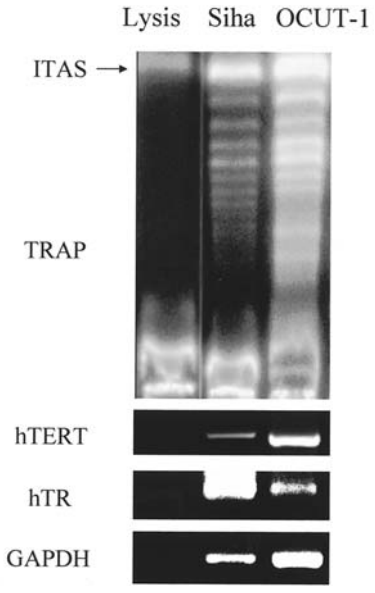

Figure 1. Telomerase activity detected by Telomeric repeat amplification protocol (TRAP) assay in the OCUT-1 cell line. The mRNAs of two catalytic subunits of telomerase, hTERT and hTR, are clearly detected on RT-PCR. An extract of Siha cells (Siha) was used as a positive control, and an internal telomerase assay standard (ITAS) was used as a positive control for PCR amplification, with lysis buffer as the negative control (Lysis).

Telomeric repeat amplification protocol (TRAP). We performed the telomeric repeat amplification protocol (TRAP) as described previously (19), with an additional 30 min of incubation at 37˚C. We used 5 μg of extract per assay. An internal telomerase assay standard (ITAS) fragment was included in the reaction as a control for PCR (20). The activity was sensitive to RNase.

Reverse-transcriptase PCR (RT-PCR). For reverse-transcriptase PCR (RT-PCR), total RNA was collected from samples using TRI-reagent (Life Technologies Inc.). Total RNA (1 μg) was reverse-transcribed in 20 μl of reaction buffer containing 1 μl (0.5 μg) of oligo dT primer, 4 μl of 5X RNA PCR buffer, 1 μl of 10 mmol/l dNTPs, 2 μl of 0.1 mol/l dithiothreitol (DTT), 0.5 μl of RNA Guard (Amersham Pharmacia Biotech, Buckinghamshire, UK), and 1 μl (20 units) of M-MLV reverse transcriptase (Life Technologies Co.) for 60 min at 37˚C. The cDNA samples were amplified in 2 μl of 10X PCR buffer (20 mmol/l Tris-HCl, pH 8.4; 100 mmol/l KCl;

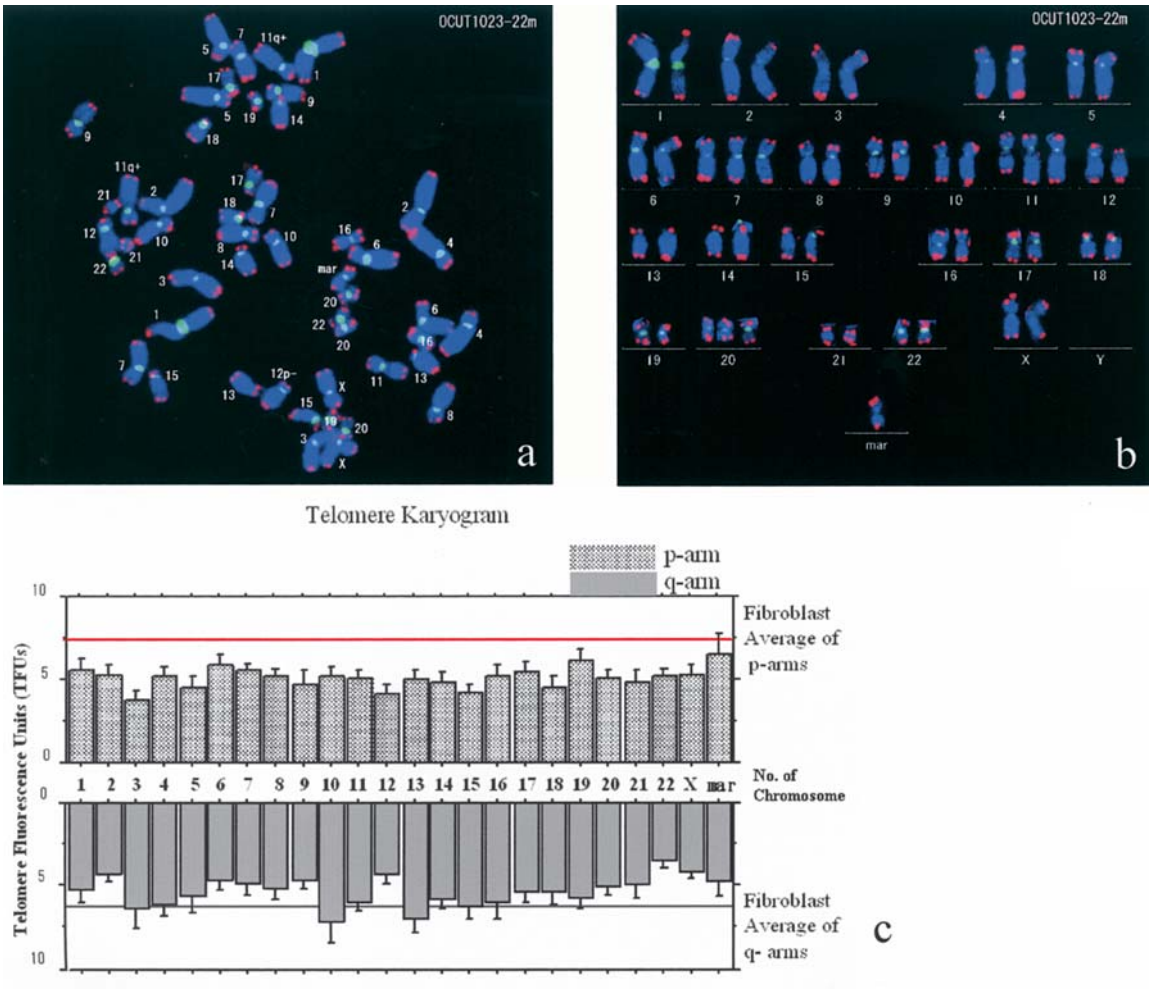

Figure 2. (a) OCUT-1 cells displayed several chromosomal abnormalities; the chromosomal count was 50 in all the 15 cell types analyzed. Digital images were acquired from DAPI-stained chromosomes, FITC-labeled centromeres, and Cy3-labeled telomeres. We combined the three images after assignment of pseudo-colors. (b) The karyotype of OCUT-1 cells was 50, XX, +7, +11, der(11)t(3;11)(q23;q23)x2, del(12)(p11.2p12), +20, +1mar. (c) The fluorescence intensity of the p- and q-arm telomeres on all chromosomes was expressed in TFUs. Most of the TFUs in the p- and q-arms on chromosomes in OCUT-1 were shorter than the average TFUs in a fibroblast cell line, except for the 3, 10, and 13 q-arms. However, there were no particularly shortened telomeres in the p- and q-arms on any chromosome in OCUT-1.

0.1 mmol/l EDTA; 0.5% Tween-20; 1 mmol/l DTT, 50% glycerol, and 15 mmol/l Mg^{2+}), 2 μl of 2.5 mmol/l dNTPs, 10 μl of ddH_2O, 1 μl of each primer set (100 μmol/l), and 0.3 μl of Taq polymerase (5 units/l; Gene Taq, Nippon Gene, Toyama, Japan). Primer sets and PCR conditions used are shown in Table I. Amplified products were electrophoresed on a 2% agarose gel (GAPDH, human telomerase RNA component, or hTERC) and a 6% polyacrylamide gel (human telomerase reverse transcriptase, or hTERT). Agarose gels were stained with ethidium bromide and analyzed by densitometry (Atto, Tokyo, Japan). Polyacrylamide gels were stained with Vistra Green (Amersham Pharmacia Biotech) and analyzed using a Fluor Imager 595 K (Molecular Dynamics Inc., Sunnyvale, CA).

Karyotype analysis. For chromosomal analysis, the cells were karyotyped from Q-FISH (DAPI-chromosomes, FITC-centromeres) samples and the standard air-drying method (21), respectively. The cells were then treated with a final concentration of 0.05 μg/ml colcemid for 2 h during the non-exponential growth phase. The cells were analyzed using the ISIS karyotyping system (Metasystem GmbH, Altlussheim, Germany) and trypsin G-banding. A total of 30 metaphase spreads were counted to determine the modal number.

Quantitative-fluorescence in situ hybridization and image analysis of telomeres. Q-FISH and image analysis were performed as described previously (22). Digital images were recorded with a CCD camera, ORCA-ER-1394 (Hamamatsu Photonics KK, Hamamatsu, Japan) on a Nikon 80i epifluorescence microscope equipped with a triple-band pass filter for Cy3/FITC/DAPI (Eclipse 80iFU-RFL, Nikon, Tokyo Japan). Microscope control and image acquisition was performed with the dedicated software, Image-Pro Plus (version 5.0, MediaCybernetics Co. Ltd., USA). Two levels of calibration were used to ensure a reliable quantitative estimation of telomere length in various samples. First, to correct for daily variations in lamp intensity and alignment, images of fluorescent beads (orange beads, size 0.2 μm, Molecular Probe) were acquired and similarly analyzed with the 'TFLTelo-V2' (35) and 'TFL Cell Analysis' software packages. Second, relative telomere fluorescence units (TFUs) were extrapolated

Table I. Subtelomeric FISH analyses in OCUT-1 by TelVysion.

Chromosome No.	The types of subtelomeric signals							Total metaphases examined
	p(0)q(6)	p(4)q(2)	p(4)q(4)	p(4)q(6)	p(6)q(2)	p(6)q(4)	p(6)q(6)	
Metaphases								
8[a]			100%					42
7[b]				3%		3%	94%	36
11[c]		5%			95%			43
12[d]			95%			5%		45
20[c]	2%		6%	2%			90%	52

Chromosome No.	p(0)q(3)	p(2)q(2)	p(2)q(3)	p(2)q(4)	p(3)q(1)	p(3)q(2)	p(3)q(3)	Total interphases examined
Interphases								
8[a]		98%				1%	1%	73
7[b]			6%			4%	90%	84
11[c]					100%			92
12[d]		96%	2%	2%				88
20[c]	4%	1%	10%				85%	73

[a]Cytogenetically normal, [b]cytogenetically normal trisomy, [c]+11, der(11)t(3;11)(q23;q23)x2, [d]del(12)(p11.2p12).

from Southern blot data for the aging cell line TIG-1, established at our institution. A linear correlation between telomere length and $(T2AG_3)_n$ length with a slope of 1.361 was determined (data not shown).

FISH analysis of subtelomeres. FISH analysis was performed as described by Bosch *et al* (23). Slides were washed in a standard 2X SSC solution, dehydrated in an ethanol series, and denatured for 3-5 min in 70% formamide solution at 73˚C. Probes were denatured at 73˚C for 5 min, and slides were hybridized in a dark chamber for 12-24 h at 37˚C. DAPI-III counterstain (Vysis Inc.) and antifade were applied to the slides prior to observation. Cell nuclei decondensation was carried out by incubating the slides in a solution of 5 mmol/l DTT at 37˚C for 8-15 min before denaturation. For subtelomeric probes, slides were treated with pepsin and post-fixed with 1% formaldehyde solution prior to hybridization. Slides were analyzed on a Nikon 80i epifluorescence microscope equipped with a triple-band pass filter for Cy3/FITC/DAPI (Eclipse 80iFU-RFL). The images were captured by a CCD camera (ORCA-ER-1394, Hamamatsu Photonics KK) and analyzed with Image-Pro plus (version 5.0, Media-Cybernetics Co).

Probes. Telomeric probes, Cy3-labeled $(CCCTAA)_3$ peptide nucleic acid (PNA) and centromeric probe (CENP1; non-specific centromere probe), and FITC-labeled CTTCGTTG GAAACGGGGTPNA were used for Q-FISH analysis. TelVysion telomeric probes 7p, 8p, 11p, and 20p (Spectrum green), respectively, and 7q, 8q, 11q, 20q, and 3q (Spectrum orange) (Vysis Inc., USA), respectively, were used for the FISH analysis.

Statistical analysis. Differences in p-values were analyzed with the Chi-squared test for independence, and Fisher's Z test was used for comparing correlations. In all comparisons, differences at $p<0.05$ were considered significant.

Results

Telomerase activity and the expression of its catalytic subunits. The TRAP assay detected a high level of telomerase activity in the OCUT-1 cell line. The mRNA of two catalytic subunits of telomerase, hTERT and hTR, were clearly detected on RT-PCR (Fig. 1).

Cytogenetic and Q-FISH analyses of telomeres in OCUT-1 cells. The OCUT-1 cells grew in a monolayer fashion. Spindle to polygonal cells grew in a cobblestone pattern with large nuclei and prominent nucleoli. The population doubling time at the exponential growth phase was 34 h. As shown in Fig. 2, the OCUT-1 cells displayed numerous chromosomal abnormalities; the chromosomal count was 50 in all the 15 cell types analyzed. The karyotype was 50, XX, +7, +11, der(11)t(3;11) (q23;q23)x2, del(12)(p11.2p12), +20, +1mar. We also analyzed metaphase chromosomes from OCUT-1 and adjacent normal fibroblast cells using Q-FISH. Digital images were acquired from DAPI-stained chromosomes, FITC-labeled centromeres, and Cy3-labeled telomeres. We combined the three images after assignment of pseudo-colors and derived a karyogram (Fig. 2b). Finally, the fluorescence intensity of the p- and q-arm telomeres on all chromosomes was expressed as TFUs (Fig. 2c). To investigate the telomere length of specific chromosome arms, we analyzed 15 metaphase spreads for both OCUT-1 and normal

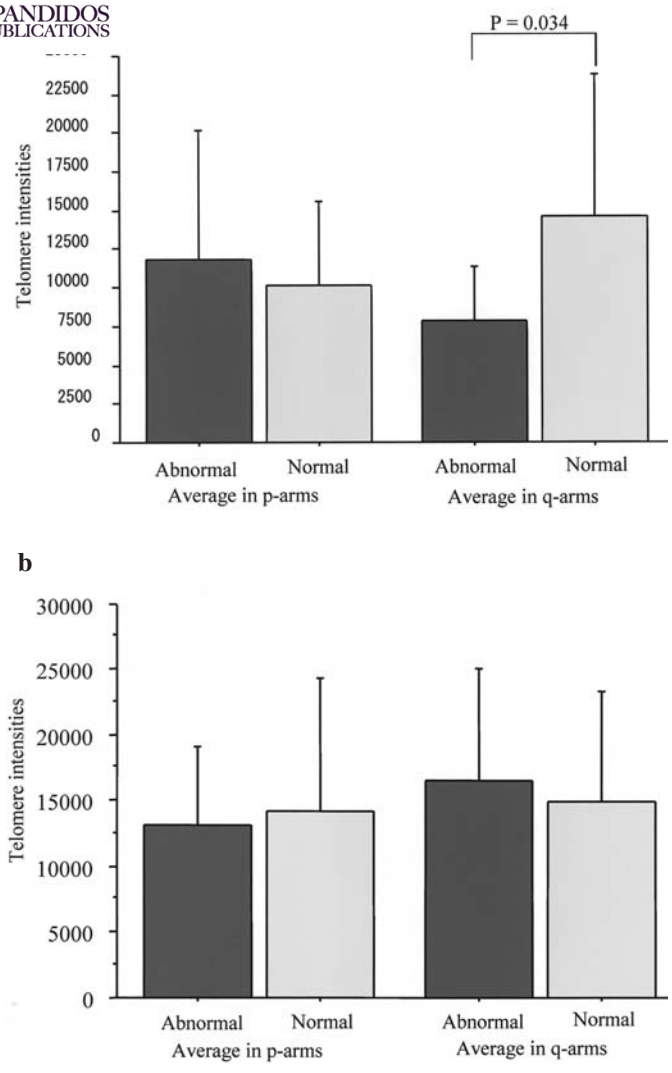

Figure 3. (a) Comparison of telomere intensities of the p- and q-arms between the abnormal chromosome del(12)(p11.2p12) and cytogenetically normal chromosome 12. TFUs in the q-arms of the abnormal chromosome del(12)(p11.2p12) were significantly shorter than the average TFUs in the q-arms of normal chromosome 12 (p=0.034). (b) Comparison of telomere intensities of p-and q-arms between the abnormal chromosome der(11)t(3;11)(q23;q23) and the cytogenetically normal chromosome 11. TFUs in the p- and q-arms of the abnormal chromosomes der(11)t(3;11)(q23;q23) were not significantly shorter than the average TFUs in the p- and q-arms of normal chromosome 11.

fibroblast cells. Most of the TFUs in the p- and q-arms of chromosomes in OCUT-1 were shorter than the median TFUs in the fibroblast cell line, except for the 3, 10, and 13 q-arms. However, there were no particularly shortened telomeres in the p- and q-arms on any chromosome of OCUT-1 (Fig. 2c). We analyzed carefully the abnormalities associated with telomere shortening and cytogenesis. TFUs in the q-arms of the abnormal chromosome del(12)(p11.2p12) were significantly shorter than the average TFUs in the q-arms of normal chromosome 12 (p=0.034) (Fig. 3a). TFUs in the p- and q-arms of the abnormal chromosomes der(11)t(3;11)(q23;q23) were not significantly shorter than the average TFUs in the p- and q-arms of normal chromosome 11 (Fig. 3b).

FISH analysis of OCUT-1 cells for subtelomeres. A total of 800 nuclei (260 metaphases and 540 interphases) were analyzed in the OCUT-1 cell line by TelVysion, and the results are shown in Table I. Subtelomeres on both abnormal chromosomes, der(11)t(3;11)(q23;q23)x2, showed a significant loss of signal number (p<0.005), but there was no signal loss in cytogenetically normal chromosomes in trisomies 7 and 20 and the abnormal chromosome del(12)(p11.2p12) (Figs. 4 and 5). The specific abnormal subtelomere on the 11q-arms was lost in 95% of metaphases and 100% of interphases (p<0.005). In the cytogenetically normal chromosome 8, subtelomere 8 had 100% perfect signals in metaphases and 98% perfect signals in interphases. In the three cytogenetically normal chromosomes in trisomy 7, subtelomere 7 had 94% and 90% perfect signals in metaphases and interphases, respectively; trisomy 20, subtelomere 20 had 90% and 85% perfect signals in metaphases and interphases, respectively. For the abnormal chromosome del(12)(p11.2p12), subtelomere 12 had 95% perfect signals in metaphases and 96% perfect signals in interphases. Subtelomeres of 3q had eight signals, and cytogenetically one pair remained in place on 3q, but another pair remained in place on the abnormal 11p (Fig. 6). The abnormal chromosome der(11)t(3;11)(q23;q23)x2, subtelomere 3q, had 88% perfect signals in metaphases and 94% perfect signals in interphases. The normal chromosome 3q, subtelomere 3q, had 100% perfect signals in metaphases and 94% perfect signals in interphases (Table II).

Discussion

The difference between the two variants of thyroid carcinoma has been discussed at length from various viewpoints ranging from clinical features to genetic alteration. Several differences in cellular and molecular characteristics that are responsible for the rapid progression or malignant potential of ATC have been reported. OCUT-1 cells, derived from ATC, grew rapidly with a doubling time of ~34 h, which was comparable with the doubling times of C643 (19-29 h) (24), BHT-101 (24 h) (25), HTC/C3 (48 h) (26), and HMCa (48 h) (27), all of which are typical ATC cell lines. The doubling times of DTC cell lines are much longer; e.g., UCLA RO82 W-1, which is a follicular carcinoma cell line, has a doubling time of 4 days (28). Therefore, the OCUT-1 cell line is considered to have very aggressive characteristics and high malignancy.

In this study, we examined the telomerase activity and two major components of human telomerase, hTERC and hTERT, in OCUT-1. Although >90% of malignant tumors have telomerase activity, thyroid cancer has a low frequency of telomerase activity. OCUT-1 cells show strong expression of hTR, hTERT, and telomerase activity, and the cell line has already been maintained for >100 passages and stably cultured for more than one year. These characteristics suggest that OCUT-1 is an aggressive immortal cell line and a very good model for studies of the telomere, subtelomere, and carcinogenesis in ATC.

To our knowledge, this is the first study of telomere measurement using Q-FISH in a thyroid carcinoma cell line. Most solid cancer cell lines have a very large number of chromosomes, sometimes >100, making it difficult to determine their karyotype. Therefore, it has not been possible to analyze the TFUs of individual chromosomes using Q-FISH. However, the karyotype of OCUT-1 is 50, XX, +7, +11, der(11)t(3;11)(q23;q23)x2, del(12)(p11.2p12), +20, +1mar,

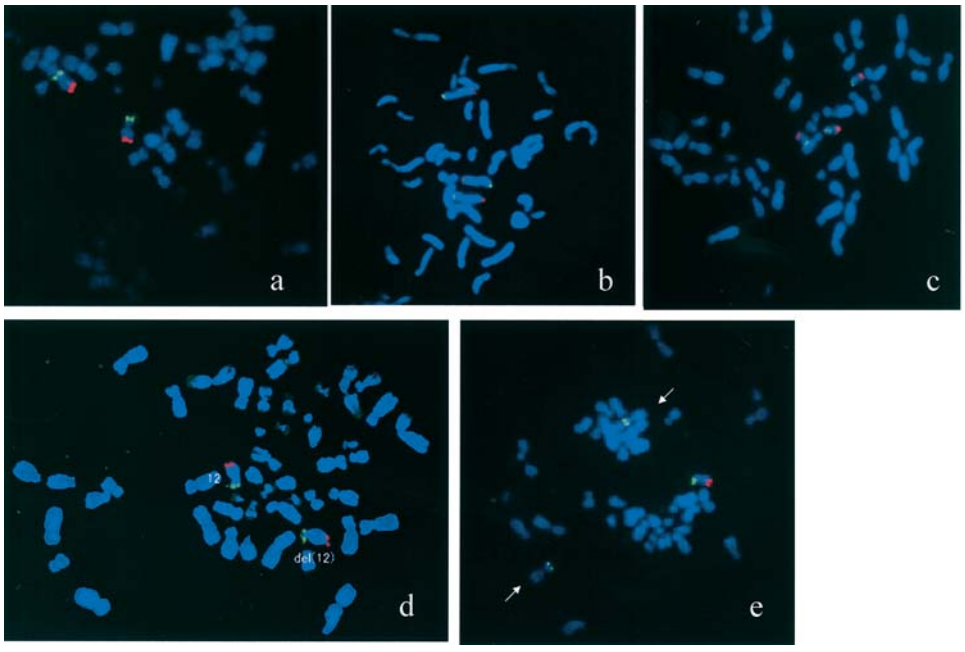

Figure 4. Subtelomere analysis of 8pq, 7pq, 20pq, 12pq, and 11pq by TelVysion in metaphases. (a) In the cytogenetically normal chromosome 8, subtelomere 8 had four red (Cy3: p-arms) and four green (FITC: q-arms) signals. (b) The three cytogenetically normal chromosomes in trisomy 7, subtelomere 7, had six red (Cy3: p-arms) and six green (FITC: q-arms) signals. (c) The three cytogenetically normal chromosomes in trisomy 20, subtelomere 20, had six red (Cy3: p-arms) and six green (FITC: q-arms) signals. (d) The abnormal chromosome in del(12)(p11.2p12), subtelomere 12, had four red (Cy3: p-arms) and four green (FITC: q-arms) signals. (e) The abnormal chromosome in trisomy der(11)t(3;11)(q23;q23), subtelomere 11, had two red (Cy3: p-arms) and six green (FITC: q-arms) signals. The arrows show loss of the 11p-arm subtelomeric signals.

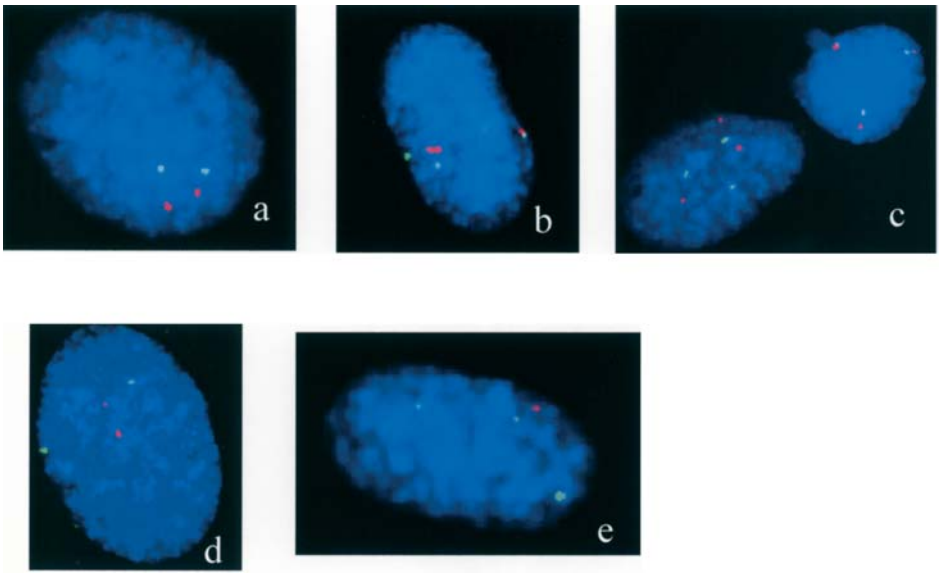

Figure 5. Subtelomere analysis of 8pq, 7pq, 20pq, 12pq, and 11pq by TelVysion in interphase. (a) We detected two red signals (Cy3: p-arms) and two green signals (FITC: q-arms) for subtelomere 8 in interphase. (b) We detected three red-signals (Cy3: p-arms) and three green-signals (FITC: q-arms) for subtelomere 7, trisomy 7, in interphase. (c) We detected three red signals (Cy3: p-arms) and three green signals (FITC: q-arms) for subtelomere 20, trisomy 20, in interphase. (d) We detected two red signals (Cy3: p-arms) and two green signals (FITC: q-arms) for subtelomere 12 in the abnormal chromosome del(12)(p11.2p12) in interphase. (e) We detected one red signal (Cy3: p-arms) and three green signals (FITC: q-arms) for subtelomere 11 in the abnormal chromosome trisomy der(11)t(3;11)(q23;q23) in interphase.

and thus is very similar to that of a normal human cell, 46, XX. Using Q-FISH, we succeeded in analyzing human metaphase chromosomes from both OCUT-1 and fibroblast cells from the stroma adjacent to the original OCUT-1 tumor. Based on studies of normal human somatic cells using Q-FISH, Martens *et al* (22) have reported that the telomeres of chromosome 17p are significantly shorter than the median telomere length and that this characteristic contributes to the frequent loss of 17p alleles in human cancers. We expected OCUT-1 cells to have shorter telomeres on specific chromosomes. To investigate the telomere lengths of specific chromosome arms, we analyzed 15 metaphase spreads for

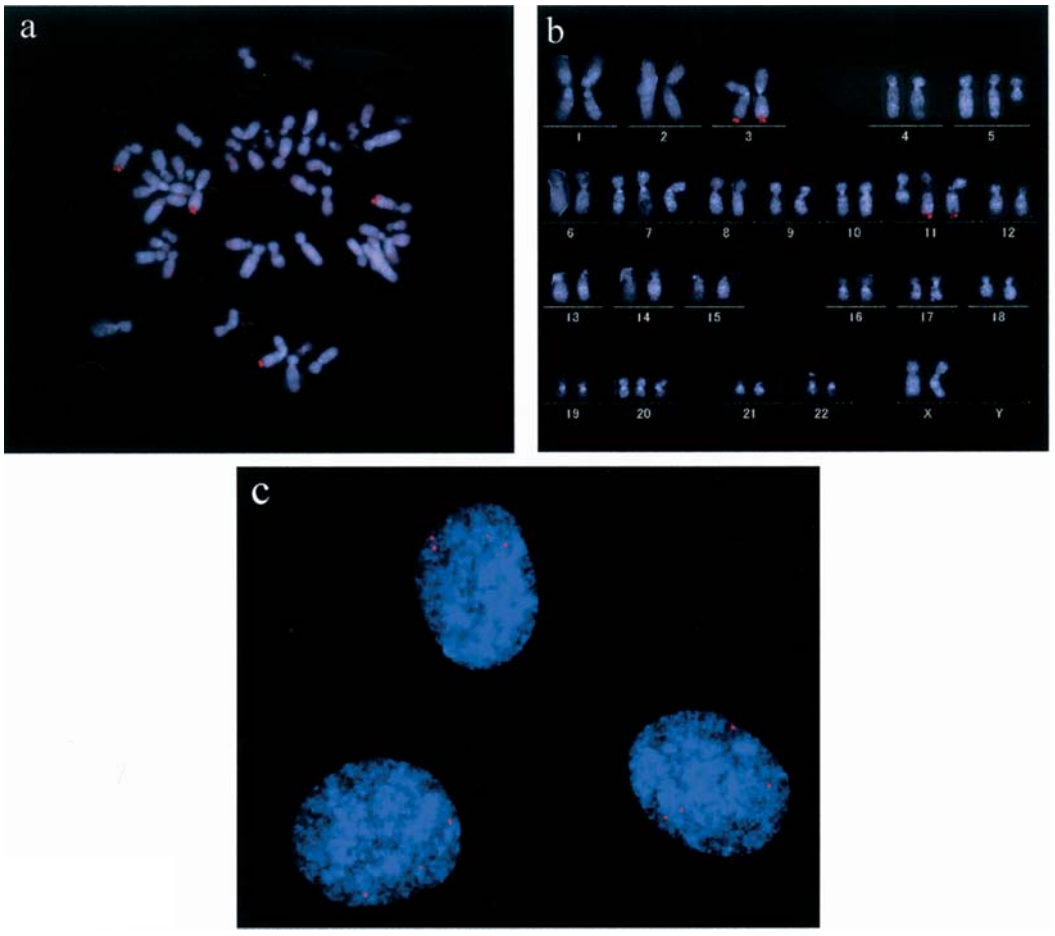

Figure 6. Subtelomere analysis for 3q by TelVysion in metaphase and interphase, and the karyotype. (a) We detected eight signals (Cy3) for subtelomere 3q in metaphase. (b) By karyotype analysis, we found that one pair (four signals) had remained in place for 3q, but another pair (four signals) replaced abnormal 11p. (c) We detected four signals (Cy3) for subtelomere 3q in interphase.

Table II. Subtelomeric FISH analyses of 3q-arms by TelVysion and karyotype.

Chromosome No. with signals	Metaphases Location of signal in chromosome		Total metaphases examined	Interphases	Total interphases examined
	3q(4)[a]	11p(4)[a]		(4)[a]	
3q	100%	88%	42	94%	130

[a]The number of signals.

each of the OCUT-1 cell types and normal fibroblasts. Most of the TFUs in the p- and q-arms of chromosomes in OCUT-1 were shorter than the median TFUs in the fibroblast cell line, except for the 3, 10, and 13 q-arms. However, there were no particular chromosomes with shortened telomeres in the p- or q-arms in OCUT-1.

We then carried out a detailed study of the abnormalities associated with telomere shortening and cytogenesis. In OCUT-1, TFUs in the q-arms of the abnormal chromosome del(12)(p11.2p12) were significantly shorter than the average TFUs in the q-arms of cytogenetically normal chromosome 12. Telomeres are thought to prevent chromosomal fusion and, as a consequence, reduce the risk of genomic instability (29). As OCUT-1 cells have already been maintained for >100 passages and stably cultured for more than a year, we did not observe any fusion in this cell line. The shorter telomere on chromosome 12q may play some role in the genomic instability of ATC.

Telomeres anchor the ends of chromosomes to the nuclear matrix and serve as sites of chromosome alignment during meiosis (30,31). This latter function, facilitating alignment of the chromosome ends, may provide a partial explanation for the increased density of genes near chromosome ends and the increased frequency of meiotic recombination in that region (32,33). Supposedly as a consequence of the sequence similarity that exists between subtelomeric regions

of non-homologous chromosomes, rearrangements with a minimum size of 3-5 MB are too small to be detected by conventional cytogenetic analysis even if experimental conditions are optimal and the banding pattern of the rearranged chromosomal material is distinctive (34). Familial subtelomeric abnormalities can cause developmental delay, idiopathic mental retardation, or non-specific congenital abnormalities (12,34). To our knowledge, the present study is the first to describe subtelomere detection using FISH in a thyroid carcinoma cell line. Subtelomeres on the abnormal chromosome der(11)t(3;11)(q23;q23)x2 showed a significant loss of signal, but there was no signal loss in cytogenetically normal trisomies 7 and 20 or the abnormal chromosome del(12)(p11.2p12). These results suggest that carcinogenesis of ATC may be associated with subtelomeric abnormality, as is the case for idiopathic mental retardation disorders.

Although this study is a preliminary report, our present findings suggest that significant telomere shortening, 12q, and subtelomeric loss of abnormal 11q may be correlated with chromosomal instability in an ATC cell line. In future studies, we plan to investigate the target gene associated with specific telomere shortening on 12q and the repetitive region among 11q haplotypes with the specific abnormal subtelomere.

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