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Original Paper

Clinical Significance of Telomerase Activation and Telomeric Restriction Fragment (TRF) in Cervical Cancer

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Telomerase activation was examined in 50 cases of cervical cancer, 27 normal cervix and five cervical cancer cell lines using the sensitive polymerase chain reaction (PCR)-based TRAP (telomeric repeat amplification protocol) assay. Telomeric restriction fragment (TRF) length of these specimens was measured by Southern hybridisation. Telomerase activation was common in cervical cancers and was detected in 46/50 cases (92%). Telomerase activity was weak in normal cervix and was detected only in 2/27 cases (7.4%). Telomerase activity was detected in all stages of cervical cancer suggesting that it is an early event in cancer progression. The clinical significance of telomerase activation was analysed in 47 squamous cell carcinoma of the cervix. High telomerase activity was more frequently detected in advanced diseases (100% in stage III and stage IV cervical cancers combined) compared with early diseases (68.6% in stage I and stage II cancers combined). The difference was statistically significant ($P < 0.02$). Telomerase activity was not statistically correlated with other clinical parameters examined. This is the first report of telomeric length in human cervical cancer. Both shortening and elongation of TRF length in cervical cancers was observed. Advanced cervical cancers tended to have a wider range of variation of TRF length compared with early disease and normal cervix. There was no obvious relationship between TRF length and the clinical parameters examined including clinical staging, differentiation status of tumour, human papilloma virus (HPV) infection, recurrence rate, tumour size and invasion depth. The clinical significance of TRF length appears to be limited in cervical cancers. Our results indicate that telomerase activity is closely associated with tumour cells and may be useful as a marker for detection of tumour cells in cervical biopsies.

Key words: telomerase, TRF, cervical cancers
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INTRODUCTION

TELOMERES ARE specialised structures at the ends of eukaryotic chromosomes with unique TTAGGG repeats [1]. These specialised structures are important in the protection and replication of chromosomes. Because of the end replication problem, telomeric repeats at the chromosomal ends are progressively shortened during cell proliferation. A critical length of telomeric repeats is required for cells to sustain their continuous proliferation. Telomerase adds the TTAGGG repeats to the chromosomal ends of proliferating cells and plays a vital role in the maintenance of telomeric length.

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Telomerase activity is present in germ cells of testis and ovary. However, telomerase activity is repressed in most somatic cells except in some somatic stem cells. Most immortal cell lines and most human cancers have strong telomerase activities. A highly sensitive polymerase chain reaction (PCR)-based method referred to as the TRAP (Telomeric Repeat Amplification Protocol) assay was developed to detect telomerase activity [2]. The PCR-based telomerase assay requires only a small quantity of cells which allows large-scale study of telomerase activity in various human cancers. Telomerase activity has been detected in most human cancers [3] including those from the brain, stomach, prostate, breast, lung, colon, liver, bladder, pancreas and recently in nasopharyngeal carcinoma [4]. Telomerase activity can be detected at high frequency in most human

cancers examined. Some reports have suggested that telomerase activity may have prognostic value in predicting patient survival in some cancers such as neuroblastoma and gastric cancers [5, 6].

Cervical cancer is a common cancer in Chinese women. In this study, we examined telomerase activation and alterations of telomeric restriction fragment (TRF) in cervical cancers. The relationship between telomerase activation and TRF with various clinicopathological features such as clinical staging, tumour grade, tumour size, depth of invasion and human papilloma virus (HPV) infection status were examined.

MATERIALS AND METHODS

Tissue samples

A total of 50 cervical cancers and 27 age-matched normal tissues of cervix were obtained at the time of surgery with patient's consent. All the samples were obtained from hysterectomy or punch biopsies which were frozen and stored at − 80°C before extraction for telomerase assay. Serial frozen sections were cut from the same specimen block and used for telomerase assay. One was stained with haematoxylin and eosin (H&E) and examined for histopathological grading using well-established criteria [7]. The histological subtypes, grades, percentage of tumour cells in section, tumour size and depth of invasion were recorded.

Cell lines

Five cervical cancer cell lines, HeLa, C33, CaSki, SiHa and C-4I, were obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A. These cells were grown in Dulbecco's Minimal Essential medium (DMEM) and supplemented with 10–20% fetal bovine serum in the presence of 5% CO_2 at 37°C. One of them, the HeLa cell line, was used as internal positive control for the telomerase assay and to adjust for interassay variation.

HPV infection

The presence of HPV DNA in the tumour samples was determined by PCR-Southern hybridisation, which was regarded as the most sensitive and specific method for the determination of HPV DNA. Detailed procedures have been previously published [8]. Briefly, the extracted DNA was amplified using specific primer pairs for HPV L1 consensus sequence, HPV16-E6 DNA and HPV 18-E6 DNA. Human globin gene amplification was amplified as internal PCR control. The amplified DNA was then blotted on to a nylon filter and hybridised to specific DNA probes of HPV 16-E6 and HPV 18-E6 DNA.

TRAP assay

The TRAP assay was carried out according to a published protocol [9, 10]. Briefly, frozen samples or cell pellets from cell lines were homogenised with disposable pestles in 20–200 µl of ice cold lysis buffer. The lysis buffer was composed of 10 mM Tris–HCl (pH 7.5), 1 mM $MgCl_2$, 1 M EGTA, 0.1 mM phenylmethylsulphonyl fluoride, 5 mM β-mercaptoethanol, 0.5% 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulphonate (Sigma, St Louis, Missouri, U.S.A.), and 10% glycerol. After 30 min of incubation on ice, the homogenated lysate was centrifuged at 14 000×*g* for 30 min at 4°C and the supernatant was aliquoted and stored frozen at − 80°C until use. Three micrograms of protein were used per reaction for the TRAP assay. For estimation of telomerase activity, each extract was assayed at three concentrations, 1×, 10× and 100× with RNAse control. Assay tubes were prepared by sequestering 0.1 µg of CX primer (5′-CCCTTACCCTTA-CCCTTACCCTTAA-3′) under a wax barrier (Ampliwax; Perkin Elmer Cetus, Foster City, California, U.S.A.). Each extract was assayed in 25 µl of reaction mixture containing 20 mM Tris–HCl (pH 8.0), 1.5 mM $MgCl_2$, 60 mM KCl, 0.05% Tween 20, 1 mM EGTA, 50 µM deoxynucleotide triphosphates, 0.1 µg of TS primer (5′-AATCCGTCGAGCAGA-GTT-3′), 0.5 µM of T4 gene 32 protein (Boehringer Mannheim, Germany), 2 µCi [$\alpha - {}^{32}$P]dCTP, 3000 Ci/mmol (Amersham, Buckinghamshire, U.K.), and 1.0 unit of Taq DNA polymerase (Boehringer Mannheim). After a 30-min incubation at room temperature for telomerase-mediated extension of the TS primer, the reaction mixture was heated at 94°C for 3 min and then subjected to 31 PCR cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 45 s. The PCR products were then electrophoresed on a 15% polyacrylamide gel. To check the specificity of the telomerase assay, RNase pretreatment of the telomerase extract (0.5 µg of RNase A for 30 min at 37°C) was always included for every sample examined. Only RNase sensitive activities were scored as positive for telomerase activation. The relative levels of telomerase in samples were scored as follows: samples with strong laddering signals in all three dilutions (1×, 10×, 100×) were scored as (+ + +); samples with only weak or no laddering signals in 100× but strong signal in 1× and 10× were scored as (+ +); samples with weak signal in 1× dilution but no or weak signal in 10× were scored as (+) (Figure 1). Negative telomerase samples were those without any detectable laddering signals in all the three dilutions of extract. Telomerase extracted from HeLa cells was included in every telomerase assay to adjust for interassay.

Southern blot analysis

Genomic DNA was extracted three times with phenol/chloroform according to a previously published protocol [11].

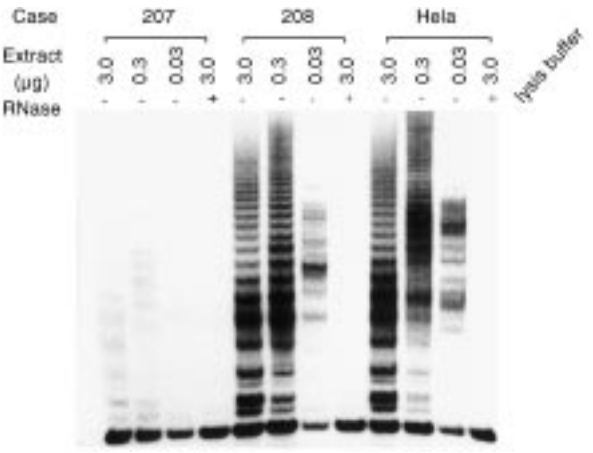

Figure 1. Telomerase activity in human cervical cancer. Telomerase activity in each sample extract was assayed at three protein concentrations (3.0, 0.3 and 0.03 µg) per reaction. Telomerase activity was assayed in the presence (+) or absence (−) of RNase. Cases 207 and 208 were sample extracts prepared from two cervical cancer specimens. HeLa is an established carcinoma cell line used as a positive control for telomerase. Case 208 and HeLa showed strong telomerase activity, whereas Case 207 demonstrated low/weak telomerase activity.

For the analysis of length of terminal restriction fragments, 5 μg of DNA was digested to completion with 25 U of *HinfI* and 25 U of *RsaI* and electrophoresed on 0.8% agarose gels. The digested DNA were then blotted on to nylon filters and hybridised to ^{32}P-labelled (TTAGGG)4 probe. The hybridised blots were washed and then autoradiographed according to published procedures [12]. We estimated the mean length of terminal restriction fragments at the peak position of hybridisation signal using the Ultroscan XL (Pharmacia, Uppsala, Sweden).

RESULTS

Telomerase activation

We examined telomerase activation in 50 surgery and punched biopsies of cervical cancers (47 squamous cell carcinoma and 3 adenocarcinomas), 27 normal cervix and 5 established cervical carcinoma cell lines (Table 1). Telomerase activation was uncommon in normal cervix and only weak telomerase activity was detected in 2 cases (7.4%). In contrast, telomerase activation was common in cervical cancer and could be detected in both squamous cell carcinoma and adenocarcinomas. In all cases, the telomerase activity in tissue extracts was assayed at three protein concentrations: 3, 0.3 and 0.03 μg per reaction (Figure 1). Positive telomerase activity could be detected in 46/50 cases (92%) of cervical carcinomas. Among these 46 positive telomerase cancers, 38 cases had high-level telomerase activity, detectable at all three concentrations of the sample extract. 8 cases had low telomerase activity where faint laddering bands were detected at higher protein concentrations (0.3 and 3 μg protein per reaction). Four cases of cervical cancers were negative in telomerase activity. Telomerase assay was repeated in these negative cases to confirm their negative telomerase status. Internal PCR controls were included in these negative cases to confirm the lack of PCR inhibitors in the telomerase extract which would result in false negatives. The use of various concentrations of telomerase extract will also dilute the effects of telomerase inhibitors if present in the extract. The lack of telomerase activity was not due to the absence of tumour cells in the specimens. The presence of cancer cells in these telomerase negative cases was confirmed by histopathological examination and was in the range of 50–70%. Two other cases were excluded from this study as a result of absence of tumour cells in the specimens by histopathological examination (excluded in Table 3). These two cancer-free specimens were negative for telomerase activity. High telomerase activities were detected in all five cervical carcinoma cell lines. These results suggest that high telomerase activity is closely associated with the presence of cancer cells in cervical tissues.

Table 1. *Telomerase activity in normal cervix and cervical cancer*

	Telomerase activity Number (frequency)		
	Negative	Weak	High
Normal cervix ($n=27$)	25 (92.6%)	2 (7.4%)	0 (0%)
Cancer			
Squamous cell carcinoma ($n=47$)	4 (8.5%)	7 (14.9%)	36 (76.6%)
Adenocarcinoma ($n=3$)	0 (0%)	1 (33.3%)	2 (66.7%)
Cancer cell lines* ($n=5$)	0 (0%)	0 (0%)	5 (100%)

*Cervical cancer cell lines: HeLa, C33A, CaSki, SiHa and C-4I.

Details of the clinico-pathological features of the 50 cases of cervical cancers are shown in Table 2. A summary of the clinical significance of telomerase activation in cervical squamous cell carcinoma is shown in Table 3. Telomerase can be detected in all stages (stages I, II, III and IV) of squamous cell carcinoma (Table 3). Telomerase activity can be detected in 90.5% of cervical cancers in stage I (66.7% with high activity and 23.8% with low activity). This suggests that telomerase activation is an early event in the cervical cancers. High telomerase activity was detected at higher frequency (100%) in advanced diseases (stage III and IV) compared with early diseases (66.7% for stage I; 72.7% for stage II) ($P=0.02$; Fisher's exact test). In addition, high telomerase activity was detected in all five patients with recurrence (100%) compared with 66.7% in patients without recurrence. However, the difference was not statistically significant (P value = 0.13; Fisher's exact test).

There was no significant relationship observed between telomerase activation and the histological grades of the cancers, as well as depth of cancer invasion of the primary tumours. Telomerase activity was detected in the smallest tumour examined in this study suggesting that telomerase activation was already present in clinically detectable cervical cancers. There were variations in the levels of telomerase activity but no significant relationship with the clinicopathological characteristics of cancers. The percentage of cancer cells in the specimen was determined by histopathological examination but no direct correlation with the level of telomerase activity was observed. There was also no significant association between telomerase activation and HPV infection in cervical cancers (Table 3). The presence of HPV DNA was detected using PCR primers for consensus sequences (L1) to detect the presence of common HPV subtypes, as well as using specific primers for the detection of the E6 ORF of HPV subtypes 16 and 18 [8]. Telomerase activity was present in cervical cancers with or without HPV infection.

Telomeric restriction fragment (TRF) length

The telomeric restriction fragment (TRF) which is an indicator of telomeric length in cervical cancers was also examined by Southern hybridisation (Table 2; Figures 2 and 3). The median lengths of TRF in normal cervix and different

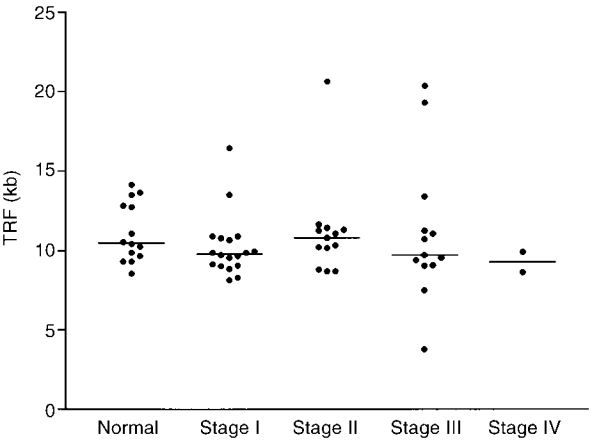

Figure 2. Distribution of telomeric restriction fragment (TRF) length in normal and cervical cancers. The TRF length was determined by the Southern blot hybridisation method using radioactively labelled TTAGGG probe.

stages of cervical cancers (squamous cell carcinoma) are shown in Figure 2. The mean ± SEM of the TRF in normal cervix and various stages of cervical cancer were: 11.13 ± 0.49 kb, normal; 10.24 ± 0.46 kb, stage I cancer; 11.16 ± 0.84 kb, stage II cancer; 11.09 ± 1.24, stage III cancer; 9.30 ± 0.65 kb, stage IV cancer. The distribution of TRF length appeared to be more scattered in cervical cancer of advanced stages (Figure 2). Stage III cervical cancer ($n = 13$) has the broadest range of distribution of TRF length, from 3.79 to 20.35 kb, whereas the range of TRF length in normal cervix ($n = 14$) was from 8.55 to 14.11 kb. However, the means of the TRF in different stages of cervical cancers were not significantly different from normal cervix ($P > 0.05$; *t*-test). Both shortening and elongation of TRF length in human cervical cancers were observed (Figure 3). When compared with normal cervical tissue, TRF length was shortened in half the cases and lengthened in the other half (Figure 4).

Table 2. Clinical significance of telomerase activation and TRF in cervical cancers

Patient No.	Age (years)	Histological subtypes	Stage	Grade	Tumour diameter (cm)	Invasion (mm)	HPV types	Recurrence	Tumour cells (%)	TRF (kbp)	Telomerase
1	33	SCC	IB				– ve	No	> 50	10.89	+++
2	34	SCC	IB				18	ND	> 75	10.67	+++
3	38	SCC	IB	1	2	6	16	No	> 65	8.13	+
4	35	SCC	IB	1			16	No	> 75	8.29	+++
5	43	SCC	IB	2		10	– ve	No	> 75	9.72	+
6	40	SCC	IB	2		7	16	No	> 75	9.88	+++
7	36	SCC	IB	2	1.5	10	18	ND	> 65	9.13	+++
8	36	SCC	IB	2		7	– ve	No	> 65	10.89	++
9	56	SCC	IB	2			ND	No	> 75	8.85	–
10	36	SCC	IB	2	4	20	16,18	No	> 50	9.55	+
11	32	SCC	IB	2		3	ND	ND	> 75	9.87	–
12	47	SCC	IB	2	2.5	16	16	Yes	> 75	ND	+++
13	70	SCC	IB	2		3	– ve	No	> 65	9.06	+++
14	45	SCC	IB	2			16	No	> 75	9.03	++
15	39	SCC	IB	2		9	16	No	> 75	9.68	+++
16	43	SCC	IB	2		15	16	No	> 75	9.96	+
17	41	SCC	IB	2	2.3	10	16	No	> 50	10.77	+++
18	38	SCC	IB	2		15	16	No	> 50	ND	+++
19	44	SCC	IB	3		4	– ve	No	> 75	16.41	+
20	29	SCC	IB	3	3	9	18	No	> 25	ND	+++
21	47	SCC	IB	3		15	16	No	> 75	ND	+++
22	56	SCC	IIA				18	No	> 65	11.63	+++
23	26	SCC	IIA	2	3	3	16	ND	> 75	11.25	+++
24	55	SCC	IIA	3		5	16	No	> 65	11.3	–
25	38	SCC	IIB				16	No	> 65	10.23	+++
26	34	SCC	IIB				16	ND	> 65	11.07	+++
27	65	SCC	IIB	1	2		16	No	> 75	10.81	+++
28	63	SCC	IIB	2		5	18	No	> 50	8.70	+
29	77	SCC	IIB	2			– ve	Yes	> 75	20.61	+++
30	73	SCC	IIB	2	2	10	– ve	No	> 75	8.70	+++
31	42	SCC	IIB	3	2		16	No	> 50	10.35	–
32	33	SCC	IIB	3		5	16,18	Yes	> 75	10.16	+++
33	65	SCC	IIIA	1			16	Yes	> 75	19.31	+++
34	76	SCC	IIIB				– ve	ND	> 75	9.05	+++
35	79	SCC	IIIB				16	ND	> 75	ND	++
36	54	SCC	IIIB	1		5	16	ND	> 50	9.55	++
37	51	SCC	IIIB	2			16	ND	> 75	11.06	+++
38	77	SCC	IIIB	2		10	16,18	No	> 65	13.40	+++
39	38	SCC	IIIB	2		8	16,18	No	> 75	9.08	+++
40	50	SCC	IIIB	2		5	18	ND	> 75	7.51	+++
41	48	SCC	IIIB	2	6	5	16	ND	> 50	20.35	+++
42	68	SCC	IIIB	2			16	No	> 65	3.79	+++
43	62	SCC	IIIB	3			– ve	No	> 65	11.25	+++
44	75	SCC	IIIB	3			– ve	No	> 75	9.40	+++
45	54	SCC	IIIB	3		9	16	ND	> 75	9.72	+++
46	70	SCC	IVB	2	1.5	5	16	No	> 50	8.65	+
47	49	SCC	IVB	3		8	18	Yes	> 25	9.94	+++
48	63	ADC	IIB	1		5	16	No	> 75	11.42	+
49	60	ADC	IIB	2		5	– ve	Yes	> 75	8.82	+++
50	68	ADC	IIIB	3				– ve	> 75	10.71	+++

*ND, not done; SCC, squamous cell carcinoma; ADC, adenocarcinoma; HPV, human papilloma virus; TRF, telomeric restriction fragment; –ve, negative.

Table 3. Summary of clinical significance of telomerase activation in squamous cell carcinoma of the cervix

	Telomerase activity No. (frequency)			
	Negative	Low	High	Total No.
Stage (FIGO):				
I	2 (9.5)	5 (23.8)	14 (66.7)	21
II	2 (18.2)	1 (9.1)	8 (72.7)	11
III	0	0	13 (100)	13
IV	0	1 (50)	1 (50)	2
Grade:				
1	0	1 (20)	4 (80)	5
2	2 (8)	5 (20)	18 (72)	25
3	2 (20)	1 (10)	7 (70)	10
HPV infection:				
Type 16/18	2 (5.7)	5 (14.3)	28 (80)	35
Absence	0	2 (20)	8 (80)	10
Recurrence:				
Yes	0	0	5 (100)	5
No	3 (10)	7 (23.3)	20 (66.7)	30
Depth of invasion (mm)				
≤ 5	2 (18.2)	3 (27.3)	6 (54.5)	11
6–10	0	2 (15.4)	11 (84.6)	13
> 10	0	2 (40)	3 (60)	5
Age:				
< 40	1 (6.3)	2 (12.5)	13 (81.3)	16
40–60	3 (16.7)	3 (16.7)	12 (66.7)	18
> 60	0	2 (15.4)	11 (84.6)	13
TRF (kbp)				
< 9	1 (12.5)	3 (37.5)	4 (50)	8
9–11	2 (8.7)	3 (13)	18 (78.3)	23
> 11	1 (9.1)	1 (19.1)	9 (81.8)	11

Figures in parentheses are percentages. HPV, human papilloma virus; TRF, telomeric restriction fragment.

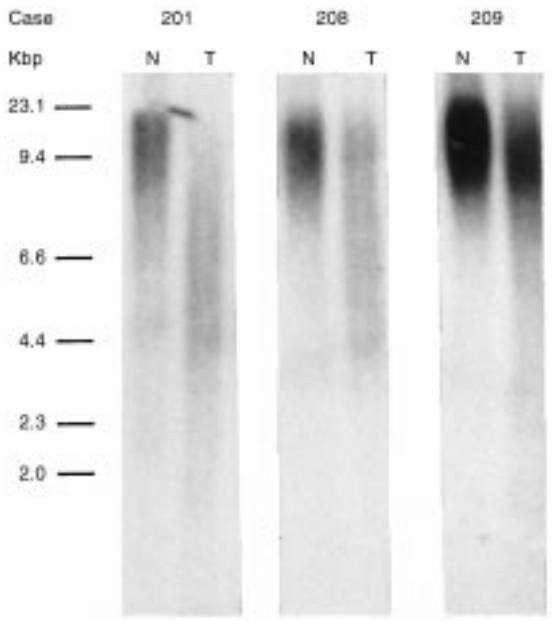

Figure 3. Alterations of TRF length in cervical cancers. TRF length was determined by Southern hybridisation.

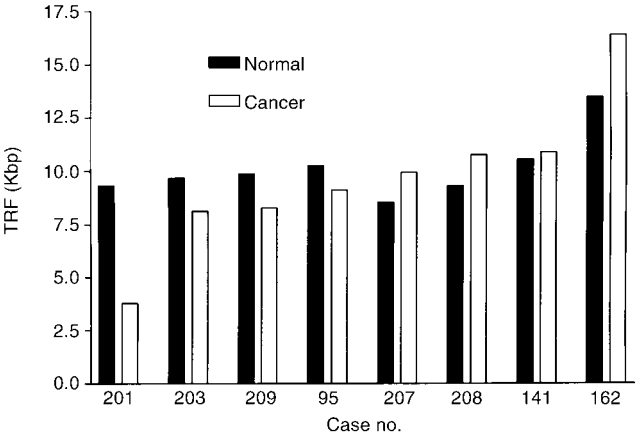

Figure 4. Alterations of TRF length in eight matched-pairs of normal and cancer tissues of cervix. TRF length was determined by Southern hybridisation.

There was no significant relationship observed between TRF and level of telomerase activities (Table 3). High telomerase activity was detected in cervical cancers with long or short TRF length. TRF length of cervical cancers was not related to the clinical parameters examined in this study including other clinicopathological features such as differentiation of cancer, recurrence rate, depth of invasion and HPV infection.

DISCUSSION

In this study, we showed that telomerase activation is a common event in cervical cancer and can be detected in 46/50 (92%) cervical cancers (Table 1), similar to previous published results [16–18]. In contrast, telomerase activation is infrequent in normal cervical tissues. There were variations in the frequency of telomerase activation detected in individual cervical cancers. Strong telomerase activity was detected at a higher frequency in advanced disease (100% in stage III and stage IV combined) compared with early diseases (68.8% in stages I and II combined) ($P = 0.02$). The association of telomerase activity with clinical staging has also been reported recently in breast cancer and other cancers [12–14]. In our study, high telomerase activity was detected in all five cases of cervical cancers which recurred, but the difference was not statistically significant ($P = 0.13$). This observation may not be conclusive because of the small sample size and the short observation period (half of them < 3 years). The higher frequency of telomerase activation in advanced cancers was not always associated with the higher percentage of tumour cells present in the cancer. In a few cases of early cancers, telomerase was not detected despite the presence of more than 70% tumour cells in the specimen. The activation of telomerase probably reflects the intrinsic biological properties of the tumour cells. These cancers may activate other non-telomerase pathways to maintain their telomeric length [15]. Other than clinical staging, there was no significant correlation observed between telomerase activation and other clinicopathological features of cancers examined including HPV infection, histological types, differentiation status, depth of invasion and tumour size.

Previous reports have shown that activation of telomerase can be achieved by the E6 and E7 proteins of HPV [19, 20]. In this study, we did not observe a significant relationship

between HPV infection and telomerase activation. There were 10 cases of HPV positive cervical cancers with undetectable telomerase activity (Table 3), and the level of telomerase activation was not related to the presence or absence of HPV infection. Telomerase activation in cervical cancer is likely to be regulated by multiple mechanisms and the understanding of these regulatory mechanism will be essential in the understanding of the basis of cellular senescence and malignant transformation.

Telomeric length alteration in cervical cancers was examined for the first time in this study. Similar to the observation in breast cancer [12], TRF length did not correlate with telomerase activity. A total of 44 cases of cervical cancers at various stages of disease were examined for telomeric restriction length using the Southern hybridisation method. All cervical cancers have a broader distribution range of TRF lengths compared with normal cervix, with advanced cases having the broadest range of distribution (Figure 2). However, the mean or median values of TRF at different clinical stages were not significantly different from that of normal cervix. Using matched-pair of cervical cancer and adjacent normal cervix, both shortening as well as elongation of telomeric length was observed in cervical cancers (Figures 3 and 4). This may be the reason for the lack of a significant difference in the mean of TRF between normal and cancerous cervix as the shortening of TRF in some cancer specimens was offset by elongation of TRF in other cancer specimens. We did not observe a significant correlation between TRF length and the clinical parameters of cervical cancers examined including clinical staging, histological types, differentiation status, HPV infection, tumour size and depth of invasion. There was an increased spread in telomeric length in advanced tumours which may be due to different levels of telomerase activation in individual cancers. Telomerase expression may be activated at different stages of tumour development in different cancers, which may also be another reason for the wider distribution of TRF observed in advanced cancers. The lack of clinical significance of TRF has also been reported in a recently published study in which telomeric length was examined in 60 primary breast cancers [12] where no relationship between the altered length of TRF and tumour stage, tumour size, lymph node status and telomerase activity was observed. The clinical significance of TRF length in human cancers is not yet established. There have been reports suggesting the clinical significance of TRF in some cancers including neuroblastoma [21], prostate cancer [13] and some haematological tumours [22]. Based on the results obtained in our study, TRF length appears to have limited prognostic significance in cervical cancers. Nonetheless, the determination of TRF by the Southern hybridisation method is probably not an accurate method for detecting alteration of TRF. Southern hybridisation only gives summative values of TRF of all chromosomes in cancer, as well as the stromal cells present in the same specimen.

There were 4 cases (8%) of telomerase negative cancers detected in this study (Table 3). The lack of telomerase activity was not due to the absence of cancer cells in the specimens nor to the insensitivity of the telomerase assay. The TRAP assay used in this study can detect telomerase activity in as few as 10–100 cancer cell equivalents per reaction (data not shown). The telomeric lengths of these 4 cases of telomerase negative cancers were 8.85 kb, 9.87 kb, 10.35 kb, 11.3 kb, which were not considerably shorter than the range of TRF of normal cervix (mean ± SEM = 11.13 ± 0.49 kb), except for the first case. A non-telomerase dependent TRF elongation mechanism may be involved in the maintenance of the TRF length in these telomerase-negative cancers [15].

Overall, our results showed that telomerase activation is a common event in cervical cancer and can be detected in early clinically staged disease. High telomerase activity was commonly associated with advanced cervical cancers. The high rate of telomerase activation (92%) in cervical cancers and its presence in all five cervical cancer cell lines tested in this study suggest that telomerase may be useful as a marker for detection of cancer cells in cervical specimens. Its role in detection of cancer cells in papanicolaou smear, which is a common procedure in early diagnosis of cervical cancer, is under current investigation.

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