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ORIGINAL ARTICLE

TERT promoter hot spot mutations are frequent in Indian cervical and oral squamous cell carcinomas

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Abstract Squamous cell carcinoma (SCC) of the uterine cervix and oral cavity are most common cancers in India. Telomerase reverse transcriptase (*TERT*) overexpression is one of the hallmarks for cancer, and activation through promoter mutation C228T and C250T has been reported in variety of tumors and often shown to be associated with aggressive tumors. In the present study, we analyzed these two hot spot mutations in 181 primary tumors of the uterine cervix and oral cavity by direct DNA sequencing and correlated with patient's clinicopathological characteristics. We found relatively high frequency of *TERT* hot spot mutations in both cervical [21.4 % (30/140)] and oral [31.7 % (13/41)] squamous cell carcinomas. In cervical cancer, *TERT* promoter mutations were more prevalent (25 %) in human papilloma virus (HPV)-negative cases compared to HPV-positive cases (20.6 %), and both *TERT* promoter mutation and HPV infection were more commonly observed in advanced stage tumors (77 %). Similarly, the poor and moderately differentiated tumors of the uterine cervix had both the *TERT* hot spot mutations and HPV (16 and 18) at higher frequency (95.7 %). Interestingly, we observed eight homozygous mutations (six 228TT and two 250TT) only in cervical tumors, and all of them were found to be positive for high-risk HPV. To the best of our knowledge, this is the first study from India reporting high prevalence of *TERT* promoter mutations in primary tumors of the uterine cervix and oral cavity. Our results suggest that *TERT* reactivation through promoter mutation either alone or in association with the HPV oncogenes (E6 and E7) could play an important role in the carcinogenesis of cervical and oral cancers.

Keywords Telomerase reverse transcriptase · Cervical cancer · Oral cancer · Promoter mutation · Human papilloma virus

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Introduction

Cervical cancer is the second most common cancer affecting women worldwide, with an estimated 528,000 cases in 2012 [1]. Almost 70 % of the global burden falls in areas with lower socioeconomic status, and more than one fifth of all new cases are diagnosed in India [2], where cervical cancer is the primary cause of cancer-related death among women. HPV infection is considered to be a major risk factor for the development of cervical cancer, and the presence of high-risk HPV 16 and 18 DNA has been found in almost all cases of invasive cervical cancer [3]. Oral squamous cell carcinoma (OSCC) of the head and neck is the sixth most common cancer in the world

[4], and in India, it ranks first among all cancers in men [4] and fifth in women [5]. The usage of smokeless tobacco products and chewing of betel leaf together with areca nut and slaked lime, a traditional and popular practice in India [6], is considered to contribute to half of the burden of oral cancer [7]. HPV infection is also an established risk factor for oral cavity cancer, with tobacco smoking and alcohol consumption having synergistic effects. Given the burden of these cancer types in India, it is essential to understand the various mechanisms underlying the tumorigenesis of cervical and oral cancers.

The upregulation of telomerase reverse transcriptase (TERT), a catalytic component of eukaryotic ribonucleoprotein complex that maintains telomere length [8], has been found in almost all tumors but not in adjacent normal cells [9, 10]. Although mutations in the coding region of *TERT* gene are infrequent in human tumors, germ line and somatic mutations in *TERT* promoter region were found to be frequent in a variety of tumors and cancer cell lines [11, 12]. Such mutations occurred in two hot spot positions, 1,295,228 C>T (C228T) and 1,295,250 C>T (C250T). These two hot spot mutations alter the −124 and −146-bp sequence upstream of translation start site of *TERT* and creates a new binding site for ETS transcription factor resulting in enhanced (two to fourfold) transcriptional activity of *TERT* promoter [11, 12]. Recently, Killela et al. (2013) studied 1230 tumors of 60 different types and reported that human cancer types including oral and cervical cancers had low (<15 %) frequency of *TERT* promoter mutations while others had relatively high (≥15 %). In view of the above findings, we investigated the frequency of these two hot spot *TERT* promoter mutations in cervical and oral SCCs of south Indian origin.

Materials and methods

Sample collection and DNA extraction

The Institutional Ethics Committee, Government Arignar Anna Memorial Cancer Hospital, Kancheepuram (No. 101041/e1/2009-2), and Madras Medical College, Chennai (No. 04092010), approved the present study. Cervical and oral cancer samples were collected following the Institutional Ethical Committee (IEC) guidelines and informed consent was obtained from each patient, after explaining about the research study. For the illiterate patients, the study was verbally explained and consent was obtained with their thumb impression. Tumor samples were collected from Government Royapettah Hospital, Institute of Social Obstetrics and Government Kasturba Gandhi Hospital for Women and Children in Chennai and Arignar Anna Memorial Cancer Hospital and Research Institute, Kancheepuram. Genomic DNA from a tissue sample was isolated by the conventional Proteinase K digestion and Phenol:Chloroform:Isoamyl alcohol (PCI) extraction method. The DNA samples were dissolved in 1× TE buffer and quantified using NanoDrop2000 UV-Vis spectrophotometer (Thermo Scientific, USA). The integrity of DNA was verified by 0.7 % agarose gel electrophoresis.

PCR amplification and sequencing of *TERT* gene promoter

A total of 181 SCC tumor DNA samples (140 uterine cervix and 41 oral cavity) were PCR amplified using the *TERT* gene promoter-specific primers *TERT* Fw: 5′-M13-GGCCGATTCGACCTCTCTC-3′ and *TERT* Rw: 5′-CAGCGCTGCCTGAAACTCG-3′ (where M13 in the *TERT* Fw is the universal sequencing primer 5′-tgtaaaacgacggccagt-3′). Polymerase chain reaction (PCR) was performed in a total of 60-μL volume containing 200 ng of genomic DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 100 μM dNTP (Takara, Japan), 2.5 units of AmpliTaq Gold (Applied Biosystems Inc., USA), 200 nM primers (Sigma, India), and 6 % 1,2-propanediol as an enhancer for amplifying GC-rich sequence [13]. Thermal cycling conditions for PCR were 5 min at 95 °C once followed by 30 s at 95 °C, 45 s at 60 °C, and 45 s at 72 °C for 10 cycles and 30 cycles of 30 s at 95 °C, 45 s at 60 °C and 30 s (with a 5-s increase in each cycle) at 72 °C and final extension for 7 min at 72 °C. The PCR products (378 bp) were electrophoresed in 2 % agarose gel and purified by QIAquick PCR purification kit (Qiagen, USA). The amplicons were then subjected to Sanger sequencing (Macrogen Inc., Seoul, South Korea).

Screening for high-risk HPV 16 and HPV 18 by real time PCR

HPV 16 and HPV 18 E6-specific primers and FAM-labeled MGB probes (Table 1) were custom designed and purchased from Invitrogen (USA). The β-globin gene served as an internal control. No template negative control was also included in all the reaction plates. The reaction mixture (10 μL) consisted of 20 ng of tissue DNA, 5 μL of 2X master mix (Roche, USA), 900 nM of each primer, and 250 nM of probe. A separate reaction was set up for HPV 16 and HPV 18 amplification, and polymerase chain reaction was carried out in ABI 7900HT Real Time PCR System (Applied Biosystems, USA) with the following thermal cycling conditions: 50 °C for 2 min followed by enzyme activation step 95 °C for 10 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 6 and the Fisher exact test with p value < 0.05 was considered as statistically significant.

Table 1 Sequence of HPV type-specific primers/probes used for HPV typing and β-globin gene specific primers/probe for control

Gene	Forward and Reverse Primers	FAM-labeled MGB probe
HPV 16	F-5′-CTGCAATGTTTCAGGACCCA-3′ R-5′-TCATGTATAGTTGTTTGCAGCTCTGT-3′	5′-CCAGAAAGTTACCACAG-3′
HPV 18	F-5′-AAACCGTTGAATCCAGCAGAA-3′ R-5′-GTCGTTCCTGTCGTGCTCG-3′	5′-CACTATAGAGGCCAGTG-3′
β-Globin	F-5′-GGATCTGTCCACTCCTGATGCTG-3′ R-5′-TCACTCAGTGTGGCAAAGGTGC-3′	5′-CTAAGGTGAAGGCTCAT-3′

Results

TERT promoter mutation in cervical cancer

TERT promoter mutations were observed in 21.4 % (30/140) of the cervical tumors (Fig. 1, Table 2). Among the mutation-positive tumors, C228T accounted for 73.3 % (22/30) while C250T accounted for 26.7 % (8/30). Out of the 22 tumors with C228T mutation, 27.2 % (6/22) were TT homozygous and 25 % (2/8) of the tumors with C250T mutations were TT homozygous. In addition, we also sequenced the parallel blood DNA samples of cases positive for *TERT* promoter mutations. No mutation was observed, indicating that the hot spot mutations were of somatic origin. The clinicopathological characteristics of cervical tumor cases were shown in Table 3 and Table S1.

We screened the cervical SCC for the presence of high-risk HPV (16 and 18) and found that 80 % (112/140) of them were positive for HPV, of which 70 % were HPV 16 and the remaining 10 % were HPV 18. *TERT* promoter mutations were found to be more prevalent in HPV-negative cases (25 %) compared to HPV-positive cases (20.6 %) (Fig. 2). A two-tailed Fisher's exact test showed that this association was not statistically significant ($p < 0.6120$).

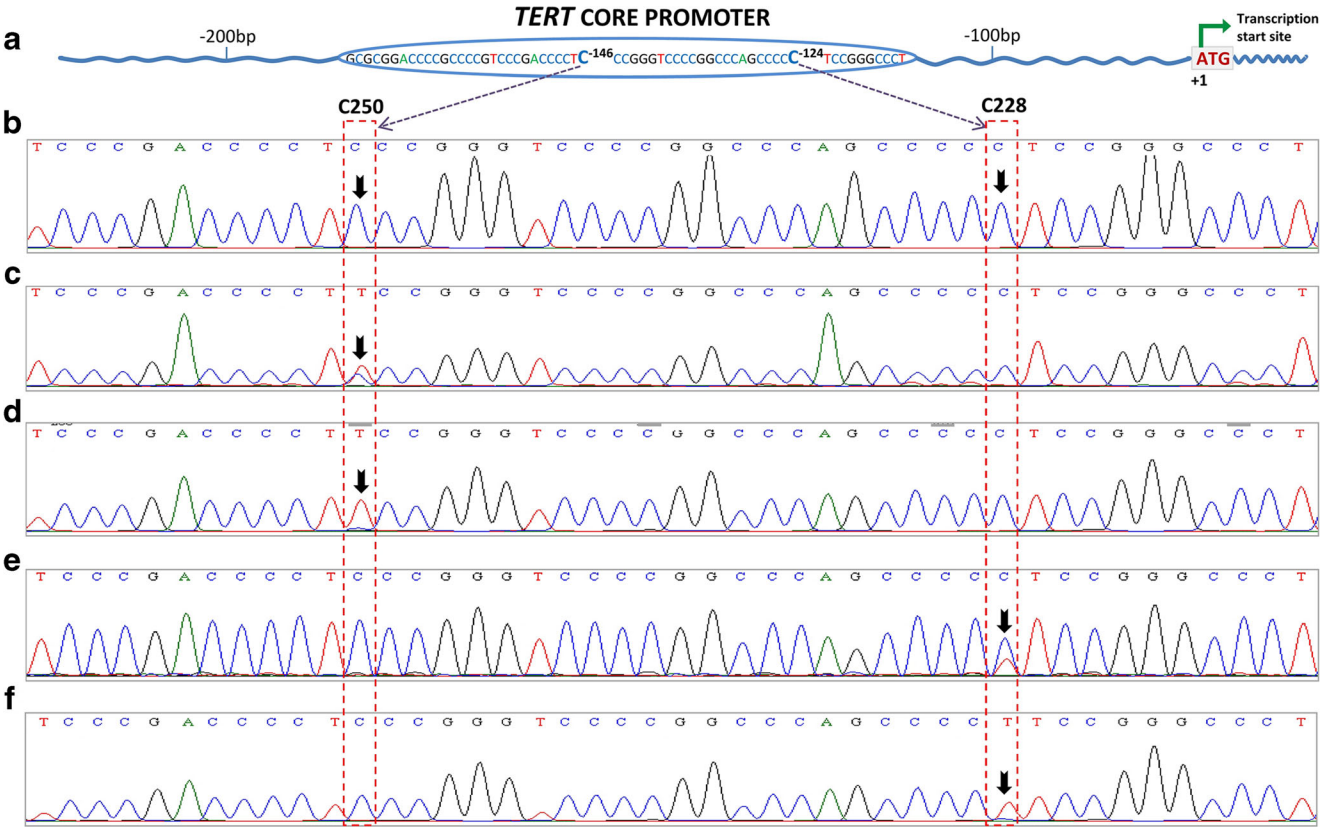

Fig. 1 *TERT* core promoter and representative sequencing chromograms showing somatic mutations in the *TERT* promoter. **a** Diagrammatic representation of *TERT* core promoter and mutations at two hot spots C228T and C250T, corresponding to −124 and −146 bp from translation start site were indicated by ↓. **b** Wild-type *TERT* promoter sequence. **c** Heterozygous mutant 250CT. **d** Homozygous mutant 250TT. **e** Heterozygous mutant 228CT. **f** Homozygous mutant 228TT

Table 2 Prevalence of *TERT* promoter hot spot mutations in squamous cell carcinoma of the uterine cervix and oral cavity

Tumor type	*TERT* promoter mutations		
	C228T *n*/*N* (%)	C250T *n*/*N* (%)	Overall *n*/*N* (%)
Squamous cell carcinoma of the uterine cervix	22/140 (15.7)	8/140 (5.7)	30/140 (21.4)
Oral squamous cell carcinoma	9/41 (21.9)	4/41 (9.7)	13/41 (31.7)
Total	31/181 (17.1)	12/181 (6.6)	43/181 (23.7)

TERT promoter mutations in oral squamous cell carcinoma

TERT gene promoter mutations were observed in 31.7 % (13/41) of oral cancer samples. Among them, C228T mutation constituted 69.2 % (9/13) while C250T constituted 30.8 % (4/13). No homozygous genotypes were observed in oral SCC. As expected, these mutations were mutually exclusive. Among the *TERT* mutations-positive cases, 12 were tobacco habitués and 3 had a history of alcohol consumption in addition to tobacco abuse. The complete clinicopathological characteristics of oral SCC were shown in Table 4 and Table S2. No significant correlation was observed between any of the genotypes and clinicopathological characteristics. None of the oral tumors were positive for HPV. Taken together, our results indicate that *TERT* promoter hot spot mutations were frequent and C228T mutation were twice frequent than C250T in the SCCs of the uterine cervix and oral cavity.

Table 3 Consolidated clinicopathological profile of one hundred eighty-one cervical cancer patients

Clinicopathological characteristics	Total (%)
Number of cases	140
Age	
Below 40 (range 26–40)	26 (18.57)
41–60	86 (61.40)
61–84	28 (20.00)
Mean age	53.18 (±11.89)
Tumor cell differentiation	
Well differentiated	22 (15.72)
Moderately differentiated	82 (58.57)
Poorly differentiated	31 (22.14)
Undifferentiated	5 (3.57)
CIN grade	
IB or IB2	8 (5.72)
IIA or IIA2	7 (5)
IIB or IIB2	45 (32.15)
IIIA	1 (0.71)
IIIB	24 (17.14)
Could not be assessed	55 (39.28)

Discussion

Telomerase reverse transcriptase (TERT) is a specialized enzyme responsible for de novo synthesis of telomeric DNA repeats present at the ends of chromosomes. In normal cell, TERT expression is downregulated as the cell divides resulting in telomere shortening and replicative senescence. To overcome this problem, in majority of the human cancers, TERT expression is reactivated and overexpressed during the late initiation phase of tumorigenesis and this is one of the hallmarks of cancer that leads to replicative immortality [10, 14, 15]. The reactivation of *TERT* through the promoter mutation was first reported in melanoma [11, 12]. *TERT* promoter

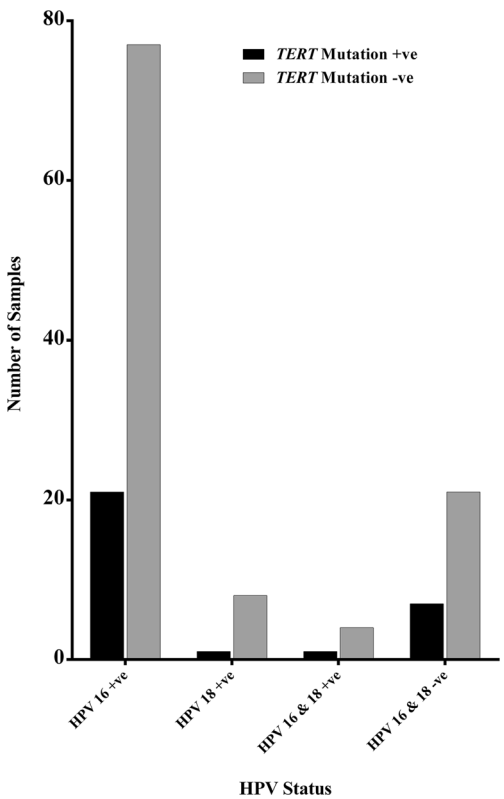

Fig. 2 Prevalence of *TERT* promoter mutations in cervical SCCs with reference to HPV infection status. One hundred eighty-one cervical cancer tumors were analyzed for the presence of both *TERT* mutation and HPV infection, and the results were presented as *bar diagram*. The *black bars* represent the mutation positive cases and *light bars* represent the mutation negative cases

Table 4 Consolidated clinicopathological profile of forty-one oral cancer patients

Clinicopathological characteristics	Total (%)
Number of cases	41
Age	
Below 40 (range 26–40)	11 (26.82)
41–60	22 (53.66)
61–84	8 (19.51)
Mean Age	50.27 (±11.84)
Sex	
Male	35 (85.37)
Female	6 (14.63)
Habit profile	
Exclusive tobacco	13 (31.7)
Exclusive betel	3 (7.3)
Exclusive alcohol	1 (2.44)
Tobacco + alcohol	7 (17.07)
Tumor grade	
Well differentiated	14 (34.15)
Moderately differentiated	20 (48.78)
Poorly differentiated	7 (17.07)
TNM-Stage	
II	6 (14.63)
III	12 (29.26)
IV	23 (56.09)

is present within the −330-bp upstream of translation start site, lacks the TATA and CAAT boxes, and has binding sites for several transcription factors [16]. Recently, two hot spot mutations in the *TERT* promoter region with varying frequencies were reported in 60 different type of human cancers ($n = 1230$), including cervical cancer (4.5 %) and head and neck cancer (17.1 %) [12, 17–19]. Several other research groups also reported these two hot spot mutations in many cancer types; melanoma, bladder cancer, and glioblastoma were reported to have very high frequency of mutation (65–71 %); and hepatocellular carcinoma, basal cell carcinomas (BCC), cutaneous squamous cell carcinoma, thyroid cancer, and central nervous system tumors reported to have high frequency (43–64 %) [12, 17, 18, 20–24]. Both these mutations altered the 11-base nucleotide stretch 5′-CCCC**T**TCCGGG-3′, creating a consensus binding site, GGAA (in reverse complement) for ETS transcription factor. The *TERT* promoter hot spot mutations were shown to increase *TERT* expression by two to fourfold [11, 12]. In the present study, we analyzed the *TERT* promoter mutations in primary tumors of the uterine cervix and oral cavity from south Indian patients and observed *TERT* promoter mutations in 21.4 % of cervical cancer and 31.7 % of oral cancer. Our results showed approximately four times higher *TERT* promoter mutations in cervical cancer and a relatively high frequency in oral cancers compared to the previous study from Western population (uterine cervix 4.5 % (1/22) and head and neck cancer including oral SCC 17.1 % (12/70)) [19]. A recent study analyzing the *TERT* promoter mutation status in SCCs of different anatomical sites reported no mutations in cervical SCC and 16.7 % mutation in head and neck SCC [25], while another study reported *TERT* promoter mutations are less frequent (3.7 %) in cervical cancer [26]. The difference in the frequency of these two hot spot mutations could be due to the ethnicity of the patient population and number of samples analyzed. Further, we observed 26.6 % (8/30) homozygous *TERT* mutations (six 228TT and two 250TT) in cervical cancer cases but were totally absent in oral cancer. All the cases with homozygous mutation were found to be positive for HPV infection. Similarly, a high frequency of homozygous mutations (62 %) was also reported in the skin SCC [27]. The loss of chromosome 5p and HPV infection was reported to be a common event in cervical carcinoma, and this could be the reason for homozygous mutations in HPV-positive cervical SCC tumors [28, 29]. The observed homozygous (TT) mutations could also be due to the presence of two or more copies of isochromosome 5p reported in carcinoma of the uterine cervix [30]. In addition, a less frequently observed *TERT* promoter mutation 242-243CC > TT in BCC, SCC, and melanoma [12, 20], resulting in gain of function via creation of another ETS binding site, was not observed in the present study. The absence of compound heterozygous mutations (C228T and C250T) in this study may be due to the mutually exclusive nature of these two hot spot mutations as reported in earlier studies.

Furthermore, *TERT* promoter mutation screening indicated that the HPV infection was more prevalent in cervical SCC negative for these two hot spot mutations compared to the mutation-positive cases. We observed the majority of the advance stage tumors (77 %) of cervical SCC harbors both *TERT* promoter mutations and HPV (16 and 18) infection. A similar trend was also observed in poor and moderately differentiated tumors (95.7 %) (Table S1). The high prevalence of HPV infection could directly activate *TERT* expression independent of the promoter mutations in cervical cancer, and together, they may confer high risk for cervical carcinogenesis. The HPV E6 protein either alone or in cooperation with E7 protein can activates the *TERT* promoter, thereby making human epithelial cells immortal and the simultaneous degradation of tumor suppressor genes p53 and Rb adding the oncogenic risk [31–38]. Besides the activation of *TERT*, E6 also interacts with various cellular proteins that regulate cell differentiation, apoptosis, gene transcription, adhesion, polarity, proliferation, and chromosomal stability [39, 40].

In summary, we have shown that *TERT* promoter mutations C228T and C250T were frequent in cervical and oral SCCs. We suggest that *TERT* promoter mutations and HPV infection together could be a biomarker for advanced stages of cervical

cancers. Unlike cervical SCC, in oral SCC, the reactivation of *TERT* by promoter mutations could be the core mechanism of oral carcinogenesis. To the best of our knowledge, this is the first study from India reporting high prevalence of *TERT* promoter mutations in SCCs of the uterine cervix and oral cavity, and also associating the HPV infection with the *TERT* mutation in primary tumors of the uterine cervix.

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Compliance with ethical standards

Conflicts of interest None

Ethics approval The Institutional Ethics Committee, Government Arignar Anna Memorial Cancer Hospital, Kancheepuram (No.101041/e1/2009-2), and the Madras Medical College, Chennai (No.04092010), approved the present study. Cervical and oral cancer samples were collected following the Institutional Ethical Committee (IEC) guidelines and informed consent was obtained from each patient, after explaining about the research study. For the illiterate patients, the study was verbally explained and consent was obtained with their thumb impression.

References

1. Ferlay JSI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, et al. GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide: IARC cancer base no. 11. Lyon: International Agency for Research on Cancer; 2012.
2. Ferlay J SI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide: IARC cancer base no. 11. International Agency for Research on Cancer 1.0. 2013.
3. Bosch FX, de Sanjose S Chapter 1: human papillomavirus and cervical cancer—burden and assessment of causality. J Natl Cancer Inst Monogr. 2003;3–13.
4. Dikshit R, Gupta PC, Ramasundarahettige C, Gajalakshmi V, Aleksandrowicz L, et al. Cancer mortality in India: a nationally representative survey. Lancet. 2012;379:1807–16.
5. Byakodi R, Byakodi S, Hiremath S, Byakodi J, Adaki S, et al. Oral cancer in India: an epidemiologic and clinical review. J Community Health. 2012;37:316–9.
6. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Betel-quid and areca-nut chewing and some areca-nut-derived nitrosamines. IARC monographs on the evaluation of carcinogenic risks to humans (2003). v. 85.
7. Gupta PC, Ray CS, Sinha DN, Singh PK. Smokeless tobacco: a major public health problem in the SEA region: a review. Indian J Public Health. 2011;55:199–209.
8. Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW. Telomerase activity in human germline and embryonic tissues and cells. Dev Genet. 1996;18:173–9.
9. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994;266:2011–5.
10. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer. 1997;33:787–91.
11. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, et al. TERT promoter mutations in familial and sporadic melanoma. Science. 2013;339:959–61.
12. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, et al. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339:957–9.
13. Zhang Z, Yang X, Meng L, Liu F, Shen C, et al. Enhanced amplification of GC-rich DNA with two organic reagents. Biotechniques. 2009;47:775–9.
14. Vogelstein B, Kinzler KW. The path to cancer—three strikes and you're out. N Engl J Med. 2015;373:1895–8.
15. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
16. Cong YS, Wen J, Bacchetti S. The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. Hum Mol Genet. 1999;8:137–42.
17. Liu X, Bishop J, Shan Y, Pai S, Liu D, et al. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocr Relat Cancer. 2013;20:603–10.
18. Rachakonda PS, Hosen I, de Verdier PJ, Fallah M, Heidenreich B, et al. TERT promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. Proc Natl Acad Sci U S A. 2013;110:17426–31.
19. Killela PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci U S A. 2013;110:6021–6.
20. Griewank KG, Murali R, Schilling B, Schimming T, Moller I, et al. TERT promoter mutations are frequent in cutaneous basal cell carcinoma and squamous cell carcinoma. PLoS One. 2013;8:e80354.
21. Landa I, Ganly I, Chan TA, Mitsutake N, Matsuse M, et al. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab. 2013;98:E1562–6.
22. Liu X, Wu G, Shan Y, Hartmann C, von Deimling A, et al. Highly prevalent TERT promoter mutations in bladder cancer and glioblastoma. Cell Cycle. 2013;12:1637–8.
23. Nault JC, Mallet M, Pilati C, Calderaro J, Bioulac-Sage P, et al. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. Nat Commun. 2013;4:2218.
24. Vinagre J, Almeida A, Populo H, Batista R, Lyra J, et al. Frequency of TERT promoter mutations in human cancers. Nat Commun. 2013;4:2185.
25. Cheng KA, Kurtis B, Babayeva S, Zhuge J, Tantchou I, et al. Heterogeneity of TERT promoter mutations status in squamous cell carcinomas of different anatomical sites. Ann Diagn Pathol. 2015;19:146–8.
26. Wu RC, Ayhan A, Maeda D, Kim KR, Clarke BA, et al. Frequent somatic mutations of the telomerase reverse transcriptase promoter in ovarian clear cell carcinoma but not in other major types of gynaecological malignancy. J Pathol. 2014;232:473–81.
27. Scott GA, Laughlin TS, Rothberg PG. Mutations of the TERT promoter are common in basal cell carcinoma and squamous cell carcinoma. Mod Pathol. 2014;27:516–23.
28. Mitra AB, Murty VV, Singh V, Li RG, Pratap M, et al. Genetic alterations at 5p15: a potential marker for progression of precancerous lesions of the uterine cervix. J Natl Cancer Inst. 1995;87:742–5.

29. Mitra AB, Murty VV, Li RG, Pratap M, et al. Allelotype analysis of cervical carcinoma. Cancer Res. 1994;54:4481–7.
30. Atkin NB. Significance of chromosome 5 and 17 changes in the development of carcinoma of the cervix uteri. Cytogenet Cell Genet. 2000;91:44–6.
31. Gewin L, Myers H, Kiyono T, Galloway DA. Identification of a novel telomerase repressor that interacts with the human papillomavirus type-16 E6/E6-AP complex. Genes Dev. 2004;18:2269–82.
32. Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA, et al. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. Nature. 1998;396:84–8.
33. Liu X, Dakic A, Chen R, Disbrow GL, Zhang Y, et al. Cell-restricted immortalization by human papillomavirus correlates with telomerase activation and engagement of the hTERT promoter by Myc. J Virol. 2008;82:11568–76.
34. Liu X, Disbrow GL, Yuan H, Tomaic V, Schlegel R. Myc and human papillomavirus type 16 E7 genes cooperate to immortalize human keratinocytes. J Virol. 2007;81:12689–95.
35. Liu X, Roberts J, Dakic A, Zhang Y, Schlegel R. HPV E7 contributes to the telomerase activity of immortalized and tumorigenic cells and augments E6-induced hTERT promoter function. Virology. 2008;375:611–23.
36. Liu X, Yuan H, Fu B, Disbrow GL, Apolinario T, et al. The E6AP ubiquitin ligase is required for transactivation of the hTERT promoter by the human papillomavirus E6 oncoprotein. J Biol Chem. 2005;280:10807–16.
37. Van Doorslaer K, Burk RD. Association between hTERT activation by HPV E6 proteins and oncogenic risk. Virology. 2012;433:216–9.
38. Oh ST, Kyo S, Laimins LA. Telomerase activation by human papillomavirus type 16 E6 protein: induction of human telomerase reverse transcriptase expression through Myc and GC-rich Sp1 binding sites. J Virol. 2001;75:5559–66.
39. Howie HL, Katzenellenbogen RA, Galloway DA. Papillomavirus E6 proteins. Virology. 2009;384:324–34.
40. Tungteakkhun SS, Duerksen-Hughes PJ. Cellular binding partners of the human papillomavirus E6 protein. Arch Virol. 2008;153:397–408.