Cellular and Molecular Life Sciences (2022) 79:110
https://doi.org/10.1007/s00018-021-04113-0

Cellular and Molecular Life Sciences

ORIGINAL ARTICLE

The association of telomere maintenance and TERT expression with susceptibility to human papillomavirus infection in cervical epithelium

Li-Na Wang[1] · Li Wang[1] · Guanghui Cheng[1] · Mingkai Dai[1] · Yunhai Yu[2] · Guoxin Teng[3] · Jingjie Zhao[1] · Dawei Xu[4]

Received: 15 October 2021 / Revised: 5 December 2021 / Accepted: 21 December 2021 / Published online: 31 January 2022
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2022

Abstract

The role of telomerase reverse transcriptase (TERT) induction and telomere maintenance in carcinogenesis including cervical cancer (CC) pathogenesis has been well established. However, it remains unclear whether they affect infection of high-risk human papillomavirus (hrHPV), an initiating event for CC development. Similarly, genetic variants at the *TERT* locus are shown to be associated with susceptibility to CC, but it is unclear whether these SNPs modify the risk for cervical HPV infection. Here we show that in CC-derived HeLa cells, TERT overexpression inhibits, while its depletion upregulates expression of Syndecan-1 (SDC-1), a key component for HPV entry receptors. The TCGA cohort of CC analyses reveals an inverse correlation between TERT and SDC-1 expression ($R=-0.23$, $P=0.001$). We further recruited 1330 females (520 non-HPV and 810 hrHPV-infected) without CC or high-grade cervical intraepithelial neoplasia to analyze telomeres in cervical epithelial cells and SNPs at rs2736098, rs2736100 and rs2736108, previously identified TERT SNPs for CC risk. Non-infected females exhibited age-related telomere shortening in cervical epithelial cells and their telomeres were significantly longer than those in hrHPV-infected group (1.31 ± 0.62 vs 1.19 ± 0.48, $P<0.001$). There were no differences in rs2736098 and rs2736100 genotypes, but non-infected individuals had significantly a higher C-allele frequency (associated with higher TERT expression) while lower T-allele levels at rs2736108 compared with those in the hrHPV group ($P=0.020$). Collectively, appropriate telomere maintenance and TERT expression in normal cervical cells may prevent CC by modulating hrHPV infection predisposition, although they are required for CC development and progression.

Keywords Cervical cancer · HPV · SDC-1 · SNP · Telomere · TERT

✉ Jingjie Zhao
zhaojingjie@sdu.edu.cn

✉ Dawei Xu
Dawei.Xu@ki.se

1 Central Research Laboratory, The Second Hospital, Cheeloo College of Medicine, Shandong University, Jinan 250033, People's Republic of China

2 Department of Gynecology, The Second Hospital, Cheeloo College of Medicine, Shandong University, Jinan 250033, People's Republic of China

3 Department of Pathology, The Second Hospital, Cheeloo College of Medicine, Shandong University, Jinan 250033, People's Republic of China

4 Division of Hematology, Bioclinicum and Center for Molecular Medicine (CMM), Department of Medicine, Karolinska Institutet and Karolinska University Hospital Solna, 171 64 Solna, Sweden

Introduction

Cervical cancer (CC) is one of the most common female malignancies worldwide and primarily caused by cervical infection of human papillomavirus (HPV), the *Papillomaviridae* family DNA virus [1, 2]. As cervical HPV infection is predominantly transmitted through sexual behavior, it mainly occurs in sexually active women. Based on their oncogenic capacity, HPVs are divided into low- and high-risk HPVs (lr and hrHPVs). hrHPV infections may resolve spontaneously, or persist, among which a small fraction eventually develop cervical cancer [3]. These data suggest that hrHPV infection alone is insufficient to drive the formation of cervical cancer and clearly, intrinsic host factors are important players to interact with hrHPV for the pathogenesis of cervical cancer [4, 5]. Indeed, it was shown that genetic heritability contributes to approximately 1/4 incidences of CC [6]. Furthermore, recent genetic analyses

including genome-wide association study (GWAS) have identified many cervical cancer susceptibility loci or single nucleotide polymorphisms (SNPs) [7–10]. However, it remains incompletely understood whether these SNPs or genetic factors are directly involved in CC formation or associated with hrHPV infection. Little has been known about the direct relationship between SNPs/genetic factors and susceptibility to hrHPV infection.

Telomerase is a ribonucleoprotein complex with the reverse transcriptase activity elongating TTAGGG telomeric DNA repeats at linear chromosome termini. These telomeric repeats and their associated proteins form protective structures to maintain chromosomal stability/integration [11]. Telomerase is silent in the majority of normal human cells resulting from the tight transcriptional repression of its rate-limiting, catalytic component *telomerase reverse transcriptase* (*TERT*) gene [11, 12]. The inability of the semiconservative DNA replication mechanism to replicate the very ends of linear chromosomes, so-called the "End replication Problem", results in progressive telomere erosion with cellular replication or with increased age [13, 14], while insufficient telomerase or TERT expression is unable to compensate for such telomere shortening [11, 12]. When telomere length becomes too short to protect chromosomes (dysfunction), the DNA damage response is thus activated, inducing replicative senescence and/or apoptosis of normal cells and preventing their unlimited proliferation [13, 14]. However, on the other hand, telomere shortening or dysfunction causes genomic instability, thereby promoting oncogenesis [11, 12, 14]. Additionally, telomere length is partially inheritable and affected by both intrinsic and external factors as well [15, 16].

In contrast to normal somatic cells, one of the key hallmarks of human malignancies is infinite cellular proliferation, which requires telomere maintenance [11, 12]. In the oncogenic process of the uterus cervix, the *TERT* gene transcription and telomerase activation are required to prevent telomere attrition and subsequently acquire immortal phenotypes [3, 11, 12, 17]. In addition, TERT is capable of acting as a co-factor to regulate gene transcription, thereby promoting carcinogenesis. Given such pivotal roles of TERT/telomerase in cervical cancer development, the genetic variants at the *TERT* locus and the disease risk have been analyzed, and SNPs rs2736100, rs2736098 and rs2736108 were observed to be associated with susceptibility to cervical cancer [15, 18]. However, all these three SNPs are associated with many types of cancer including other female-specific malignancies (breast and/or ovarian cancer) [15, 19–24]. Thus, it remains unclear whether they are associated with HPV infection or participate in other processes in CC development.

In addition to its oncogenic effect, telomere maintenance and TERT are essential to human health, too [25]. For instance, telomere dysfunction or TERT/telomerase dysregulation induces immune cell senescence or impaired immune response [26]. It has also been shown that shorter telomeres in immune cells are associated with a lower activity against viral infection [27]. Moreover, we have recently observed that the genetic variants at rs2736098 and rs2736100 significantly modify the susceptibility to hepatitis B virus infection and the auto-immune disease primary glomerulonephritis, respectively [28, 29]. Given these findings, together with our earlier results showing detectable TERT expression and telomerase activity in normal cervical epithelial cells [30], we hypothesize a link between the TERT/*TERT* variants and/or telomere length and susceptibility to HPV infection. Our results reveal that TERT inhibits the expression of syndecan-1 (SDC-1), a major epithelial form of heparan sulfate proteoglycans (HSPGs) for HPV entry receptors [31, 32]. Telomere length in cervical epithelial cells derived from hrHPV-infected women is significantly shorter than in non-hrHPV-infected ones. Moreover, the *TERT* rs2736108_TT genotype is associated with an increased risk of cervical HPV infection. These findings suggest that appropriate telomere maintenance and physiologically regulated TERT expression/telomerase activity in cervical cells may prevent CC by modulating hrHPV infection predisposition, although aberrant telomerase reactivation via oncogenic events is required for the CC pathogenesis.

Materials and methods

Cell culture, siRNA and vector transfection

CC-derived HeLa cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific), 100 U/ml penicillin, 100 µg/ml streptomycin and 4 mM L-glutamine. Control and TERT siRNAs were purchased from Thermo Fisher Scientific and transfected into HeLa cells with Lipofectamine2000 (Thermo Fisher Scientific) according to the protocol provided by the manufacturer. siRNA sequences are as follows: Control: CCU ACA UCC CGA UCG AUG AUG UUG A, and TERT: AGG CAC UGU UCA GCG UGC UCA ACU A. pBabe and pBabe-hTERT retroviral vectors (kindly provided by Dr. R Weinberg) were used to infect HeLa cells.

RNA extraction, reverse transcription and qPCR

Total RNA was extracted from HeLa cells, normal cervical epithelial cell and primary CC tumors with Trizol-Reagent (Thermo Fisher Scientific), and reversely transcribed using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). qPCR was performed in QuantStudio 7 Flex Real-Time PCRSystem using SYBRGreen (Thermo

Fisher Scientific). Levels of TERT and SDC-1 mRNA were calculated based on the ΔCT values and normalized to human GAPDH expression. Primers used in this study are: TERT: 5′-CGGAAGAGTGTCTGGAGCAA-3′ (Forward) and 5′-GGATGAAGCGGAGTCTGGA-3′ (reverse); SDC-1: 5′-ggc tca ggt gca ggt gct t-3′ (Forward) and 5′-ggg ttc tgg aga cgt ggg aa-3′ (reverse); GAPDH: 5′-GGGAGCCAA AAGGGTCATCA-3′ (Forward) and 5′-TGATGGCATGGA CTGTGGTC-3′ (reverse).

Western blot analysis

Cellular proteins were extracted using Pierce RIPA Buffer (Thermo Scientific) with 1% Phenylmethanesulfonyl fluoride (Sigma-Aldrich) and quantified with DC Protein Assay (Bio-Rad). Thirty µg of proteins were separated in Mini-PROTEAN TGX Gels (Bio-Rad) and transferred to PVDF membranes using Trans-Blot Turbo Transfer Pack (Bio-Rad). Membranes were blocked with 5% non-fat milk diluted in TBST, and then incubated with primary antibodies and secondary antibodies before being imaged with Clarity Max Western ECL Substrate (Bio-Rad, 1,705,062) and ChemiDoc MP Imaging System (Bio-Rad). Primary antibodies include TERT (Abcam, #ab32020), SDC-1 (Proteintech, 66,009–1-Ig) and Actin (Proteintech, 10,593–1-AP). Secondary antibodies include Goat Anti-Mouse IgG (H + L)-HRP Conjugate (Bio-Rad, 170–6516) and Goat Anti-Rabbit IgG (H + L)-HRP Conjugate (Bio-Rad, 170–6515).

TCGA and GSE63514 cohorts of normal cervical specimens and cervical squamous cell carcinoma

The TCGA cohort includes 275 patients with cervical squamous cell carcinoma [33, 34]. RNA sequencing data were available for tumors from these patients and downloaded from The Cancer Genome Atlas Legacy Archieve (http://cancergenome.nih.gov) in March 2021 for analyses. Levels of mRNAs were arbitrarily expressed as RSEM. There are 24 normal cervical specimens and 28 CC tumors with expression profiling of array in GSE63514 [35](GEO Accession viewer, http://www.ncbi.nih.gov/geo), and the data were downloaded on December 1, 2021.

HPV screening and participants

The participants were recruited from the cervical HPV screening program at Shandong University Second Hospital from July 2019 to March 2020, using Cobas4800 HPV assay kits (Roche Diagnostics GmbH, Mannheim, Germany) [36]. A total of 1330 female adults were included in the present study, among which non- and hrHPV-infected individuals were 520 and 810, respectively. Age in these two groups was 39 ± 14 (range 19–83, median 39), and 37 ± 11 (range 18–83, median 38), respectively. Cervical exfoliated cells were collected by removing cervical secretions and rotating clinician-sampling brush a few of times, and the cells on the brush were immediately transferred into a tube containing 10 ml of SurePath® Solution (BD Diagnostics-TriPath, Franklin Lakes, NJ) for cytology/HPV tests. DNA extraction and PCR amplification of HPV genomic DNA was performed using a cobas® 4800 HPV Test kit according to the manufacture's protocol. Briefly, a pool of HPV primers present in the Master Mix is designed to amplify hrHPV DNA from 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The part of collected cells was used for AutoCyte® thin-layer liquid-based cytology test (TriPath Imaging, Inc). Cytology slides were reviewed by the pathologist GT to exclude cervical cancer or high-grade CIN. The study was approved by Shandong University 2nd Hospital Ethics Committee (#KYLL-2016(LW)-0012 and #KYLL-2020(LW)-062) and informed consent was obtained from all participants. The study was performed in accordance with the Declaration of Helsinki.

Assessment of telomere length in cervical epithelial cells

DNA derived from cervical epithelial cells was analyzed for telomere length using real-time PCR as previously described [37, 38]. Briefly, 10 ng of DNA were used for each PCR reaction in triplicate. The primer sequences for human telomere (named Tel 1b and Tel 2b, respectively) and β-globin (named HBG3 and HBG4, respectively) were: Tel1b: 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTT-TGGGTT TGGGTT-3′; Tel2b: 5′-GGCTTGCCTTACCCTTACCCT TACCCTTACCC-TTACCCT-3′; HBG3: 5′-TGTGCTGGC CCATCACTTTG-3′, and HBG4: 5′-ACCAGCCA-CCACTT TCTGATAGG-3′. *T*/HBG (*S*) values (*T*: telomere repeat copy number, and *S*: single-copy gene number) were determined using the formula $T/S = 2 - \Delta Ct$, where ΔCt = average $Ct_{telomere}$ – average $Ct_{\beta\text{-globin}}$. The *T*/*S* ratio was arbitrarily expressed as telomere length. Age-adjusted telomere length for each non-HPV infected control and an infected individual was done by subtracting the subject's linear predicted telomere length from the observed one.

Genotyping of the *TERT* rs2736098, rs2736100 and rs2736108 variants

The *TERT* rs2736098 (C/T), rs2736100 (A/C) and rs2736108 (C/T) variants were genotyped using a MassARRAY® System. The Agena Bioscience (Agena Bioscience, Nanjing, China) Assay Design Suite V2.0 software (https://agenacx.com/online-tools/) was used to design following amplification and extension primers: rs2736098: 5′-ACG TTGGATGCGTGGTTTCTGTGTGGTGTC-3′ (1st PCR),

5′-ACGTTGGATGAGAGGAAGTGCTTGGTCTCG-3′ (2nd PCR) and 5′-GCCATCCGTGGGCCGCCAGCACCA CGC-3′ (unextended mini-sequencing primer); rs2736100: 5′-ACGTTGGATGAGAACCACGCAAAGGACAAG-3′ (1st PCR), 5′-ACGTTGGATGGCTGTTTTCCCTGCTGA CTT-3′ (2nd PCR) and 5′-CGGTGTTGAGTGTTTCT-3′ (unextended mini-sequencing primer); rs2736108: 5′-ACG TTGGATGAAGGGGCTCAATCCCAGTAG-3′ (1st PCR), 5′-ACGTTGGATGCAGGCTTCAGGTCCCAGTG-3′ (2nd PCR) and 5′-AGATGTAGAATCAGGGCGC-3′ (Unextended mini-sequencing primer). The Agena MassARRAY platform (Agena Bioscience, San Diego, CA, USA) and Agena Bioscience TYPER version 4.0 were used for the SNPs genotyping and data analysis, respectively.

Telomerase activity assessment

Protein extraction and telomerase activity assay were performed using TeloTAGGG™ Telomerase PCR ELISA kit Roche (Merck SA, Darmstadt, Germany) according to the manufactory protocol. For each assay, 1.0 µg proteins were used, and 28 cycles were run for PCR amplification. The level of telomerase activity was expressed as absorbance [optimal density (OD) in arbitrary units].

Statistical analyses

The relationship between telomere length and age, or between TERT and SDC-1 expression was assessed using Pearson’s test. Differences in telomere length between non-infected controls and HPV-infected individuals were assessed using Student *T*-test. Differences in SDC-1 expression between HPV-positive and negative tumors were assessed using Student *T*-test. The evaluation of distribution differences in alleles of the *TERT* rs2736098, rs2736100 and rs2736108 between different groups were done using χ^2 test. Unconditional univariate and multivariate logistic regression analyses were used to estimate ORs for risk of HPV infection and their 95% confidence intervals (CIs). All the tests were computed using SPSS17.0 software. P values of <0.05 were considered as statistically significant.

Results

TERT-mediated inhibition of SDC-1 expression, a critical component for the HPV entry receptor

Cell surface HSPGs are known to function as HPV entry receptors, an essential first step for HPV infection, while SDC-1 is the major epithelial form of HSPGs and its depletion prevents HPV infection from CC-derived cells [31, 32]. Because TERT has been shown to regulate gene expression [11], we thus determined whether TERT up-regulates SDC-1 expression to promote CC pathogenesis by enhancing SDC-1 expression. Toward this end, we depleted TERT expression in CC-derived HeLa cells using TERT-specific siRNA and then examined SDC-1 expression. Unexpectedly, TERT inhibition led to robust up-regulation of SDC-1 mRNA compared with that in control siRNA-transfected HeLa cells, coupled with an increased protein level (Fig. 1A, B). In contrast, when TERT was over-expressed in these cells by introducing the TERT expression vector, significant down-regulation of SDC-1 expression was observed (Fig. 1A, B). These results collectively demonstrate that TERT inhibits SDC-1 expression in CC cells.

Correlation between TERT and SDC-1 expression in primary CC tumors

Given the findings above, we sought to determine whether there was a relationship between TERT and SDC-1 in primary CC tumors. The TCGA cohort of 275 patients with cervical squamous cell carcinoma was thus analyzed for their TERT and SDC-1 expression. Levels of TERT and SDC-1 mRNAs were expressed as RNA-Seq by Expectation Maximization (RSEM). Consistent with the results obtained from cellular experiments, TERT mRNA levels were inversely correlated with SDC-1 expression ($R^2=-0.23$, $P=0.001$) (Fig. 1C). Moreover, significantly higher levels of SDC-1 expression were observed in HPV-positive tumors than in HPV-negative ones (positive vs negative tumors: 2.53 ± 0.08 vs 0.30 ± 0.75, RSEM) (Fig. 1D).

Telomere shortening in cervical epithelial cells with age

It is well established that telomeres in leukocytes and other types of cells become progressively short with increased age [11, 12, 38], however, the direct determination of telomere length in normal cervical epithelial cells from healthy women of different ages has not been reported. Thus, we first assessed cervical telomere length in 520 healthy individuals. Age in this group was 39 ± 14 (range 19–83, median 39). Telomere length in 508 of 520 individuals was evaluable. Similar to the findings in leukocytes from healthy adults, telomere length varied significantly among examined healthy women with a mean value 1.30 ± 0.60. Nevertheless, an inverse correlation between telomere length and age was observed ($R^2=0.09334$, $P<0.0001$) (Fig. 2A). These results demonstrate that telomeres in cervical epithelial cells shorten progressively with age, exactly as seen in human leukocytes.

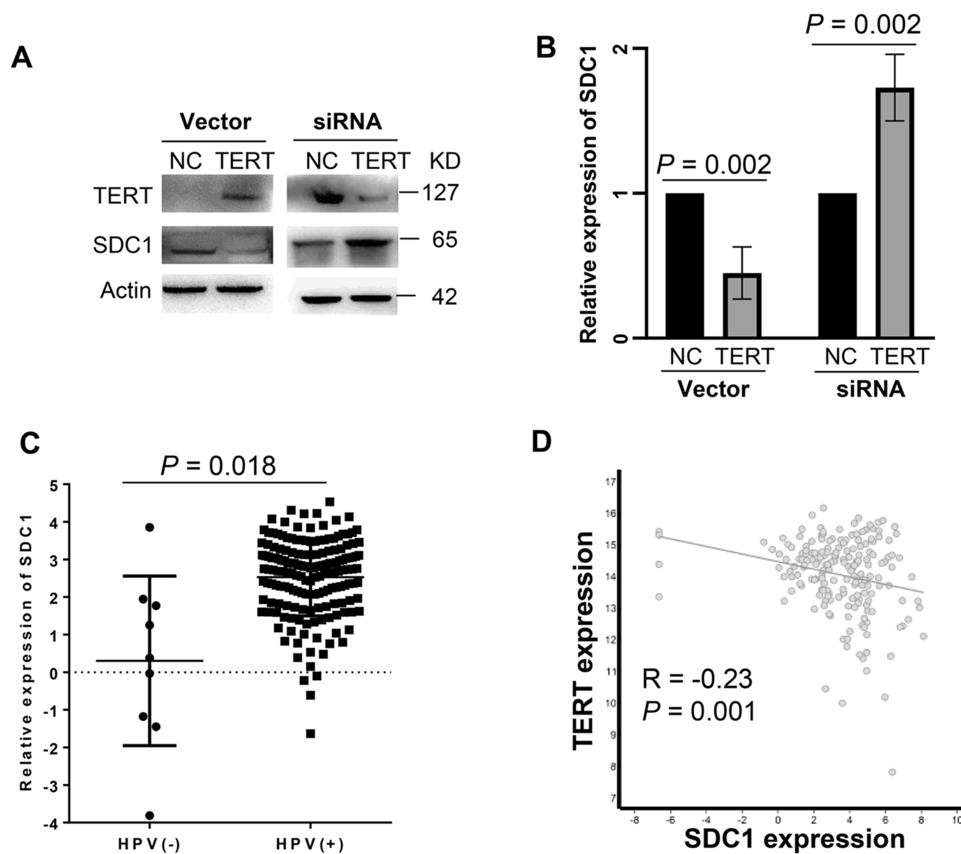

Fig.1 The association between TERT and SDC-1 expression in HeLa cells and primary tumors from patients with cervical cancer. **A**, **B** TERT regulation of SDC-1 expression in HeLa cells. **A** Immunoblotting assessments show efficient overexpression and knockdown of TERT in HeLa cells, which leads to inhibition and upregulation of SDC1 expression, respectively. **B** qPCR analyses show down- and upregulation of SDC-1 mRNA expression in TERT-overexpressed and depleted HeLa cells, respectively. *NC* nonspecific control vector or siRNA, *TERT* nonspecific control vector or siRNA. **C** Higher expression of SDC1 in HPV-positive tumors than in PHV-negative ones from the TCGA cohort of cervical cancer patients. **D** The inverse correlation between TERT and SDC-1 expression in primary tumors from the TCGA cohort of cervical cancer patients. mRNA levels of SDC-1 were expressed as RSEM (RNA-Seq by expectation maximization)

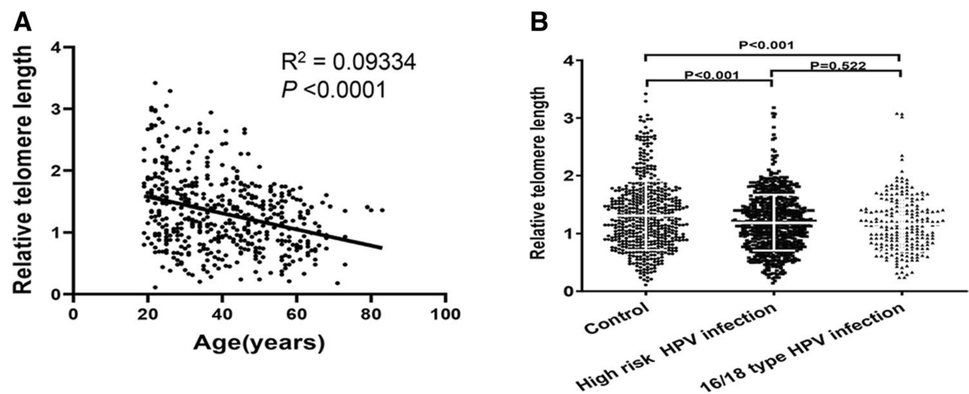

Fig. 2 Telomere length in cervical epithelial cells from non-HPV- and hrHPV-infected women. Telomere length was assessed using qPCR. **A** The inverse correlation between telomere length and age in non-HPV-infected healthy women. **B** Differences in telomere length in cervical epithelial cells between non-HPV- and hrHPV-infected women

Shorter telomeres in cervical epithelial cells from hrHPV-infected females

We then determined telomere length in cervical epithelial cells from 810 hrHPV-infected individuals. Their age was 37 ± 11 (range 18–83, median 38), which was largely matched with that of healthy females. As seen in non-HPV-infected women, telomere length varied significantly among infected individuals and the mean length was 1.19 ± 0.48, which was significantly shorter compared

to that (1.30 ± 0.60) in non-infected group ($P < 0.001$) (Fig. 2B). When divided into HPV16/18 and other hrHPV-infected groups, telomere length was 1.17 ± 0.47 and 1.19 ± 0.48, respectively. There is no difference in telomere length between two HPV-infected groups ($P = 0.522$), however, telomere length in either hrHPV group was significantly shorter than that in the non-infected group (other hrHPV or HPV16/18 vs non-infected: $P < 0.001$) (Fig. 2B). Because significantly shorter telomeres were previously observed in cervical precursor lesions. To rule out this influence, all the recruited hrHPV-infected individuals were excluded from patients with CC or high-grade cervical intraepithelial neoplasia (CIN), as determined using cytological and/or other examinations (Fig. 3).

TERT rs2736098, rs2736100 and rs2736108 genotypes and hrHPV infection risk

The SNPs at *TERT* rs2736098, rs2736100 and rs2736108 have been shown to be associated with the susceptibility to CC. Therefore, we sought to determine whether this link is attributable to the SNP-mediated HPV infection. The genotyping results revealed that the allele distribution of both rs2736098 and rs2736100 did not differ significantly between HPV non-infected and infected groups, or between non-infected group and hrHPV-infected one (Tables 1, 2). In contrast, the allele distribution of rs2736108 was significantly different between the healthy group and HPV-infected one (Table 3): the

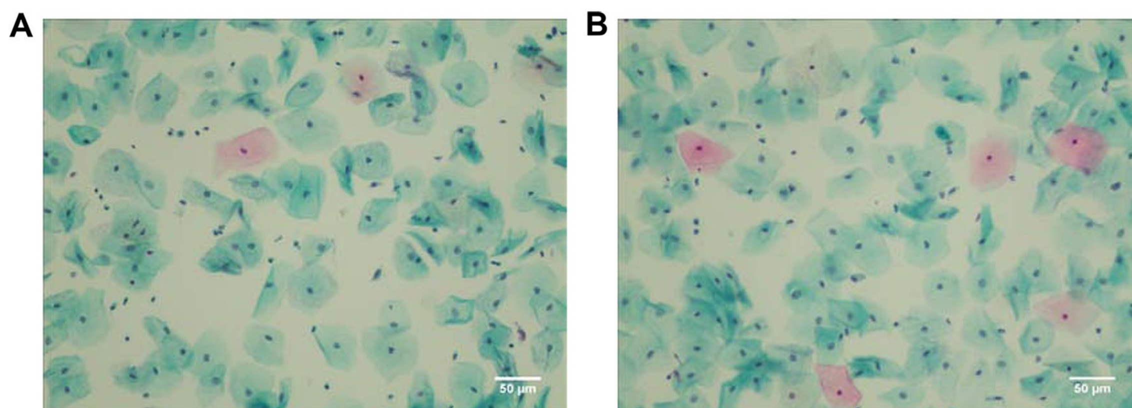

Fig. 3 Morphological examination of cervical smears using Pap Test. Papanicolaou staining was performed and shown are representative images. Magnifications: 200×. Scale bars: 50 µm. **A**, **B** The images from non-HPV- and HPV16-infected women, respectively. There are no abnormal epithelial cells. Cells from basal layers were stained in blue while keratinocytes were in pink

Table 1 The difference in rs2736098 genotypes between non-HPV infected and infected females

	Non-infected controls	hrHPV-infected (total)	Odds ratio (95% CI)	*P* value
Genotype	365 (100%)	625 (100%)		
CC	203 (55.6)	313 (50.1)	1.0 (ref.)	
CT	90 (24.7)	185 (29.6)	1.333 (0.980—1.813)	0.067
TT	72 (19.7)	127 (20.3)	1.144 (0.815—1.605)	0.436
C	496 (67.9)	811 (64.9)	1.0 (ref.)	
T	234 (32.1)	439 (35.1)	1.147 (0.945—1.393)	0.165
	Non-infected controls	HPV16/18-infected	Odds ratio (95% CI)	*P* value
Genotype	365 (100%)	230 (100%)		
CC	203 (55.6)	116 (50.4)	1.0 (ref.)	
CT	90 (24.7)	66 (28.7)	1.283(0.868—1.897)	0.211
TT	72 (19.7)	48 (20.9)	1.167 (0.758—1.795)	0.483
C	496 (67.9)	298 (64.8)	1.0 (ref.)	
T	234 (32.1)	162 (35.2)	1.152 (0.901—1.474)	0.260

hrHPV high-risk human papillomavirus, *CI* confidence interval

Table 2 The difference in rs2736100 genotypes between non-HPV infected and infected females

	Non-infected controls	hrHPV-infected (total)	Odds ratio (95% CI)	*P* value
Genotype	430 (100%)	698 (100%)		
AA	179 (41.6)	283 (40.5)	1.0 (ref.)	
CC	81 (18.8)	130 (18.6)	1.015 (0.726—1.419)	0.930
CA	170 (39.5)	285 (40.8)	0.943 (0.722—1.231)	0.667
A	528 (61.4)	851 (61.0)		
C	332 (38.6)	545 (39.0)	1.019 (0.856—1.213)	0.837
	Non-infected controls	HPV16/18-infected	Odds ratio (95% CI)	*P* value
Genotype	436 (100%)	262 (100%)		
AA	175 (40.1)	108 (41.2)	1.0 (ref.)	
CC	87 (20.0)	43 (16.4)	0.801 (0.517—1.240)	0.319
CA	174 (39.9)	111 (42.4)	1.034 (0.737—1.449)	0.848
A	524 (60.1)	327 (62.4)	1.0 (ref.)	
C	348 (39.9)	197 (37.6)	0.907 (0.726—1.133)	0.391

hrHPV high-risk human papillomavirus, *CI* confidence interval

former with 76.3% C and 23.7% T, respectively, while the later with 72.0% C and 28.0% T, respectively [odd ratios (ORs) = 1.255, 95% CI 1.036–1.519, $P = 0.020$]. The rs2736108_CC genotype was significantly higher in the healthy group than in hrHPV-infected one (60.9% vs 55.6%, OR = 1.547, 95% CI 1.021–2.343, $P = 0.039$) (Table 3). When the comparison was made between non-infected and HPV16/18-infected groups, a similar result was obtained (non-infected vs HPV16/18: CC: 60.9% vs 55.3%, TT: 8.2% vs 13.5%, OR = 1.803, 95% CI 1.097–2.963, $P = 0.019$) (Table 3).

TERT expression and telomerase activity in normal cervical epithelial cells

Finally, we sought to determine whether TERT expression and telomerase activity were present in normal cervical epithelium. Five specimens from non-hrHPV-infected women were available for TERT mRNA and telomerase activity measurement, and HeLa cells and 5 primary CC tumors were included for a comparison. TERT mRNA levels were 0.324 ± 0.100 and 0.838 ± 0.171 in normal cervical and CC specimens, respectively ($P = 0.03$), and telomerase activity was 0.168 ± 0.020 and 0.24 ± 0.022, respectively ($P = 0.06$)

Table 3 The difference in rs2736108 genotypes between non-HPV infected and infected females

	Non-infected controls	hrHPV-infected (total)	Odds ratio (95% CI)	*P* value
Genotype	450 (100%)	732 (100%)		
CC	274 (60.9)	407 (55.6)	1.0 (ref.)	
CT	139 (30.9)	240 (32.8)	1.162 (0.897–1.506)	0.255
TT	37 (8.2)	85 (11.6)	1.547 (1.021–2.343)	**0.039**
C	687 (76.3)	1054 (72.0)	1.0 (ref.)	
T	213 (23.7)	410 (28.0)	1.255 (1.036–1.519)	**0.020**
	Non-infected controls	HPV16/18-infected	Odds ratio (95% CI)	*P* value
Genotype	450 (100%)	275 (100%)		
CC	274 (60.9)	152 (55.3)	1.0 (ref.)	
CT	139 (30.9)	86 (31.3)	1.115(0.799–1.558)	0.522
TT	37 (8.2)	37 (13.5)	1.803 (1.097–2.963)	**0.019**
C	687 (76.3)	390 (70.9)	1.0 (ref.)	
T	213 (23.7)	160 (29.1)	1.323 (1.041–1.682)	**0.022**

hrHPV high-risk human papillomavirus, *CI* confidence interval

P value in bold: statistically significant

(Figs. 4A, B). Both TERT expression and telomerase activity in primary cells were much lower than those in HeLa cells (Fig. 4A, B right panels). In addition, the GSE63514 data analyses showed even comparable levels of TERT expression in normal cervical specimens and CC tumors (1.73 ± 0.007 vs 1.72 ± 0.004, $P = 0.365$) (Fig. 4C).

Discussion

The role of TERT induction and telomere maintenance in carcinogenesis including CC pathogenesis has been well established [3, 11]. However, it remains poorly understood whether they affect infection of HPV, a master event initiating CC development. Similarly, a number of the genetic variants including rs2736098, rs2736100 and rs2736108 at the *TERT* locus were shown to be associated with susceptibility to cervical cancer and other female-specific malignancies [15, 18–23], but it is unclear whether these SNPs modify risk for cervical HPV infection, a key factor to drive cervical cancer pathogenesis. In the present study, we show that TERT inhibits the expression of SDC-1, a key HPV entry receptor. We further demonstrate significantly higher frequencies of the rs2736108 T allele and TT genotype, coupled with shorter telomeres in cervical cells, from HPV-infected women than those in non-infected healthy controls.

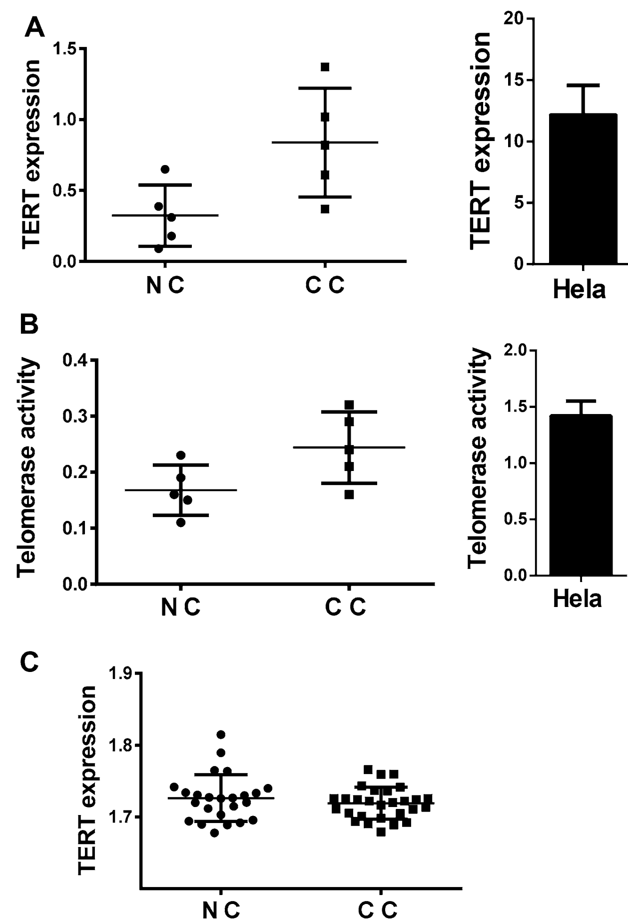

Fig. 4 TERT expression and telomerase activity in normal cervical epithelial cells. TERT mRNA expression and telomerase activity were determined using qPCR and using (**A**) TERT mRNA expression in normal cervical epithelial specimens ($N = 5$), primary CC tumors ($N = 5$) and HeLa cells. TERT mRNA expression was determined using qPCR and GAPDH served as an internal control for normalization. **B** Telomerase activity in the same sets of materials above assessed using a TeloTAGGG™ Telomerase PCR ELISA kit. The level of telomerase activity was expressed as absorbance [optimal density (OD) in arbitrary units]. **C** TERT mRNA expression in normal cervical specimens ($N = 24$) and primary CC tumors ($N = 28$) derived from GSE63514 based on Affymetrix microarray data

HPV entry is initiated by binding to HSPG cell surface attachment factors, whereas SDC-1, the predominant form of HSPG in keratinocytes, serves as a primary HPV-cellular interacting partner [31, 32]. When SDC-1 was knocked down in CC-derived HaCAT cells, HPV infection of these cells was almost completely blocked. Intriguingly, we observed that TERT strongly inhibited SDC-1 expression. Conceivably, TERT-mediated SDC-1 down-regulation prevents HPV entry into target cells. This is supported by a significantly higher level of SDC-1 expression in HPV-positive tumors than in HPV-negative ones, based on the TCGA data analyses. It has long been characterized that TERT acts as a transcription co-factor independently of its telomere lengthening function, thereby regulating gene expression; moreover, by doing so, TERT also changes DNA methylation and other epigenetic statuses, leading to widespread alterations in the transcriptome [39–43].

Our findings above were obtained from CC-derived cells where TERT is substantially expressed [30]. It may be argued that the scenario is totally different in normal cervical cells because human differentiated cells, in general, do not express TERT. However, different levels of TERT expression are detectable in stem/progenitor cells, activated lymphocytes, proliferating keratinocytes, and normal cervical cells [11, 25, 26, 30, 44–46]. Our present results further demonstrate that normal cervical epithelial cells do express TERT and telomerase activity, although lower than in CC tumors and HeLa cells, and GSE data analyses even show equal enrichments of TERT expression between normal cervical tissues and primary CC tumors. As cervical stem/progenitor cells are HPV target cells [47], ideally, experiments should be performed on these cells to determine TERT and SDC-1 expression and their effect on HPV infection. In addition, it is currently unclear how exactly TERT regulates SDC-1 expression. All these issues call for detailed investigations in the future.

Among >200 types of HPVs, approximately 60 exhibit affinity for cervical epithelial cells and they are classified into low and high-risk groups based on their oncogenic capacity [3]. The low-risk group includes HPV6, 11, 42,

43, and 44, while the high-risk group consists of HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66, and 68, with HPV16 and 18 being the most frequent and strongest carcinogenic drivers [3]. Women with cervical infection of hrHPVs frequently experience spontaneous virus clearance due to efficient cell-mediated immune response. Failure to do so (persistent HPV infection) has been shown to occur in individuals carrying mutations in a panel of genes including EVER1/TMC6, EVER2/TMC8 RHOH, MST-1, CORO1A and CXCR4, with all these mutations leading to a defective immune system for HPV clearance [48]. In addition, the genetic variants at HLA loci are significantly associated with cervical HPV infection and cervical cancer risk [49, 50]. Moreover, it has also been well characterized that telomerase or TERT and telomeres play pivotal parts in regulating immunological activity [26]. Activation of telomerase via induction of TERT expression occurs in activated lymphocytes for their expansion in response to infectious challenge [26, 51]. Shorter leukocyte telomere length was associated with increased susceptibility to experimentally induced acute upper respiratory virus infection and clinical illness in adults [27]. Consistently, much shorter telomeres impair proliferation potential of immune cells, compromising immune response to pathogens or host resistance to infection. Thus, TERT variants may modify the risk of HPV infection by influencing host immune function. In support of this hypothesis, the rs2736108_TT genotype has been shown to be correlated with shorter telomere length in leukocytes [15, 52]. Moreover, the TERT promoter bearing rs2736108_TT exhibits significantly lower activity in breast and ovary cancer cells under certain settings [19]. In the present study, we did not determine leukocyte or lymphocyte telomere length in control and hrHPV-infected women, but the healthy group exhibited significantly longer telomeres than did the hrHPV-infected group.

Telomere length is affected by both intrinsic and external factors, and shortened telomeres have been shown to be associated with age-related diseases including cancer [38, 53]. Mechanistically, shorter telomeres lead to telomere dysfunction, thereby inducing genomic instability and chromosome aberrations to promote cellular transformation [12]. Therefore, shorter telomeres together with HPV infection in cervical epithelial cells may synergistically drive cervical cancer formation. This scenario has been observed in HPV-related oropharyngeal carcinoma [54]. On the other hand, it is well characterized that the HPV E6 protein interacts with intrinsic factors such as c-MYC to activate *TERT* gene transcription and telomerase activity [3, 55]. In addition, HPV DNA is capable of integrating into the human genome and may activate *TERT* transcription by inserting into the TERT promoter or enhancer regions [11, 56]. Taken together, *TERT* genetic variants, shorter telomeres and hrHPV may orchestratedly cooperate to contribute to the CC pathogenesis.

It is well established that telomeres shorten with increased age, but most results are obtained from analyses of blood leukocytes or other types of cells. The present findings for the first time demonstrate that telomere erosion occurs in normal cervical epithelial cells with age. It is evident from our results that telomere length in cervical epithelial cells varies significantly among both hrHPV-infected and non-infected individuals. It will be interesting to probe whether shorter telomeres interact with HPV infection to synergistically promote cervical cancer development.

The present study has limitations. First, we show the association between hrHPV-infection and shorter telomere length in the cervical epithelium, however, TERT expression and telomerase activity were not determined in all cervical specimens from individuals with and without hrHPV infection for a direct comparison. It is also possible that shorter telomeres observed in the hrHPV-infected group are consequences of increased cell proliferation driven by hrHPV infection. Therefore, direct evidence is required to ascertain a causal relationship between them. Second, our findings obtained from TERT knockdown and TCGA primary tumors suggest that TERT may prevent hrHPV infection by inhibiting SDC-1 expression. This hypothesis remains to be tested by performing HPV infection experiments on TERT-manipulated cervical epithelial stem/progenitor cells. In summary, the present findings unexpectedly reveal a strong inhibition of SDC-1 expression mediated by TERT. Moreover, telomere length is significantly shorter in hrHPV-infected cervical epithelial cells than in non-infected ones. Thus, appropriate telomere maintenance and TERT expression in normal cervical epithelium may prevent HPV infection. The variants at the *TERT* locus associated with lower TERT expression may weaken immune response to HPV infection. These results collectively suggest a dual role of TERT and telomere maintenance: they are required for the CC pathogenesis on one hand, but reduce CC risk by blocking HPV infection on the other hand. These results reveal a complicated relationship among telomere biology, HPV infection and the carcinogenesis of the uterus cervix.

Acknowledgements We thank Dr. Robert Weinberg (Massachusetts Institute of Technology) For the TERT expression vector.

Author contributions L-NW, JZ and DX performed study concept and design; L-NW, LW, MD, GC and YY performed development of methodology and experiments; GT performed cytology and histology examination; L-NW, LW, GC and MD provided acquisition, analysis and interpretation of data, and statistical analysis; L-NW, JZ and DX wrote the manuscript. All authors read and approved the final manuscript.

Funding The study was supported by grants from Shandong Provincial Natural Science Foundation, China (2016ZDJS07A09 and ZR201702160271), Key R & D projects of Shandong Province, China (2017GSF201065), Natural Science Foundation of China (No.31571421), the Swedish Cancer Society, Swedish Research

Council, the Cancer Society in Stockholm and Karolinska Institutet Foundation.

Data availability The TCGA datasets can be found at http://cancergenome.nih.gov. GSE63514 data can be downloaded from GEO Accession viewer, http://www.ncbi.nih.gov/geo.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethics approval and consent to participate Shandong University 2nd Hospital Ethics Committee approved the study (#KYLL-2016(LW)-0012 and #KYLL-2020(LW)-062) and informed consent was obtained from all participants. The study was performed in accordance with the Declaration of Helsinki.

References

1. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV (2002) The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 55(4):244–265
2. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Eng J Med 348(6):518–527
3. Panczyszyn A, Boniewska-Bernacka E, Glab G (2018) Telomeres and telomerase during human papillomavirus-induced carcinogenesis. Mol Diagn Ther 22(4):421–430
4. Chen D, Gyllensten U (2015) Lessons and implications from association studies and post-GWAS analyses of cervical cancer. Trends Genet 31(1):41–54
5. Magnusson PK, Lichtenstein P, Gyllensten UB (2000) Heritability of cervical tumours. Int J Cancer 88(5):698–701
6. Chen D, Cui T, Ek WE, Liu H, Wang H, Gyllensten U (2015) Analysis of the genetic architecture of susceptibility to cervical cancer indicates that common SNPs explain a large proportion of the heritability. Carcinogenesis 36(9):992–998
7. Chen D, Juko-Pecirep I, Hammer J, Ivansson E, Enroth S, Gustavsson I, Feuk L, Magnusson PK, McKay JD, Wilander E et al (2013) Genome-wide association study of susceptibility loci for cervical cancer. J Nat Cancer Inst 105(9):624–633
8. Takeuchi F, Kukimoto I, Li Z, Li S, Li N, Hu Z, Takahashi A, Inoue S, Yokoi S, Chen J et al (2019) Genome-wide association study of cervical cancer suggests a role for ARRDC3 gene in human papillomavirus infection. Hum Mol Genet 28(2):341–348
9. Shi Y, Li L, Hu Z, Li S, Wang S, Liu J, Wu C, He L, Zhou J, Li Z et al (2013) A genome-wide association study identifies two new cervical cancer susceptibility loci at 4q12 and 17q12. Nat Genet 45(8):918–922
10. Leo PJ, Madeleine MM, Wang S, Schwartz SM, Newell F, Pettersson-Kymmer U, Hemminki K, Hallmans G, Tiews S, Steinberg W et al (2017) Defining the genetic susceptibility to cervical neoplasia—a genome-wide association study. PLoS Genet 13(8):e1006866
11. Yuan X, Larsson C, Xu D (2019) Mechanisms underlying the activation of TERT transcription and telomerase activity in human cancer: old actors and new players. Oncogene 38(34):6172–6183
12. Daniel M, Peek GW, Tollefsbol TO (2012) Regulation of the human catalytic subunit of telomerase (hTERT). Gene 498(2):135–146
13. Olovnikov AM (1996) Telomeres, telomerase, and aging: origin of the theory. Exp Gerontol 31(4):443–448
14. Linskens MH, Harley CB, West MD, Campisi J, Hayflick L (1995) Replicative senescence and cell death. Science 267(5194):17
15. Bojesen SE, Pooley KA, Johnatty SE, Beesley J, Michailidou K, Tyrer JP, Edwards SL, Pickett HA, Shen HC, Smart CE et al (2013) Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. Nat Genet 45(4):371–384
16. Codd V, Wang Q, Allara E, Musicha C, Kaptoge S, Stoma S, Jiang T, Hamby SE, Braund PS, Bountziouka V et al (2021) Polygenic basis and biomedical consequences of telomere length variation. Nat Genet 53(10):1425–1433
17. Salamati A, Majidinia M, Asemi Z, Sadeghpour A, Oskoii MA, Shanebandi D, Alemi F, Mohammadi E, Karimian A, Targhazeh N et al (2020) Modulation of telomerase expression and function by miRNAs: anti-cancer potential. Life Sci 259:118387
18. Wang S, Wu J, Hu L, Ding C, Kan Y, Shen Y, Chen X, Shen H, Guo X, Hu Z (2012) Common genetic variants in TERT contribute to risk of cervical cancer in a Chinese population. Mol Carcinog 51(Suppl 1):E118-122
19. Beesley J, Pickett HA, Johnatty SE, Dunning AM, Chen X, Li J, Michailidou K, Lu Y, Rider DN, Palmieri RT et al (2011) Functional polymorphisms in the TERT promoter are associated with risk of serous epithelial ovarian and breast cancers. PLoS ONE 6(9):e24987
20. de Martino M, Taus C, Lucca I, Hofbauer SL, Haitel A, Shariat SF, Klatte T (2016) Association of human telomerase reverse transcriptase gene polymorphisms, serum levels, and telomere length with renal cell carcinoma risk and pathology. Mol Carcinog 55(10):1458–1466
21. McKay JD, Hung RJ, Gaborieau V, Boffetta P, Chabrier A, Byrnes G, Zaridze D, Mukeria A, Szeszenia-Dabrowska N, Lissowska J et al (2008) Lung cancer susceptibility locus at 5p15.33. Nat Genet 40(12):1404–1406
22. Melin BS, Nordfjall K, Andersson U, Roos G (2012) hTERT cancer risk genotypes are associated with telomere length. Genet Epidemiol 36(4):368–372
23. Wolpin BM, Rizzato C, Kraft P, Kooperberg C, Petersen GM, Wang Z, Arslan AA, Beane-Freeman L, Bracci PM, Buring J et al (2014) Genome-wide association study identifies multiple susceptibility loci for pancreatic cancer. Nat Genet 46(9):994–1000
24. Lu Y, Yan C, Du J, Ji Y, Gao Y, Zhu X, Yu F, Huang T, Dai J, Ma H et al (2017) Genetic variants affecting telomere length are associated with the prognosis of esophageal squamous cell carcinoma in a Chinese population. Mol Carcinog 56(3):1021–1029
25. Saretzki G (2018) (2012) Telomeres, telomerase and ageing. Subcell Biochem 90:221–308
26. Weng NP (2012) Telomeres and immune competency. Cur Opin Immunol 24(4):470–475
27. Cohen S, Janicki-Deverts D, Turner RB, Casselbrant ML, Li-Korotky HS, Epel ES, Doyle WJ (2013) Association between telomere length and experimentally induced upper respiratory viral infection in healthy adults. JAMA 309(7):699–705
28. Cheng G, Yuan X, Wang F, Sun Q, Xin Q, Li K, Sun C, Lin Z, Luan Y, Xu Y et al (2017) Association between the telomerase rs2736098_TT genotype and a lower risk of chronic hepatitis B and cirrhosis in Chinese males. Clin Transl Gastroenterol 8(3):e79
29. Sun Q, Liu J, Cheng G, Dai M, Qi Z, Zhao J, Li W, Kong F, Liu G, Bjorkholm M et al (2020) The telomerase gene polymorphisms, but not telomere length, increase susceptibility to primary glomerulonephritis/end stage renal diseases in females. J Transl Med 18(1):184
30. Liu L, Liu C, Lou F, Zhang G, Wang X, Fan Y, Yan K, Wang K, Xu Z, Hu S et al (2011) Activation of telomerase by seminal

plasma in malignant and normal cervical epithelial cells. J Pathol 225(2):203–211
31. Surviladze Z, Dziduszko A, Ozbun MA (2012) Essential roles for soluble virion-associated heparan sulfonated proteoglycans and growth factors in human papillomavirus infections. PLoS Pathog 8(2):e1002519
32. Koganti R, Suryawanshi R, Shukla D (2020) Heparanase, cell signaling, and viral infections. Cell Mol Life Sci 77(24):5059–5077
33. Ojesina AI, Lichtenstein L, Freeman SS, Pedamallu CS, Imaz-Rosshandler I, Pugh TJ, Cherniack AD, Ambrogio L, Cibulskis K, Bertelsen B et al (2014) Landscape of genomic alterations in cervical carcinomas. Nature 506(7488):371–375
34. Cancer Genome Atlas Research Network (2017) Integrated genomic and molecular characterization of cervical cancer. Nature 543(7645):378–384
35. den Boon JA, Pyeon D, Wang SS, Horswill M, Schiffman M, Sherman M, Zuna RE, Wang Z, Hewitt SM, Pearson R et al (2015) Molecular transitions from papillomavirus infection to cervical precancer and cancer: Role of stromal estrogen receptor signaling. Proc Nat Acad Sci USA 112(25):E3255-3264
36. Song F, Du H, Xiao A, Wang C, Huang X, Liu Z, Zhao M, Men H, Wu R (2020) Type-specific distribution of cervical hrHPV infection and the association with cytological and histological results in a large population-based cervical cancer screening program: baseline and 3-year longitudinal data. J Cancer 11(20):6157–6167
37. Cawthon RM (2002) Telomere measurement by quantitative PCR. Nucl Acids Res 30(10):e47
38. Yuan X, Kronstrom M, Hellenius ML, Cederholm T, Xu D, Sjogren P (2018) Longitudinal changes in leukocyte telomere length and mortality in elderly Swedish men. Aging 10(10):3005–3016
39. Luo Z, Wang W, Li F, Songyang Z, Feng X, Xin C, Dai Z, Xiong Y (2019) Pan-cancer analysis identifies telomerase-associated signatures and cancer subtypes. Mol Cancer 18(1):106
40. Ding D, Xi P, Zhou J, Wang M, Cong YS (2014) Human telomerase reverse transcriptase regulates MMP expression independently of telomerase activity via NF-κB-dependent transcription. FASEB J 27(11):4375–4383
41. Liu Z, Li Q, Li K, Chen L, Li W, Hou M, Liu T, Yang J, Lindvall C, Bjorkholm M et al (2013) Telomerase reverse transcriptase promotes epithelial-mesenchymal transition and stem cell-like traits in cancer cells. Oncogene 32(36):4203–4213
42. Yu J, Yuan X, Sjoholm L, Liu T, Kong F, Ekstrom TJ, Bjorkholm M, Xu D (2018) Telomerase reverse transcriptase regulates DNMT3B expression/aberrant DNA methylation phenotype and AKT activation in hepatocellular carcinoma. Cancer lett 434:33–41
43. Liu N, Guo XH, Liu JP, Cong YS (2020) Role of telomerase in the tumour microenvironment. Clin Exp Pharmacol Physiol 47(3):357–364
44. Kang MK, Guo W, Park NH (1998) Replicative senescence of normal human oral keratinocytes is associated with the loss of telomerase activity without shortening of telomeres. Cell Growth Differ 9(1):85–95
45. Harle-Bachor C, Boukamp P (1996) Telomerase activity in the regenerative basal layer of the epidermis inhuman skin and in immortal and carcinoma-derived skin keratinocytes. Proc Nat Acad Sci USA 93(13):6476–6481
46. Ge Z, Liu C, Bjorkholm M, Gruber A, Xu D (2006) Mitogen-activated protein kinase cascade-mediated histone H3 phosphorylation is critical for telomerase reverse transcriptase expression/telomerase activation induced by proliferation. Mol Cell Biol 26(1):230–237
47. Doorbar J, Griffin H (2019) Refining our understanding of cervical neoplasia and its cellular origins. Papillomavirus Res 7:176–179
48. Leiding JW, Holland SM (2012) Warts and all: human papillomavirus in primary immunodeficiencies. J Allergy Clin Immunol 130(5):1030–1048
49. Del Rio-Ospina L, Camargo M, Soto-De Leon SC, Sanchez R, Moreno-Perez DA, Patarroyo ME, Patarroyo MA (2020) Identifying the HLA DRB1-DQB1 molecules and predicting epitopes associated with high-risk HPV infection clearance and redetection. Sci Rep 10(1):7306
50. Balasubramaniam SD, Wong KK, Oon CE, Balakrishnan V, Kaur G (2020) Comparative transcriptomic profiling in HPV-associated cervical carcinogenesis: implication of MHC class II and immunoglobulin heavy chain genes. Life Sci 256:118026
51. Luiten RM, Pene J, Yssel H, Spits H (2003) Ectopic hTERT expression extends the life span of human CD4+ helper and regulatory T-cell clones and confers resistance to oxidative stress-induced apoptosis. Blood 101(11):4512–4519
52. Do SK, Yoo SS, Choi YY, Choi JE, Jeon HS, Lee WK, Lee SY, Lee J, Cha SI, Kim CH et al (2015) Replication of the results of genome-wide and candidate gene association studies on telomere length in a Korean population. Korean J Intern Med 30(5):719–726
53. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA (2003) Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 361(9355):393–395
54. Zhang Y, Sturgis EM, Dahlstrom KR, Wen J, Liu H, Wei Q, Li G, Liu Z (2013) Telomere length in peripheral blood lymphocytes contributes to the development of HPV-associated oropharyngeal carcinoma. Cancer Res 73(19):5996–6003
55. Liu X, Dakic A, Chen R, Disbrow GL, Zhang Y, Dai Y, Schlegel R (2008) Cell-restricted immortalization by human papillomavirus correlates with telomerase activation and engagement of the hTERT promoter by Myc. J Virol 82(23):11568–11576
56. Li W, Tian S, Wang P, Zang Y, Chen X, Yao Y (2019) The characteristics of HPV integration in cervical intraepithelial cells. J Cancer 10(12):2783–2787

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.