

High expression of TMEM33 predicts poor prognosis and promotes cell proliferation in cervical cancer

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Research Article

Keywords: CESC, TMEM33, prognosis, immune infiltration, cell proliferation

Posted Date: January 14th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1252853/v1>

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Abstract

Background

As one of the most common cancer among women worldwide, the prognosis of patients with advanced cervical cancer remains unsatisfactory. A study indicated that transmembrane protein 33 (TMEM33) was implicated in tumor recurrence, while its role in cervical cancer has not been elucidated.

Methods

TMEM33 expression in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) was primarily screened in The Cancer Genome Atlas (TCGA), and further validated in Gene Expression Omnibus (GEO) database. The Kaplan–Meier plotter analysis and Cox regression were constructed to evaluate the prognostic value of TMEM33 in CESC. Functional enrichment analysis was performed with GO, KEGG and GSEA tools. Protein-protein interaction analysis and correlated gene networks were conducted using STING and GEPIA2 websites, respectively. The expression of TMEM33 in cervical cancer cells were examined by immunoblotting and RT-qPCR. Finally, CCK-8 assay and colony formation assay were performed to investigate the role of TMEM33 in cervical cancer cell proliferation.

Results

TMEM33 expression was significantly elevated in CESC compared with normal tissues. High expression of TMEM33 was associated with poor prognostic clinical characteristics in CESC patients. KM-plotter analysis revealed that patients with increased TMEM33 had shorter overall survival (OS), progress free interval (PFI), and disease specific survival (DSS). Moreover, Multivariate Cox analysis further confirmed that high TMEM33 expression was an independent risk factor for OS in patients with CESC. TMEM33 was associated with immune cell infiltration, and its expression was correlated with tumorigenesis-related genes RNF4, OCIAD1, TMED5, DHX15, MED28 and LETM1. More importantly, knockdown of TMEM33 in cervical cancer cells decreased the expression of those genes and inhibited cell proliferation.

Conclusions

Increased TMEM33 in cervical cancer can serve as an independent prognostic marker and might play a role in tumorigenesis by promoting cell proliferation.

Background

Cervical cancer is the fourth most common cancer among women, with approximately 600,000 new cases and 300,000 deaths reported every year[1]. Although the incidence and mortality rates are decreasing due to early diagnosis and vaccines, these methods do not affect regression of advanced

cervical cancer. More than 80% of cervical cancer is the result of human papillomavirus (HPV) infection, of which 70% are positive for high-risk HPV type 16 or 18[2]. Persistent infection with HPV induces high grade cervical intraepithelial neoplasia (CIN), which may progress to invasive cervical cancer if left untreated[3]. The International Federation of Gynecology and Obstetrics (FIGO) system is commonly used for cervical cancer staging[4]. However, significant differences in survival are observed in the same FIGO staging, suggesting that more prognostic markers are needed for reflecting cancer biodiversity, improving individual risk stratification, and for directing a personalized treatment regimen.

Transmembrane protein 33 (TMEM33) is a three-pass transmembrane domain protein conserved throughout evolution[5]. Human TMEM33 has been shown to locate in the nuclear envelope and endoplasmic reticulum (ER) in vitro, serve as a regulator of the tubular ER network by suppressing the membrane-shaping activity of reticulons[6]. At present, limited functional studies of TMEM33 in multicellular organisms have been reported. Savage AM. et. al. characterized TMEM33 is required in an EC-specific manner for Vegfa-mediated Ca²⁺ oscillations, to promote angiogenesis in zebrafish embryos[7]. TMEM33 also regulates intracellular calcium homeostasis in a polycystin-2 (PC2)-dependent manner, causes cathepsins translocation and sensitization to apoptosis in renal tubular epithelial cells[8]. Recently, TMEM33 was shown as a downstream effector of PKM2 in regulating the activation of SREBPs and lipid metabolism[9]. Although the role of TMEM33 is gradually revealed in different aspects, its expression in cancer and implications in tumorigenesis are largely unknown, warranting further exploration.

In the present study, we identified TMEM33 was markedly overexpressed in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) dataset. The aberrant expression was verified by Immunoblotting and RT-qPCR in cervical cancer cells. The common biological pathways and enrichment analysis of the differentially expressed genes were performed by GO, KEGG and GSEA. The interacted protein network and correlated genes were explored utilizing the STRING and the GEPIA2 websites, respectively. Subsequently, the prognostic value of TMEM33 in cervical cancer and its role in immune infiltration and cell proliferation were evaluated.

Methods

TCGA, GTEx and GEO dataset

Clinical information of CESC patients and gene expression quantification data were downloaded from the TCGA dataset. Gene expression data with clinical information on healthy cervix tissue were obtained from the Genotype-Tissue Expression (GTEx) database. A total of 306 cervical cancer samples and 13 healthy cervix controls were enrolled in the present study. GSE63514 (with 24 normal cervix tissues and 28 cervical cancer tissues) was used as a validation dataset[10]. Gene expression and clinical data of patients in GSE63514 were downloaded from the GEO database. No extra Ethics committee approval was necessary since the data were downloaded from public databases.

Data processing

R programming language (version 3.6.3) was the principal tool for analyzing data throughout the study. The differentially expressed genes between cervical cancer samples and healthy controls with different clinical stages were obtained using the ggplot2 package (version 3.3.3)[11]. GO and KEGG analysis were conducted by the clusterProfiler package (version 3.14.3), and visualized by the ggplot2 package. GSEA analysis was performed using the clusterProfiler package (version 3.14.3)[12]. Survival analysis and survival curves were done by the survminer packages (version 0.4.9). Survival pROC package (version 1.17.0.1) was used to create a ROC curve. Nomogram and calibration analysis were generated using rms package (version 6.2-0)[13]. Immune infiltration analysis was performed by the GSVA package (version 1.34.0)[14].

STRING and GEPIA2 websites

STRING website (<https://string-db.org/>) hosts a large collection of integrated and consolidated protein-protein interaction data. We imported TMEM33 with the main settings such as meaning of network edges “evidence”, active interaction sources “experiments”, the minimum required interaction score “Low confidence (0.150)”, and max number of interactors to show “no more than 50 interactors” operated.

GEPIA2 website (<http://gepia2.cancer-pku.cn/>) is a web server supporting normal and cancer gene expression profiling and interactive analysis. The top 200 TMEM33 correlated genes in CESC were obtained via “similar gene detection” module.

Cell culture, RNAi and cell transfection

Cervical cancer cell lines HeLa, SiHa, CaSki, C33A and normal cervix-derived cell line H8 were purchased from ATCC. All cell lines were cultured in a complete medium with 10% FBS (Gibco, USA) as previously described[15]. For cell transfection, cells were seeded onto a 60 mm dish and transfected with a final concentration of 20 nM siRNA per target gene using Lipofectamine 2000 transfection reagents according to the manufacturer’s instruction (Invitrogen, USA). The siRNA sequences (Ruibo, China) were shown in Supplementary Table 1.

Real-time quantitative PCR and Immunoblotting

RT-qPCR and immunoblotting were performed as described previously[16]. All the primers were synthesized by Biosune, with the primer sequences listed in Supplementary Table 1. The TMEM33 polyclonal antibody (Bethyl, USA) was used at 1:1000.

CCK-8 assay and colony formation assay

The cell viability was determined by the Cell Counting Kit-8 (Apexbio, USA) according to the manufacturer’s instruction and read by the Multiskan GO microplate reader (ThermoFisher, USA) at 450

nm. An amount of 800 cells per well was seeded in six-well plates and incubated for 14 days until colonies appeared. The colonies were fixed with methanol and stained with 0.5% crystal violet solution.

Statistical analysis

R programming language and GraphPad prism 7.0 software were used for statistical analysis. Unpaired Student t-test was used to analyze the differences between 2 groups. Univariate Cox regression and multivariate Cox regression analysis were performed to evaluate the prognostic value of TMEM33 with CESC. Kaplan-Meier curve was used to assess differences in overall survival, disease specific survival and progress free interval between high-risk score group and low-risk score group, the significance was determined by log-rank test. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated to evaluate variables associated with overall survival. $P < 0.05$ was used as a cutoff value for significance.

Results

1. TMEM33 is highly expressed in cervical squamous cell carcinoma and endocervical adenocarcinoma

Based on the TCGA and GTEx databases, we examined the expression profile of TMEM33 in pan-cancer analysis. As shown in Figure 1a, among 33 cancer types, TMEM33 was significantly highly expressed in 24 cancers compared with normal tissues. For TCGA tumors and cancer-adjacent normal tissues, TMEM33 expression was significantly up-regulated in 14 cancer types (Fig. 1b). Specifically, TMEM33 expression was increased in CESC (Fig. 1c), and was further validated in the GSE63514 dataset (Fig. 1d). The expression of TMEM33 was higher in adenosquamous compared with squamous cell carcinoma of CESC (Fig. 1e). In order to explore the clinical value of TMEM33 in CESC diagnosis, we performed ROC curve analysis. As the area under the curve (AUC) was 0.881, TMEM33 showed significantly high sensitivity and specificity for CESC diagnosis (Fig. 1f).

2. Association of TMEM33 expression and clinicopathological characteristics in CESC

We examined the clinicopathological characteristics of CESC patients based on the mean value of TMEM33 expression. As shown in Table 1, the high expression of TMEM33 was significantly associated with Height ($P=0.048$), N stage ($P=0.003$) and Histological type ($P=0.003$), while it was not associated with other features. The results of univariate analysis using logistic regression indicated that TMEM33 expression was correlated with poor prognostic clinical characteristics in CESC patients (Table 2). High TMEM33 expression was correlated with N stage (OR=0.372, $P=0.002$) and Histological type (OR=0.366, $P=0.002$).

3. Identification of TMEM33 as an independent prognostic indicator in CESC

Next, Kaplan-Meier analysis was used to assess the predictive value of TMEM33 on clinical outcomes. As shown in Figures 2a-c, high expression of TMEM33 predicted poor prognosis in both overall survival (OS, HR: 1.97, $P=0.01$), progress free interval (PFI, HR: 3.13, $P=0.001$) and disease specific survival (DSS, HR: 2.40, $P=0.006$). In addition, we conducted univariate and multivariate analysis using the Cox proportional

hazards regression model. As shown in Table 3 and Supplementary Figure 1, the univariate cox regression analysis revealed TMEM33, clinical T, N and M stages were positive prognostic factors informing worsen CESC patient overall survival. However, only T stage, N stage and TMEM33 were independent risk factors for overall survival in multivariate regression cox analysis.

Moreover, a nomogram was constructed taking into account the statistically significant prognostic factors in multivariate Cox regression analysis to predict 3, 5 and 7-year overall survival (Fig. 2d). The calibration plots demonstrated good agreement between prediction and observed events (Fig. 2e). These data illustrate that the high expression of TMEM33 predicts poor prognosis of CESC.

4. Functional enrichment analysis of differentially expressed genes of TMEM33

To better understand the potential function and molecular pathways of the TMEM33 gene in CESC, we analyzed the differentially expressed genes (DEGs) based on the TCGA dataset. A total of 1,178 genes related to TMEM33 expression were altered (711 up-regulated, 467 down-regulated, $|\log_2(\text{FC})| > 1$ & $p.\text{adj} < 0.05$), which may reflect the potential value of TMEM33 on CESC pathogenesis (data not shown).

Subsequently, TMEM33 and its DEGs were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis. The top 10 enrichment pathways from each analysis result were exhibited. As shown in Figure 3a, the gene clusters were involved in epidermal cell differentiation, production of the molecular mediator of immune response, immunoglobulin production, and complement activation in the category of biological processes. For molecular function, the DEGs were enriched at receptor ligand activity, glycosaminoglycan binding, immunoglobulin receptor binding and antigen binding, etc. (Fig. 3b). In the category of cellular components, the DEGs were mostly distributed in immunoglobulin complex, apical part of cell, apical plasma membrane, intermediate filament cytoskeleton and blood microparticle (Fig. 3c). The KEGG pathway analysis showed that the DEGs were enriched in neuroactive ligand-receptor interaction, estrogen signaling pathway, vascular smooth muscle contraction, etc. (Fig. 3d). The GO and KEGG analysis demonstrate that TMEM33 might participate in signal transduction pathways and immune regulation.

5. GSEA identifies a TMEM33-related signaling pathway

Using the low- and high-TMEM33 expression datasets, we performed GSEA to identify the most significantly altered pathways based on the gene sets from the MSigDB (c2.cp.kegg.v6.2.symbols.gmt). As shown in Figure 4a-f, gene sets related to genes encoding extracellular matrix proteins, cellular response to external stimulus, calcium mobilization, innate immune system, adaptive immune system and complement cascade, with the threshold of NES > 1.5 and $p.\text{adj} < 0.05$. Interestingly, in addition to the pathways that have been reported to reflect the biological functions of TMEM33 as a transmembrane protein, some immune-related signaling pathways were enriched, which provided additional evidence that TMEM33 might be implicated in immune response.

6. Association of TMEM33 and immune cell infiltration in CESC

To confirm our hypothesis, we determined the infiltration of 24 immune cell types in CESC using the ssGSEA method, and subsequently the association between TMEM33 and immune cell infiltration was investigated by Spearman's correlation analysis. As shown in Figure 5a, T helper cells and Eosinophils were positively correlated with TMEM33 expression. However, Th1 cells, Treg, iDC, DC, Cytotoxic cells, B cells, T cells, CD56dim NK cells, pDC and aDC showed a significant negative association with TMEM33. More specifically, we assessed the infiltration levels of the top two relevant immune cells of each group, T helper cells, Eosinophils, Th1 cells and Treg in distinct TICRR groups, which showed consistent trends with the forest plots (Fig. 5b). Further, the correlation between TMEM33 expression and T helper cells, Th1 cells, Treg, DC, Cytotoxic cells and B cells in CESC were exhibited by scatter plots (Fig. 5c).

7. Functional inference of TMEM33 in CESC

In order to investigate the internal mechanism of TMEM33 in tumorigenesis, the PPI network analysis was performed by utilizing the STRING database. As shown in Figure 6a, the interaction network of 50 TMEM33-binding proteins with the experimental evidence identification was visualized. In addition, we analyzed the similar genes of TMEM33 in CESC using the "similar gene detection" module of GEPIA2. Corresponding hierarchical clustering analysis of the top 20 similar genes was displayed by the heatmap (Fig. 6b). Through comparing the 50 TMEM33-interacted genes and the 200 TMEM33-similar genes, we screened out the common molecule RNF4 (Fig. 6c), which plays a role in protein ubiquitination[17]. Moreover, the TMEM33 expression level was remarkably positively correlated with similar genes of RNF4, OCIAD1, TMED5, DHX15, MED28 and LETM1 (Fig. 6d), which have been reported to be implicated in tumorigenesis of various cancers[18-23].

We further performed enrichment analysis using the above 250 TMEM33-interacted and similar genes. The top 5 enrichment pathways of MF, CC and BP were shown in Figure 6e. However, there was no pathway enriched with KEGG owing to the few number of genes selected.

8. TMEM33 promotes cell proliferation of cervical cancer cells in vitro

To investigate the expression level of TMEM33 in cervical cancer cells, we performed RT-qPCR and immunoblotting in cervical cancer cell lines HeLa, SiHa, CaSki, H8 and C33A. TMEM33 was up-regulated in SiHa at the mRNA level (Fig. 7a), while highly expressed in HeLa and SiHa at the protein level (Fig. 7b). Based on that analysis, HeLa (HPV 18+) and SiHa (HPV 16+) cells were chosen for further functional studies. TMEM33 knockdown was performed via RNAi and the transfection efficiency was shown in Figure 7c.

Since six tumorigenesis related TMEM33-correlated genes were filtered by bioinformatics tools (Fig. 6c), we verified their association with TMEM33 in cervical cancer cells. As shown in Figure 7d and e, the mRNA expression of RNF4, OCIAD1, TMED5, MED28 and LETM1 were significantly decreased in HeLa-siTMEM33 cells compared with control cells, while all six genes were significantly down-regulated in SiHa-siTMEM33 cells, indicating a certain correlation existed.

Next, cell viability was assessed by CCK-8 assay. HeLa-siTMEM33 and SiHa-siTMEM33 cells showed a significant reduction of cell viability compared with control cells after transfection in 96 hours (Fig. 7f-g). In addition, colony formation assay showed that TMEM33 knockdown reduced the clonogenicity of HeLa cells (Fig. 7h) and SiHa cells (Fig. 7i). The above results suggest that TMEM33 contributes to cell proliferation in cervical cancer HeLa and SiHa cells, which provide evidence of the carcinogenesis role of TMEM33 in CESC.

Discussion

The incidence and mortality rates of cervical cancer are still high in developing or least-developed economies. The strategy of cancer treatment depends on pretreatment disease status according to FIGO stage. Despite the early stage of cervical cancer can be treated with surgery or chemoradiotherapy, the survival of patients with advanced cervical cancer is suboptimal, and varying clinical outcomes of cervical cancer patients with the same FIGO stage are also common[24]. Fortunately, reliable biomarkers can be used to assist tumor diagnosis, prognosis, and predict the response to therapy or outcome. Thanks to the progress of bioinformatics techniques, the seeking of promising biomarkers for CESC is more effective. For instance, decreased expression of NUSAP1 and TP73 predicts poor overall survival in cervical cancer[25, 26]. Additionally, some autophagy-related genes are confirmed as survival prediction signatures in CESC[27]. Despite the prognosis value of these biomarkers has been revealed, those studies are short of molecular networking analysis, and have no further functional experimental validation, an understanding of the comprehensive molecular mechanisms is lacking. In our study, we found TMEM33 was up-regulated in CESC and most other cancer types. TMEM33 was confirmed as a potential marker for the diagnosis of CESC with high sensitivity and specificity using ROC curve analysis. We determined the predictive value of TMEM33 for OS, PFI, and DSS in CESC patients. Multivariate Cox analysis further confirmed that the high TMEM33 expression was an independent risk factor for OS in patients with CESC. Pathway analysis revealed DEGs were enriched in immune response pathways. Finally, we examined the expression of TMEM33 in cervical cancer cells and uncovered its role in facilitating cell proliferation.

TMEM33 is a conserved protein harboring three transmembrane domains, regulating the tubular ER network[28]. TMEM33 is highly expressed in breast cancer cells and up-regulated in early recurrent breast cancer specimens compared with non-recurrent breast tumors[6], however, its expression in the majority of cancers has not been well characterized. Through bioinformatics analysis on TCGA and GTEx datasets, we found TMEM33 was up-regulated in 24 of 33 cancer types compared with normal tissues, indicating TMEM33 might be involved in tumorigenesis. The ROC curve for TMEM33 discrimination of CESC diagnosis had an AUC of 0.881, strongly suggesting that TMEM33 was a potential biomarker for CESC diagnosis. High TMEM33 expression was correlated with height, N stage and histological type. Moreover, cervical cancer patients with higher TMEM33 levels showed strikingly worse OS, PFI, and DSS. The nomogram and calibration curve indicated a possibility that it could represent a prognostic biomarker for cervical cancer.

Given limited previous research with regard to TMEM33 in cancer, we performed functional analysis based on GO, KEGG and GESA in CESC. Interestingly, the DEGs were enriched in immune responses and immunoregulation, in addition to pathways in signal transduction, small molecule biosynthesis, and transmembrane transport. It has been reported that RNF26 co-assembles with TMEM43, TMED1, TMEM33, and ENDOD1 to form a complex, modulating innate immune signaling through the cGAS-STING pathway[29]. Here, we revealed the underlying relationship between TMEM33 expression and immune cell infiltration. Tumor-infiltrating immune cells are implicated in tumor progression, metastasis and chemoradiotherapy resistance[30]. We found TMEM33 expression was positively correlated with T helper cells and eosinophils, but negatively correlated with DCs, Th1 cells, cytotoxic cells, B cells and T cells. As the antigen presenting cells, DCs activate CD8+ T cells and then initiate anti-tumor responses[31]. Th1 cells play a vital role in anti-cancer immunity. Th1 polarization enhance CTLs-mediated killing of tumor cells and tumor regression[32]. T cells, especially the CD8+ cytotoxic T cells mediated cellular immunity participant in anti-tumor immunity[33]. Therefore, highly expressed TMEM33 in cervical cancer may potentially impact tumor immunity thereby contributing to oncogenesis.

To further elucidate the underlying biological function of TMEM33, we analyzed TMEM33-interacted and similar genes using STRING and GEPIA2 websites, respectively. The common gene RNF4, is an E3 ubiquitin-protein ligase involved in chromosome alignment, spindle assembly, hypoxia and heat shock responses, and regulation of transcription. Studies found that up-regulated RNF4 stabilizes certain oncoproteins and potentiates tumor cell properties[21]. Other five similar genes OCIAD1, TMED5, DHX15, MED28 and LETM1 were also selected according to their role in tumorigenesis, and their correlation with TMEM33 were analyzed. Increased TMED5 expression facilitates cell proliferation, invasion and migration in cervical cancer cells[34]. LETM1 is a target of miR-613 in suppressing cervical cancer progression[19]. In summary, the molecular networking analysis provided more evidence to deeply understand the potential mechanism of TMEM33 in cervical cancer.

The last issue of interest we addressed was the role of TMEM33 in cervical cancer cells. In zebrafish, TMEM33 mediates the effects of VEGF during angiogenesis and is required for VEGF-mediated cytosolic Ca²⁺ signaling within endothelial tip cells. TMEM33 knockdown inhibits endothelial cells proliferation in response to VEGF[7]. After we examined the expression of TMEM33 in cervical cancer cells, HeLa and SiHa cells were further selected for the TMEM33 functional study. Inhibition of TMEM33 decreased cell viability and colony formation in HeLa and SiHa cells, indicating TMEM33 was involved in cell proliferation regulation. In addition, we examined the mRNA expression of RNF4, OCIAD1, TMED5, DHX15, MED28, and LETM1 in TMEM33 knockdown cervical cancer cells. Five of these genes were significantly decreased in HeLa cells while all six genes were decreased in SiHa cells. Consequently, TMEM33 dysregulation may contribute to the tumorigenesis of cervical cancer.

Conclusions

Taken together, this is the first study to investigate the oncogenic role of TMEM33 in CESC. High expression of TMEM33 predicted poor prognosis and was considered as an independent prognostic

factor and potential therapeutic target for cervical cancer. Increased TMEM33 expression correlated with immune cell infiltration and promoted cell proliferation in cervical cancer. Our findings revealed a novel role of TMEM33 in tumorigenesis of CESC.

Abbreviations

TMEM33

Transmembrane protein 33

CESC

Cervical squamous cell carcinoma and endocervical adenocarcinoma

HPV

Human papillomavirus

CIN

Cervical intraepithelial neoplasia

TCGA

The Cancer Genome Atlas

GEO

Gene Expression Omnibus

GTE_x

Genotype-Tissue Expression

DEGs

Differentially expressed genes

AUC

Area under the curve

OS

Overall survival

DSS

Disease specific survival

PFI

Progress free interval

IQR

Interquartile range

CI

Confidence interval

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the National Natural Science Foundation of China (82002755), Natural Science Foundation of Shandong Province (ZR2020QH206), and Medical and Health Science and Technology Development Project of Shandong Province (2019WS502).

Authors' contributions

LW and WM designed the research; HC and SZ performed the bioinformatics analysis; YL carried out the cell proliferation assay; HC and LW wrote the paper; YW and WM revised the manuscript.

Acknowledgements

Not applicable.

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Tables

Table 1. Clinicopathological characteristics of CESC patients with differential TMEM33 expression.

Characteristic	levels	Low expression of TMEM33	High expression of TMEM33	P value
n		153	153	
Age, n (%)	<=50	99 (32.4%)	89 (29.1%)	0.291
	>50	54 (17.6%)	64 (20.9%)	
Weight, n (%)	<=70	61 (22%)	77 (27.8%)	0.063
	>70	78 (28.2%)	61 (22%)	
Height, n (%)	<=160	59 (22.5%)	75 (28.6%)	0.048*
	>160	73 (27.9%)	55 (21%)	
T stage, n (%)	T1	71 (29.2%)	69 (28.4%)	0.121
	T2	36 (14.8%)	36 (14.8%)	
	T3	14 (5.8%)	7 (2.9%)	
	T4	2 (0.8%)	8 (3.3%)	
N stage, n (%)	N0	58 (29.7%)	76 (39%)	0.003**
	N1	41 (21%)	20 (10.3%)	
M stage, n (%)	M0	53 (41.7%)	63 (49.6%)	0.410
	M1	7 (5.5%)	4 (3.1%)	
Clinical stage, n (%)	Stage I	77 (25.8%)	85 (28.4%)	0.234
	Stage II	34 (11.4%)	35 (11.7%)	
	Stage III	29 (9.7%)	17 (5.7%)	
	Stage IV	9 (3%)	13 (4.3%)	
Histological type, n (%)	Adenosquamous	16 (5.2%)	37 (12.1%)	0.003**
	Squamous cell carcinoma	137 (44.8%)	116 (37.9%)	
Age, median (IQR)		45 (38, 56)	47 (39, 56)	0.221

Table 2. Logistic regression analysis of the association between clinicopathological characteristics and TMEM33 expression in CESC patients.

Characteristics	Total(N)	Odds Ratio(OR)	P value
T stage (T2&T3&T4 vs. T1)	243	1.009 (0.606-1.680)	0.972
N stage (N1 vs. N0)	195	0.372 (0.194-0.695)	0.002**
M stage (M1 vs. M0)	127	0.481 (0.120-1.681)	0.263
Clinical stage (Stage II&Stage III&Stage IV vs. Stage I)	299	0.818 (0.518-1.289)	0.387
Histological type (Squamous cell carcinoma vs. Adenosquamous)	306	0.366 (0.189-0.681)	0.002**

Table 3. Cox regression analysis for clinical outcomes in CESC patients.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	306				
<=50	188	Reference			
>50	118	1.289 (0.810-2.050)	0.284		
Weight	277				
<=70	138	Reference			
>70	139	0.761 (0.463-1.248)	0.279		
Height	262				
<=160	134	Reference			
>160	128	1.094 (0.635-1.888)	0.746		
T stage	243				
T1	140	Reference			
T2	72	1.152 (0.562-2.359)	0.700	0.000 (0.000-Inf)	0.992
T3	21	2.710 (1.167-6.292)	0.020*	0.904 (0.117-6.994)	0.923
T4	10	8.088 (3.419-19.135)	<0.001***	84.580 (7.056-1013.898)	<0.001***
N stage	195				
N0	134	Reference			
N1	61	2.844 (1.446-5.593)	0.002**	2.760 (1.023-7.442)	0.045*
M stage	127				
M0	116	Reference			
M1	11	3.555 (1.187-10.641)	0.023*	0.925 (0.000-Inf)	1.000
TMEM33	306				
Low	153	Reference			

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
High	153	1.692 (1.053-2.718)	0.030*	3.739 (1.189-11.758)	0.024*

Figures

Figure 1

TMEM33 expression in pan-cancers and CESC. Box plots assessing the expression profiles of TMEM33 between tumor and normal tissue (a) or between tumor and pericarcinous tissue (b) in different types of cancers based on TCGA and GTEx datasets. (c) The expression of TMEM33 in normal cervical tissue and CESC tissue. (d) The validation of TMEM33 expression in GSE63514 dataset. (e) The expression profile of TMEM33 based on histological type of CESC. (f) Receiver operating characteristic (ROC) curve analysis evaluating the performance of TMEM33 for CESC diagnosis. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 2

Prognostic value of TMEM33 in CESC based on the TCGA database. Kaplan–Meier analyses comparing overall survival (a), progression-free interval (b), and disease-specific survival (c) between low- and high-TMEM33 expression groups. (d) A nomogram for predicting probability of patients with 3-, 5- and 7-year overall survival. (e) Calibration plots validating the efficiency of nomograms for overall survival.

Figure 3

Enrichment analysis of TMEM33-correlated DEGs in CESC. Bubble plots display the functional enrichment results by TMEM33 expression correlated DEGs in terms of biological processes (a), molecular functions (b), cellular components (c), and KEGG signaling pathways. The functional categories and pathways were annotated with color gradient bubbles of different sizes.

Figure 4

Gene set enrichment analysis (GSEA) results based on the TMEM33 mRNA expression in CESC. (a-f) Significantly enriched pathways with $p_{adj} < 0.05$, such as Matrisome, Cellular responses to external stimuli, Role of LAT2/NTAL/LAB on calcium mobilization, Innate immune system, Adaptive immune system, and Complement cascade.

Figure 5

Immune infiltration analysis of TMEM33 in CESCC. (a) The forest plot shows the correlation between TMEM33 expression level and 24 immune cells. (b) The enrichment scores of TMEM33 expression in T helper cells, Eosinophils, Th1 cells, and TReg. (c) The correlation between TMEM33 expression and T helper cells, Th1 cells, TReg, DC, Cytotoxic cells, and B cells.

Figure 6

Analysis of TMEM33 correlated genes and PPI network. (a) The visualizing interaction network of TMEM33-binding proteins was generated base on STRING database. (b) Top 20 genes positively related to TMEM33 obtained from GEPIA2 were displayed in heatmap. (c) An intersection analysis of 200 TMEM33-similar genes and 50 TMEM33-interacted genes was performed. (d) Correlation analysis between TMEM33 expression and screened tumorigenesis-related genes, including RNF4, OCIAD1, TMED5, DHX15, MED28, and LETM1. (e) GO enrichment analysis by TMEM33-similar and interacted genes.

Figure 7

TMEM33 promotes cell proliferation in cervical cancer. (a) RT-qPCR analysis of relative mRNA level of TMEM33 in cervical cancer cell lines (HeLa, SiHa, CaSki, H8 and C33A). (b) Immunoblotting analysis of TMEM33 expression in cervical cancer cell lines. (c) siTMEM33 (20 nM) and siCon (20 nM) were transfected with Lipofectamine 2000 according to the manufacturer's protocol. At 48 h post-transfection, the efficiency was determined by RT-qPCR. (d and e) RT-qPCR analysis of relative mRNA level of RNF4, OCIAD1, TMED5, DHX15, MED28 and LETM1 in HeLa and SiHa cells transfected with TMEM33 siRNA. (f and g) CCK-8 assay of HeLa and SiHa cells transfected with TMEM33 siRNA. (h and i) Colony formation assay of HeLa and SiHa cells transfected with TMEM33 siRNA. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Supplementary Files

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