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Original Article

Silencing PDCD4 Mediates TFEB Overexpression Promoting Proliferation, Migration and Invasion of Cervical Cancer HeLa Cells

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ABSTRACT

Aim/Background: Cervical cancer is a common gynecologic malignant tumor, its occurrence and development are related to genetic and environmental factors. Recent studies have shown that Programmed Cell Death 4 (PDCD4) and Transcription Factor EB (TFEB) plays crucial roles in the pathogenesis of cervical cancer. The interaction between PDCD4 and TFEB and their regulatory mechanism on cellular functions in cervical cancer have not been fully explored. **Materials and Methods:** Therefore, this study utilized the Hela cell line as a cervical cancer model to investigate the changes in TFEB expression levels and the proliferation, migration, invasion and EMT processes of cervical cancer cells through the silencing of PDCD4. Real-time quantitative PCR and Western blot were employed to assess the expression levels of PDCD4 and TFEB, while CCK-8, scratch assay, Transwell invasion assay and Western blot were used to evaluate changes in cell proliferation, migration, invasion capabilities and EMT processes. **Results:** The experimental results demonstrated that silencing PDCD4 significantly increased the expression level of TFEB. Simultaneously, silencing PDCD4 also significantly accelerated the proliferation rate of Hela cells, enhanced the cells' migration, invasion capabilities and promoted the EMT processes. Further experimental results showed that silencing TFEB could partially reverse the promoting effects of PDCD4 silencing on cell proliferation, migration and invasion. In cervical cancer, silencing PDCD4 can lead to TFEB overexpression, thereby promoting the proliferation, migration and invasion of Hela cells. **Conclusion:** These findings provide crucial clues for the in-depth study of molecular mechanisms in cervical cancer and indicate that the PDCD4-TFEB pathway could potentially serve as a target for the treatment and prevention of this disease.

Keywords: Cervical Cancer, PDCD4, TFEB, Proliferation, Migration, Invasion.

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INTRODUCTION

Cervical cancer remains a leading cause of cancer-related mortality among women in developing countries, ranking as the second most common malignancy worldwide among females.[1] Although Human Papillomavirus (HPV) has been identified as a primary pathogen, evidence suggests that HPV infection alone is insufficient to induce malignant transformation, indicating the importance of other genetic alterations in the development of cervical cancer.[2]

The characterization of established molecular markers should aid in unraveling the molecular mechanisms underlying cervical cancer, with implications for diagnosis, prognosis and treatment. The pathogenesis of cervical cancer is associated with the regulation of various genes within tumor cells.

Transcription Factor EB (TFEB), a member of the transcription factor family, plays pivotal roles in cellular processes like autophagy, lysosome formation and clearance.[3] Aberrant expression of TFEB in various tumor types is closely related to malignant behaviors such as cell proliferation, migration and invasion.[4] In previous studies, TFEB is mainly observed when investigating the autophagy and lysosomal activities of cervical cancer cells.[5,6] Recent study suggested that TFEB-mediated lysosome biogenesis promoted PD-L1 protein degradation and inhibited cervical cancer progression.[7] However, the understanding of TFEB's mechanistic role and its regulatory factors in cervical cancer require further investigation.

Programmed Cell Death 4 (PDCD4), a protein inhibiting transcription and translation, exerts significant influence over the cell cycle, proliferation and transformation processes.[8]

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Additionally, PDCD4 has been identified as a tumor suppressor gene with its expression level being downregulated or even absent in tumor tissues.[9] PDCD4 not only directly exerts anti-tumor effects but also participates in the functional deployment of various anti-cancer drugs. An increasing body of research has shown that abnormal expression of PDCD4 in tumors is closely associated with promoting malignant behaviors like cancer cell proliferation, invasion and metastasis.[10] Interestingly, tumor suppressor PDCD4 decreases nuclear levels of TFEB, PDCD4 deficiency promotes the anti-tumor effect of macrophages by enhancing TFEB expression in the tumor microenvironment, indicating the potential interaction of TFEB and PDCD4.[11] Nonetheless, in cervical cancer, the interaction between PDCD4 and TFEB and their regulatory mechanism on cellular functions have not been fully explored.[12] Hence, this study aims to delve into the impact of PDCD4 silencing-mediated TFEB overexpression on the proliferation, migration and invasion of cervical cancer Hela cells. We anticipate that a detailed investigation of the interaction between TFEB and PDCD4 will unveil novel molecular mechanisms involved in cervical cancer progression, providing a robust theoretical foundation for devising more effective treatment modalities and inhibiting cervical cancer metastasis. Concurrently, the insights garnered from this study could potentially offer fresh perspectives and references for the development of treatment strategies for other types of tumors.

MATERIALS AND METHODS

Cell Culture

HeLa and H8 cells were obtained from Hunan Fenghui (WC0019 and SC20210512008). The recovery culture flasks were retrieved, the old culture medium was aspirated and the cells were collected by centrifugation. Cells were washed twice with PBS, followed by the addition of trypsin-EDTA solution (Hyclone, South Logan, UT, USA, SH30042) to disperse the cells. The trypsin action was terminated by adding an adequate amount of fresh culture medium containing serum (Wuhan Puno Sai, Wuhan, China, 164210) (Wuhan Puno Sai, Wuhan, China, PM150210). The cells were then centrifuged and the cell clumps were disrupted by pipetting up and down several times. Once thoroughly mixed, cells were transferred to new culture flasks according to the dilution ratio and placed in a CO_2 incubator under culture conditions of 5% CO_2, saturated humidity and 37°C.

Detection of PDCD4 and TFEB mRNA Expression by RT-qPCR

Cells were seeded in 12-well plates and cultured until the end of the cultivation period. Total RNA from each group of cells was extracted following the instructions of the TRIzol reagent manual (TaKaRa, Tokyo, Japan, 9108). The RNA was reverse transcribed into cDNA according to the instructions of the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Tokyo, Japan, RR047A). Primers for RT-PCR were designed using Primer software, with the primer sequences listed in Table 1. The primer sequences were verified for non-specific amplification through NCBI n Blast alignment. The reaction systems were prepared and the amplification and melting curves for RT-PCR were carried out using the ABI VII a7 Real-Time PCR System. After the reaction, the specificity of the PCR products was confirmed by melting curve analysis, checking for non-specific bands and primer dimerization. The Ct values obtained for each sample were used to calculate the relative mRNA expression levels using the formula $2^{-\Delta\Delta Ct}$. Here, ΔCt=Ct value of the target gene -Ct value of the reference gene and -ΔΔCt=average ΔCt value of the blank control group -ΔCt value of each sample group.

Detection of PDCD4, TFEB and EMT-related protein expression by Western blotting

Cells were seeded in a 12-well plate and upon the completion of culturing, cells were harvested, washed, lysed and centrifuged to collect the supernatant. The protein concentration was determined using a BCA protein assay kit (Beyotime, P0010, Shanghai, China). The extracted proteins were mixed with sample buffer and boiled at 100°C for 10 min, followed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a PVDF membrane (Millipore, USA, ISEQ00010). The membrane was blocked with 5% non-fat milk at room temperature for 1 hr and primary antibodies were added followed by overnight incubation at 4°C. The membrane was then washed with TBST for 30 min, changing the solution every 10 min. Secondary antibodies were added and incubated at room temperature for 2 hr, followed by another wash with TBST for 30 min, changing the solution every 10 min. The membrane was exposed using ECL detection in a dark room (Tanon, 180-5001, Shanghai, China) and images were captured under Tanon 5200 Chemiluminescent Imaging System. The images were analyzed using Image J software.

CCK-8 Assay for Cell Proliferation Evaluation

Cell concentration was adjusted to 5×10^3 cells/well and seeded in a 96-well plate, followed by overnight culturing. Three replicate wells were set for each group, with the following treatment groups: (1) NC group; (2) si-PDCD4 group; (3) si-TFEB group; (4) si-PDCD4+si-TFEB group. After incubating the cells in a 37°C, 5% CO_2 cell culture incubator for 48 hr, cell proliferation

Table 1: Primer Sequences.

Gene Name	Primer Sequence (5′-3′)	
PDCD4	F	GTATGATGTGGAGGAGGTGGAT
	R	CCCTCCAATGCTAAGGATACTG
TFEB	F	ATTTCTGTCCAGCAACATGA
	R	ATGGTAGGATGTGGGATTCT
GAPDH	F	GAAGGTGAAGGTCGGAGT
	R	CATGGGTGGAATCATATTGGAA

was assessed using the CCK-8 assay. Initially, 10% CCK-8 solution (Beyotime, C0037, Shanghai, China) of the culture medium volume was added to each well and the plate was placed back in the 37°C, 5% CO_2 incubator for an additional 2-3 hr. 100 µL from each well was transferred to a fresh 96-well plate and the absorbance at 450 nm wavelength was measured using a microplate reader to obtain the Optical Density (OD) values, which were then recorded and analyzed. Finally, the relative cell viability (%) was calculated using the formula: Relative cell viability (%)=(OD450'/avg (OD450C')) × 100%. Here, OD450' represents the absorbance value of the experimental group minus the zeroing group and avg (OD450C') represents the corrected average absorbance value of the control group.

Scratch Assay

Cells were seeded in a 24-well plate and assigned to the following treatment groups: (1) NC group; (2) si-PDCD4 group; (3) si-TFEB group; (4) si-PDCD4+si-TFEB group. A pipette tip was used to create a scratch perpendicular to the cell culture plate, ensuring the width of the scratches was consistent across all wells. The cell culture medium was then removed and the wells were washed three times with PBS to remove cell debris generated from the scratch. Fresh culture medium was added and the cells were placed back into the incubator at 37°C with 5% CO_2. Images of the scratches were captured under an inverted microscope at 0 and 24 hr post-scratch creation to measure the width of the scratches and the image data were collected for analysis of the experimental results.

Transwell Assay for Cell Migration

Cells were assigned to the following treatment groups: (1) NC group; (2) si-PDCD4 group; (3) si-TFEB group; (4) si-PDCD4+si-TFEB group. Cells were digested and adjusted to a concentration of 5×10^5 cells/ml in the cell suspension. According to the grouping, 100 µL of cell suspension was added to the upper chamber of the Transwell, while 500 µL of culture medium containing 10% FBS was added to the lower chamber (ensuring no bubbles formed between the lower medium and the chamber). The setup was incubated at 37°C, 5% CO_2 for 48 hr. The Transwell chamber was then dismantled and the culture medium inside was aspirated. The membrane on the lower side of the chamber was washed thrice with PBS and the Matrigel matrix inside the chamber was wiped off using a dry cotton swab. It was followed by fixation with 4% paraformaldehyde for 20 min. After three washes with PBS, the chamber was stained with 1% crystal violet solution (Beijing Solarbio, G1062) at room temperature for 20 min. The crystal violet solution was removed and the chamber was carefully rinsed three times with PBS, then air-dried upside down at room temperature. Under a microscope, four fields of view on the cross lines were chosen for each group, photographs were taken under 20× objective lens and the average number of cells that migrated through the membrane in the four fixed fields of view for each group was calculated.

Statistical Analysis

The data obtained from the experiments are represented as mean±Standard Error of the Mean (Mean±SEM) and all data were processed and statistically analyzed using GraphPad Prism 6 (La Jolla, CA, USA). Comparisons between the means of two groups were performed using the t-test, while comparisons among the means of multiple groups were conducted using ANOVA. A p-value of <0.05 was considered statistically significant.

RESULTS

Expression levels of PDCD4 and TFEB in Hela cells and normal cervical epithelial cells H8

The comparative expression levels of PDCD4 and TFEB in the two cell lines are shown in Figure 1, all experiments are conducted in triple duplications. The results of the qRT-PCR experiment showed that compared with Hela cells, the mRNA expression level of TFEB in H8 cells was significantly reduced, while the mRNA expression level of PDCD4 was significantly increased (Figure 1a-b, $p<0.01$). Western Blot experiment results showed that compared with Hela cells, the protein expression level of PDCD4 in H8 cells was significantly increased, while the protein expression level of TFEB was significantly reduced (Figure 1c-e, $p<0.0001$).

Inhibition of PDCD4 Alters Expression of PDCD4 and TFEB in HeLa Cells

The comparison of PDCD4 and TFEB expression levels in different groups of HeLa cells is depicted in Figure 2. qRT-PCR results indicated that compared to the NC group (A), the si-PDCD4 group (B) exhibited a significant decrease in PDCD4 mRNA expression and a significant increase in TFEB mRNA expression (Figure 2a-b, $p<0.01$). In contrast, the si-TFEB group (C) showed a significant increase in PDCD4 mRNA expression and a significant decrease in TFEB mRNA expression. The si-PDCD4+si-TFEB group (D) displayed a decrease in mRNA expression levels for both PDCD4 and TFEB (Figure 2a-b, $p<0.01$). Western Blot results echoed the mRNA expression trends. Relative to the NC group (A), the si-PDCD4 group (B) showed a significant reduction in PDCD4 protein expression and a significant elevation in TFEB protein expression. Conversely, the si-TFEB group (C) exhibited a significant increment in PDCD4 protein expression and a significant decrement in TFEB protein expression. In the si-PDCD4+si-TFEB group (D), both PDCD4 and TFEB protein expression levels were decreased (Figure 2c-e, $p<0.0001$).

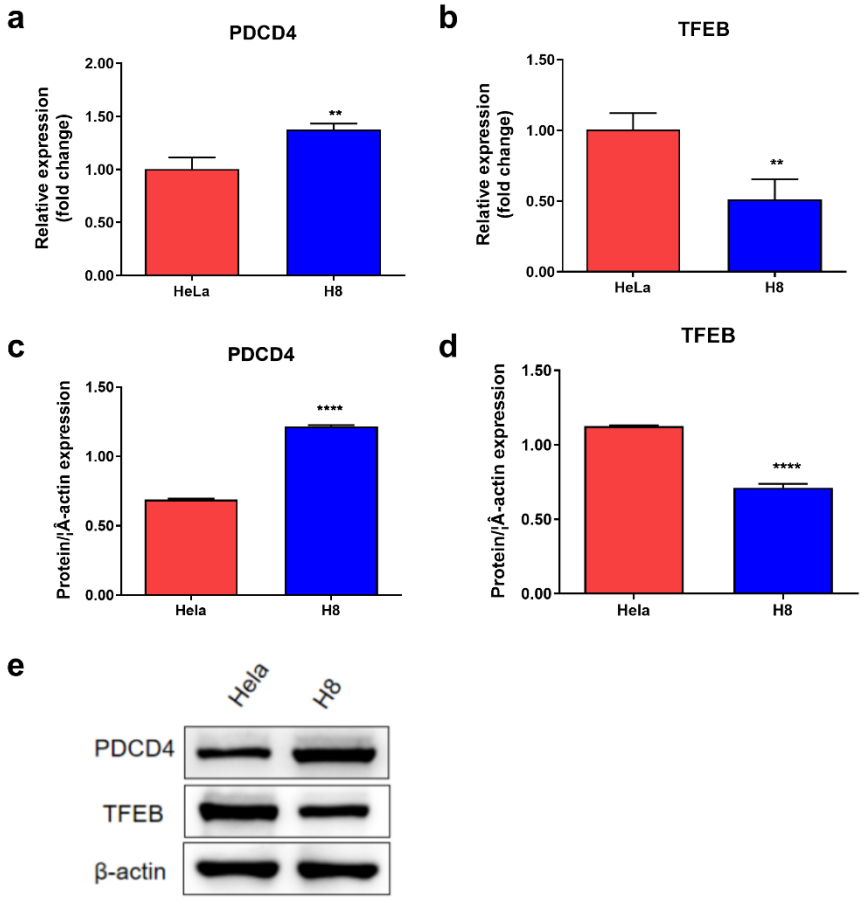

Figure 1: Comparison of PDCD4 and TFEB expression levels in the two cell strains. (a-b) mRNA expression levels of PDCD4 and TFEB in the two cell strains; (c-d) protein expression levels of PDCD4 and TFEB in the two cell strains; (e) WB protein exposure bands (** $p<0.01$, **** $p<0.0001$ vs. HeLa). $n=3$.

Inhibition of PDCD4 Promotes Proliferation, Migration and Invasion of HeLa Cells

As illustrated in Figure 3, the results from the CCK-8 assay demonstrated that compared to the NC group (A), the cell proliferation levels were significantly elevated in both si-PDCD4 group (B) and si-PDCD4+si-TFEB group (D), while a significant reduction in cell proliferation level was observed in the si-TFEB group (C) (Figure 3a-c, $p<0.001$ and $p<0.0001$). Scratch assay results showed a similar trend where, compared to the NC group (A), both si-PDCD4 group (B) and si-PDCD4+si-TFEB group (D) exhibited significantly increased levels of cell migration, while the si-TFEB group (C) showed a significant reduction in cell migration level (Figure 3d). The Transwell assay results corroborated these findings, indicating a significant increase in cell migration levels in both si-PDCD4 group (B) and si-PDCD4+si-TFEB group (D) compared to the NC group (A), whereas a significant decrease was observed in the si-TFEB group (C) (Figure 3e). In summary, silencing PDCD4 mediated overexpression of TFEB, promoting proliferation, migration and invasion of cervical cancer HeLa cells.

Effect of PDCD4 Silencing on EMT-Related Protein Expression

The comparison of protein expression levels of Vimentin, N-cadherin and E-cadherin among different HeLa cell groups is shown in Figure 4. Western Blot assay results demonstrated that compared to the NC group (A), cells in the si-PDCD4 group (B) exhibited significantly increased expression levels of Vimentin and N-cadherin and a significantly decreased expression level of E-cadherin (Figure 4b-d, $p<0.01$, $p<0.001$ and $p<0.0001$). Conversely, the si-TFEB group (C) showed significantly decreased expression levels of Vimentin and N-cadherin, while E-cadherin expression was significantly increased (Figure 4b-d, $p<0.001$, $p<0.01$ and $p<0.01$). In the si-PDCD4+si-TFEB group (D), the expression levels of Vimentin and N-cadherin were elevated, whereas the expression level of E-cadherin was reduced (Figure 4b-d, $p<0.001$, $p<0.01$ and $p<0.0001$).

DISCUSSION

The present study provides compelling evidence that the expression levels of the tumor suppressor PDCD4 and the transcription factor TFEB are inversely correlated in cervical

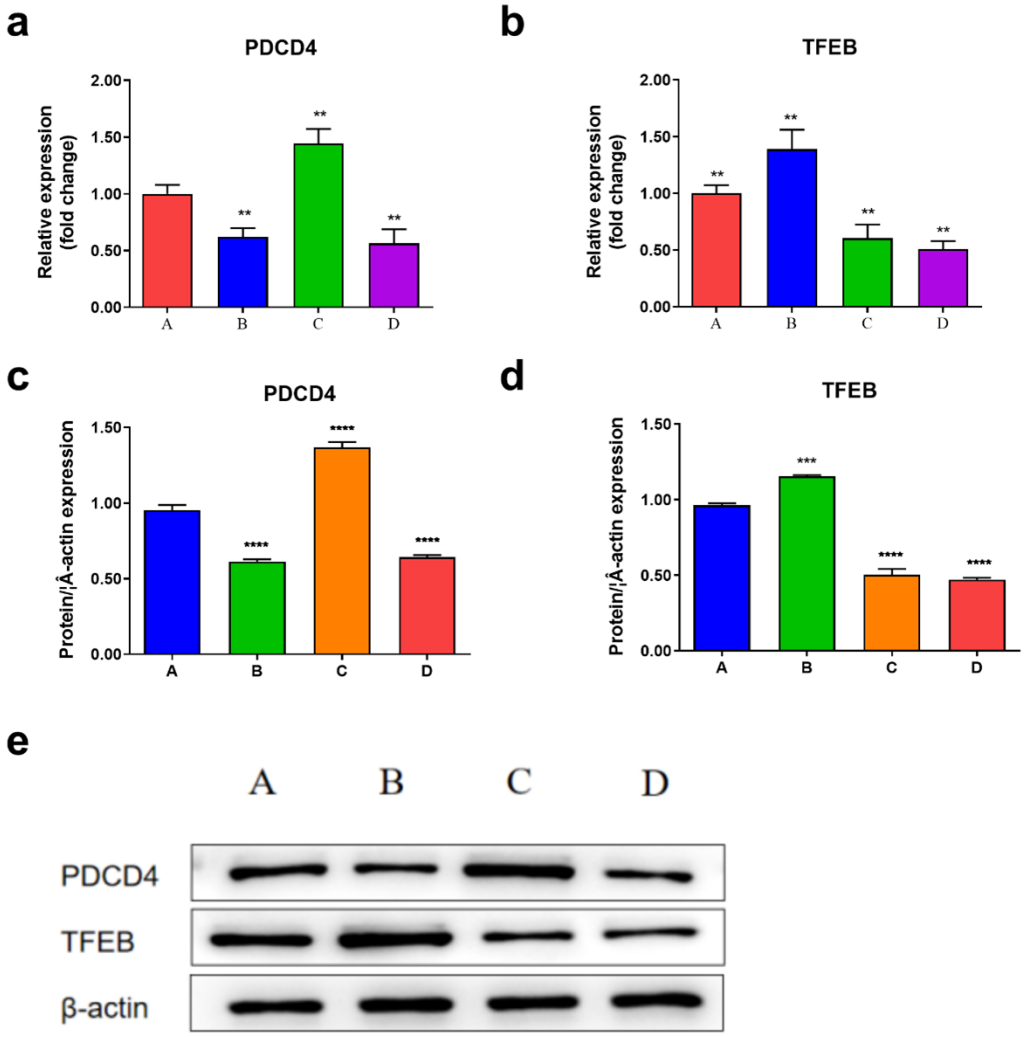

Figure 2: Comparison of PDCD4 and TFEB expression levels in different HeLa cell groups. (a-b) mRNA expression levels of PDCD4 and TFEB in HeLa cells; (c-d) protein expression levels of PDCD4 and TFEB in HeLa cells; (e) WB protein exposure bands (** $p<0.01$, **** $p<0.0001$ vs. A). Note: A: NC group; B: si-PDCD4 group; C: si-TFEB group; D: si-PDCD4+si-TFEB group. $n=3$.

cancer HeLa cells compared to normal cervical epithelial H8 cells. Notably, silencing PDCD4 in HeLa cells led to a significant downregulation of PDCD4 expression and a concomitant upregulation of TFEB expression, both at the mRNA and protein levels. Conversely, silencing TFEB resulted in increased PDCD4 expression. Furthermore, the functional experiments demonstrated that PDCD4 silencing promoted the proliferation, migration and invasion of HeLa cells, while TFEB silencing had the opposite effect. Mechanistically, PDCD4 silencing also induced epithelial-mesenchymal transition in HeLa cells, as evidenced by the increased expression of the mesenchymal markers Vimentin and N-cadherin and the decreased expression of the epithelial marker E-cadherin. Collectively, these findings suggest that PDCD4 may act as a critical regulator of TFEB expression and the PDCD4-TFEB axis plays a pivotal role in modulating the malignant phenotypes of cervical cancer cells, providing new insights into the molecular mechanisms of cancer progression and may hold significant implications for future therapeutic strategies.

PDCD4, the protein encoded by PDCD4, contains an α-helical structure domain at its amino-terminal which can bind with eukaryotic translation initiation factors, exerting inhibitory effects on protein synthesis and ribosomal complex formation .[13-17] Furthermore, PDCD4 can interact with PolyA binding proteins to directly inhibit gene transcription, participating in protein synthesis and transportation processes.[18-21] PDCD4 is a well-known tumor suppressor factor associated with various types of cancer, including cervical cancer. Acting as a translational regulator, it inhibits the translation of specific genes involved in cell growth, apoptosis and migration. Previous studies have demonstrated that reduced expression of PDCD4 is correlated with advanced stages of cancer, poor prognosis and increased tumor invasiveness.[12-24] Consistent with these findings, our study

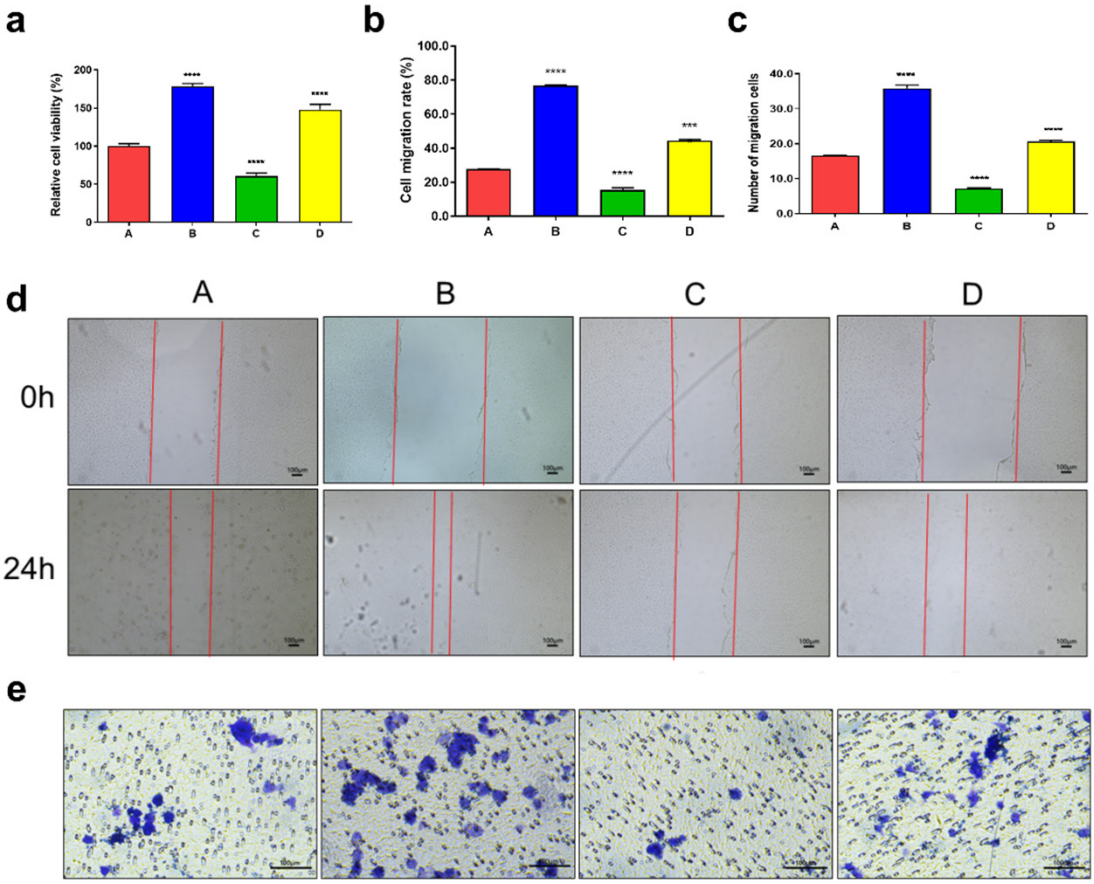

Figure 3: Comparison of cell proliferation and migration levels among different HeLa cell groups. (a) CCK8 assay for cell proliferation; (b) Observation of cell scratch under the microscope (40×); (d) Statistical results of cell migration rate; (c) Observation of migrated cells under the microscope (200×); (e) Statistical results of the number of migrated cells (*** $p<0.001$, **** $p<0.0001$ vs. A). Note: A: NC group; B: si-PDCD4 group; C: si-TFEB group; D: si-PDCD4+si-TFEB group. $n=3$.

revealed significant downregulation of PDCD4 in HeLa cells, suggesting its potential role in the pathogenesis of cervical cancer.

TFEB, a member of the MICROPHTHALMIA-ASSOCIATED TRANSCRIPTION FACTOR (MITF) family, plays a crucial role in lysosome biogenesis and autophagy.[25] Recent research has highlighted novel roles of TFEB in cancer biology. TFEB promotes cancer cell survival, proliferation and invasion by regulating various target genes involved in these processes .[26] In our investigation, we observed significant upregulation of TFEB in HeLa cells, indicating its possible involvement in driving the invasive behavior of cervical cancer cells. Study have indicated that inhibiting PDCD4 promotes the anti-tumor effect of macrophages by enhancing TFEB expression in the tumor microenvironment, indicating the potential interaction of TFEB and PDCD4.[11] In order to elucidate the functional relationship between PDCD4 and TFEB in cervical cancer, we conducted functional gain experiments by overexpressing TFEB in HeLa cells with or without PDCD4 knockdown. Our research findings demonstrated that overexpression of TFEB significantly enhanced the proliferation, migration and invasion of HeLa cells. Interestingly, the impact of TFEB overexpression on cellular behaviors was further amplified when PDCD4 was concurrently knocked down. These discoveries suggest that PDCD4 might exert its tumor-suppressive functions by negatively regulating the processes mediated by TFEB. These results are in agreement with the previous findings, who reported that certain compounds can inhibit growth and metastasis of HeLa cells by modulating similar molecular pathways.[27,28]

The identification of PDCD4 as a negative regulator of TFEB-mediated processes in cervical cancer cells holds significant therapeutic relevance. Targeting TFEB or its downstream effectors could potentially disrupt the oncogenic signaling cascade and inhibit the invasive behaviors of cervical cancer cells. Additionally, strategies aimed at restoring PDCD4 expression to counter the overactivation of TFEB in cervical cancer could be explored. Future research should focus on developing specific inhibitors or regulators for TFEB or its downstream targets to enhance the therapeutic efficacy in treating cervical cancer. While our study provides valuable insights into the roles of PDCD4 and TFEB in the progression of cervical cancer, several limitations need to be acknowledged. Firstly, our experiments were conducted *in vitro* using HeLa cells. Although HeLa cells

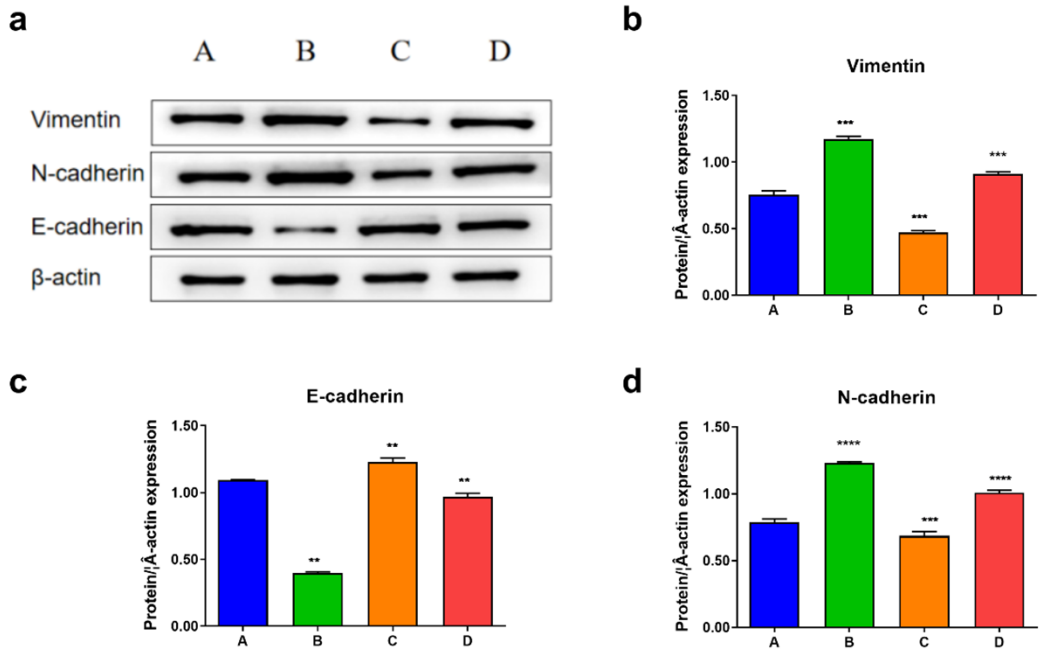

Figure 4: Comparison of Vimentin, N-cadherin and E-cadherin protein expression levels among different HeLa cell groups. (a) WB protein exposure strips; (b-d) Semi-quantitative analysis of protein expression levels. (** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ vs. A). Note: A: NC group; B: si-PDCD4 group; C: si-TFEB group; D: si-PDCD4+si-TFEB group. $n=3$.

are a widely used model for cervical cancer research, they may not fully recapitulate the complex tumor microenvironment and heterogeneity observed *in vivo*. To validate and extend the findings of this study, future investigations should utilize more physiologically relevant models, such as patient-derived xenograft systems or genetically engineered mouse models of cervical cancer. Secondly, the downstream targets and signaling pathways regulated by TFEB in cervical cancer remain largely unknown. While our study demonstrates the critical role of the PDCD4-TFEB axis in modulating the malignant phenotypes of cervical cancer cells, a more detailed understanding of the TFEB-mediated transcriptional program and its effector molecules is necessary to uncover the underlying mechanisms. Future studies should employ advanced techniques, such as RNA-sequencing, chromatin immunoprecipitation sequencing (ChIP-seq) and pathway analysis, to systematically identify the TFEB target genes and elucidate the broader signaling networks influenced by this transcription factor in cervical cancer. Elucidating these molecular pathways would provide valuable insights into potential therapeutic vulnerabilities that could be exploited for targeted interventions.

CONCLUSION

In summary, our investigation elucidates the inhibitory role of PDCD4 in modulating TFEB-mediated processes, thereby influencing the pivotal aspects of cervical cancer progression, including cell proliferation, migration and invasion in HeLa cells. This novel regulatory axis uncovered in our study provides valuable insights into the molecular mechanisms governing cervical cancer. The identified interplay between PDCD4 and TFEB offers a potential therapeutic target for disrupting the signaling cascade implicated in aggressive cancer phenotypes. Targeted therapies aimed at manipulating TFEB signaling may hold promise for the development of more effective treatment strategies for cervical cancer. Further research into the downstream targets and signaling pathways governed by TFEB in this context is warranted, offering prospects for advancing our understanding and refining therapeutic interventions for the benefit of cancer patients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

PDCD4: Programmed Cell Death 4; **TFEB:** Transcription Factor EB; **EMT:** Epithelial-mesenchymal transition; **HPV:** Human Papillomavirus; **RT-qPCR:** Real-time reverse transcriptase-polymerase chain reaction; **CCK-8:** Cell Counting Kit-8; **MITF:** Microphthalmia-associated transcription factor.

SUMMARY

This study explored the impact of silencing PDCD4, a tumor suppressor gene, on the progression of cervical cancer in HeLa cells, focusing on the overexpression of Transcription Factor EB (TFEB) and its effects on cell behaviors. Results demonstrated that silencing PDCD4 led to increased TFEB expression, significantly enhancing the proliferation, migration and invasion of HeLa cells. Additionally, the study suggested that the PDCD4-TFEB pathway could serve as a potential therapeutic target for cervical cancer. The findings offered insights into the molecular mechanisms of cervical cancer progression and pointed towards novel intervention strategies.

REFERENCES

1. Bosch FX, de Sanjosé S. Human papillomavirus and cervical cancer-burden and assessment of causality. J Natl Cancer Inst Monogr. 2003;Chapter 1(31):3-13. doi: 10.1093/oxfordjournals.jncimonographs.a003479, PMID 12807939.
2. Martin CM, Astbury K, O'Leary JJ. Molecular profiling of cervical neoplasia. Expert Rev Mol Diagn. 2006;6(2):217-29. doi: 10.1586/14737159.6.2.217, PMID 16512781.
3. Zhou R, Lin X, Liu D, Li Z, Zeng J, Lin X, *et al.* Research hotspots and trends analysis of TFEB: A bibliometric and Scientometric analysis. Front Mol Neurosci. 2022;15:854954. doi: 10.3389/fnmol.2022.854954, PMID 35531069.
4. Do M, Park J, Chen Y, Rah SY, Nghiem TT, Gong JH, *et al.* PERK activation by SB202190 ameliorates amyloidogenesis via the TFEB-induced autophagy-lysosomal pathway. Aging (Albany, NY). 2022;14(3):1233-52. doi: 10.18632/aging.203899, PMID 35166693.
5. Burton TD, Fedele AO, Xie J, Sandeman LY, Proud CG. The gene for the lysosomal protein LAMP3 is a direct target of the transcription factor ATF4. J Biol Chem. 2020;295(21):7418-30. doi: 10.1074/jbc.RA119.011864, PMID 32312748.
6. Sun W, Yue J. TPC2 mediates autophagy progression and extracellular vesicle secretion in cancer cells. Exp Cell Res. 2018;370(2):478-89. doi: 10.1016/j.yexcr.2018.07.013, PMID 29990474.
7. Wang JY, Jiang MW, Li MY, Zhang ZH, Xing Y, Ri M, *et al.* Formononetin represses cervical tumorigenesis by interfering with the activation of PD-L1 through MYC and STAT3 downregulation. J Nutr Biochem. 2022;100:108899. doi: 10.1016/j.jnutbio.2021.108899, PMID 34748924.
8. Rahtes A, Geng S, Lee C, Li L. Cellular and molecular mechanisms involved in the resolution of innate leukocyte inflammation. J Leukoc Biol. 2018;104(3):535-41. doi: 10.1002/JLB.3MA0218-070R, PMID 29688584.
9. Huang Z, Zhu S, Han Z, Li C, Liang J, Wang Y, *et al.* Proteome-wide analysis reveals TFEB targets for establishment of a prognostic signature to predict clinical outcomes of colorectal cancer. Cancers. 2023;15(3):744. doi: 10.3390/cancers15030744, PMID 36765702.
10. Guo X, Li R, Bai Q, Jiang S, Wang H. TFE3-PD-L1 axis is pivotal for sunitinib resistance in clear cell renal cell carcinoma. J Cell Mol Med. 2020;24(24):14441-52. doi: 10.1111/jcmm.16066, PMID 33145941.
11. Chen X, Guan Y, Zhang Y, Jia Y, Li W, Guo C, *et al.* Programmed cell death 4 modulates lysosomal function by inhibiting TFEB translation. Cell Death Differ. 2021;28(4):1237-50. doi: 10.1038/s41418-020-00646-2, PMID 33100324.
12. Meric-Bernstam F, Chen H, Akcakanat A, Do KA, Lluch A, Hennessy BT, *et al.* Aberrations in translational regulation are associated with poor prognosis in hormone receptor-positive breast cancer. Breast Cancer Res. 2012; 14(5):R138. doi: 10.1186/bcr3343, PMID 23102376.
13. Manirujjaman M, Ozaki I, Murata Y, Guo J, Xia J, Nishioka K, *et al.* Degradation of the tumor suppressor PDCD4 is impaired by the suppression of p62/SQSTM1 and autophagy. Cells. 2020;9(1):218. doi: 10.3390/cells9010218, PMID 31952347.
14. Nicolè L, Cappellesso R, Sanavia T, Guzzardo V, Fassina A. MiR-21 over-expression and Programmed Cell Death 4 down-regulation features malignant pleural mesothelioma. Oncotarget. 2018;9(25):17300-8. doi: 10.18632/oncotarget.24644, PMID 29707109.
15. Schmid T, Bajer MM, Blees JS, Eifler LK, Milke L, Rübsamen D, *et al.* Inflammation-induced loss of Pdcd4 is mediated by phosphorylation-dependent degradation. Carcinogenesis. 2011;32(10):1427-33. doi: 10.1093/carcin/bgr131, PMID 21771721.
16. Wang Q, Zhu J, Wang YW, Dai Y, Wang YL, Wang C, *et al.* Tumor suppressor Pdcd4 attenuates Sin1 translation to inhibit invasion in colon carcinoma. Oncogene. 2017;36(45):6225-34. doi: 10.1038/onc.2017.228, PMID 28692058.
17. Braun C, Katholnig K, Kaltenecker C, Linke M, Sukhbaatar N, Hengstschläger M, *et al.* p38 regulates the tumor suppressor PDCD4 via the TSC-mTORC1 pathway. Cell Stress. 2021;5(12):176-82. doi: 10.15698/cst2021.12.260, PMID 34917890.
18. Liao J, Liu R, Shi YJ, Yin LH, Pu YP. Exosome-shuttling microRNA-21 promotes cell migration and invasion-targeting PDCD4 in esophageal cancer. Int J Oncol. 2016;48(6):2567-79. doi: 10.3892/ijo.2016.3453, PMID 27035745.
19. Zhen Y, Li D, Li W, Yao W, Wu A, Huang J, *et al.* Reduced PDCD4 expression promotes cell growth through PI3K/Akt signaling in non-small cell lung Cancer. Oncol Res. 2016;23(1-2):61-8. doi: 10.3727/096504015X14478843952861, PMID 26802652.
20. Li C, Deng L, Zhi Q, Meng Q, Qian A, Sang H, *et al.* MicroRNA-183 functions as an oncogene by regulating. Gastric Cancer Anticanc Agent Me. 2016;16(4);447-55:PDCD4.
21. Liang X, Xu Z, Yuan M, Zhang Y, Zhao B, Wang J, *et al.* MicroRNA-16 suppresses the activation of inflammatory macrophages in atherosclerosis by targeting PDCD4. Int J Mol Med. 2016;37(4):967-75. doi: 10.3892/ijmm.2016.2497, PMID 26936421.
22. Wan J, Yang J, Huang Y, Deng L. MicroRNA-150 inhibitors enhance cell apoptosis of melanoma by targeting PDCD4. Oncol Lett. 2018;15(2):1475-82. doi: 10.3892/ol.2017.7445, PMID 29434838.
23. Wang D, Hou Q, Zhao L, Gao J, Xiao Y, Wang A. Programmed cell death factor 4 enhances the chemosensitivity of colorectal cancer cells to Taxol. Oncol Lett. 2019;18(2):1402-8. doi: 10.3892/ol.2019.10398, PMID 31423204.
24. Li JZ, Gao W, Lei WB, Zhao J, Chan JY, Wei WI, *et al.* MicroRNA. 744-3p promotes MMP-9-mediated metastasis by simultaneously suppressing PDCD4 and PTEN in laryngeal squamous cell carcinoma. Oncotarget. 2016;7(36):58218-33.
25. Asrani K, Murali S, Lam B, Na CH, Phatak P, Sood A, *et al.* mTORC1 feedback to AKT modulates lysosomal biogenesis through MiT/TFE regulation. J Clin Invest. 2019;129(12):5584-99. doi: 10.1172/JCI128287, PMID 31527310.
26. Jeong S, Byun JK, Cho SJ, Chin J, Lee IK, Choi YK, *et al.* Transcription factor Eb is required for macropinocytosis-mediated growth recovery of nutrient-deprived kras-mutant cells. Nutrients. 2018;10(11):1638. doi: 10.3390/nu10111638, PMID 30400219.
27. Panigrahi R, Parida R. Preliminary Screening of *in vitro Kaempferia galanga* Oil for anti-proliferative Activities against HeLa and MCF-7 Cell. Pharmacogn Mag. 2023;19(3):660-6. doi: 10.1177/09731296231172544.
28. Buranrat B. *Cratoxylum formosum* leaf extracts inhibit growth, induce apoptosis, and decrease metastasis of hela human cervical cancer cells. Pharmacogn Mag. 2022;18(78):296-303.

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