



OPEN

ANGPTL4 suppresses progression and improves cisplatin sensitivity in cervical cancer

Waraporn Chan-on¹✉, Methawadee Turinthorn¹, Arkom Chaiwongkot^{2,3},
Teerasit Techawiwattanaboon^{2,4}, Prasong Khaenam⁵ & Chaniya Leepiyasakulchai⁶

Cervical cancer (CC) remains a leading cause of cancer-related deaths worldwide and still requires effective interventions to improve patient outcomes. Angiopoietin-like 4 (ANGPTL4) is a multifaceted glycoprotein that plays crucial roles in lipid metabolism and tumor progression. ANGPTL4 exhibits both tumor-promoting and tumor-suppressing effects and has been proposed as a promising target for cancer therapy. This study investigated the role and potential of ANGPTL4 in enhancing therapeutic efficacy in CC using cell line models *in vitro*. Our analysis revealed a decreased expression of ANGPTL4 in CC samples from the GSE dataset and in the CC cell lines examined. Functional assays demonstrated that ANGPTL4 overexpression suppressed CC cell proliferation, migration, and invasion. Notably, overexpression of ANGPTL4 resulted in decreased cell viability and increased levels of apoptosis, cleaved caspase-3, and cleaved PARP under cisplatin treatment. Furthermore, these analyses were also conducted in ANGPTL4-knockdown cells, and results supporting the tumor-suppressive roles of ANGPTL4 were observed. Taken together, our study elucidates the critical role of ANGPTL4 in modulating progression and chemosensitivity of CC cells, suggesting ANGPTL4 as a potential target for CC treatment.

Keywords Cervical cancer, Angiopoietin-like 4 (ANGPTL4), Chemosensitivity, Cisplatin, Apoptosis

Cervical cancer (CC) ranks as the fourth most common cancer and the fourth leading cause of cancer-related deaths among women globally. In 2022, an estimated 660,000 women were diagnosed with CC, and 350,000 women died from the disease worldwide¹. Persistent infection with high-risk human papillomavirus (HPV) such as HPV 16 and HPV 18 is the primary etiological factor for CC². Treatment options including surgery, radiotherapy, and chemotherapy are used either alone or in combination to improve treatment outcomes of patients³. Cisplatin has been the most effective single chemotherapeutic agent⁴, and a platinum-based combination regimen is the standard first-line chemotherapy for recurrent and advanced disease^{5,6}. Despite advances in therapeutic strategies, CC patients with advanced disease still have unsatisfactory survival rates. This underscores the need for alternative approaches to improve treatment effectiveness. In addition, understanding the critical functions of key genes that modulate cancer progression may reveal opportunities for CC treatment.

Angiopoietin-like 4 (ANGPTL4) is a secreted glycoprotein that has been well recognized as an inhibitor of lipoprotein lipase, thereby regulating lipid metabolism⁷. ANGPTL4 is cleaved into an N-terminal coiled-coil domain (nANGPTL4) and a C-terminal fibrinogen-like domain (cANGPTL4), which participates in lipid metabolism and non-lipid-related processes, respectively⁸. Numerous studies have elucidated the dual role of ANGPTL4 as both oncogenic and tumor-suppressive factor in human malignancies⁹. It influences various signaling pathways and cellular processes, including cell proliferation, migration, and metastasis^{10–15}. For instance, ANGPTL4 enhanced proliferation and migration of ovarian cancer cells through ERK1/2 pathway¹⁰, and increased proliferation of papillary thyroid cancer through AKT phosphorylation¹³. In colorectal cancer, it increased cell proliferation, migration and invasion via STAT1 signaling¹⁶ and BMP7¹⁷. Additionally, ANGPTL4 facilitates brain metastases of triple-negative breast cancer in response to TGF-β2¹⁸. Conversely, its tumor-

¹Center for Research Innovation and Biomedical Informatics, Faculty of Medical Technology, Mahidol University, Nakhon Pathom 73170, Thailand. ²Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. ³Center of Excellence in Applied Medical Virology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. ⁴Chula Vaccine Research Center (Chula VRC), Center of Excellence in Vaccine Research and Development, Chulalongkorn University, Bangkok 10330, Thailand. ⁵Center for Standardization and Product Validation, Faculty of Medical Technology, Mahidol University, Nakhon Pathom 73170, Thailand. ⁶Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Nakhon Pathom 73170, Thailand. ✉email: waraporn.cho@mahidol.ac.th

suppressive role was found to inhibit progression of renal cell carcinoma¹¹ and osteosarcoma¹² by regulating lysosomal acid lipase activity and branched-chain amino acid metabolism, respectively. Moreover, ANGPTL4 was involved in suppression of gastric cancer cells¹⁹.

The evidence regarding ANGPTL4 involvement in tumor progression is accumulating. However, ANGPTL4 information in CC remains limited. Previously, upregulation of ANGPTL4 and its association with poor prognosis was reported in CC²⁰; however, functional investigations are warranted to elucidate its potential role in the context of CC. The present study aimed to assess the impact of ANGPTL4 in CC by analyzing the expression patterns of ANGPTL4 in dataset obtained from the Gene Expression Omnibus (GEO) database and determining its effects on proliferation, migration, invasion, and apoptosis of CC cells. In addition, the impact of ANGPTL4 on chemotherapeutic response of CC cells was also investigated.

Results

Expression of ANGPTL4 in CC samples and cell lines

To understand the expression patterns of ANGPTL4 in CC, we analyzed the GSE63514 dataset²¹ which allowed us to compare ANGPTL4 expression levels in normal cervical epithelia, cervical intraepithelial neoplasms (CIN), and cervical squamous epithelial cancers. The GSE63514 dataset showed that ANGPTL4 expression remained relatively unchanged in the precancerous lesions or CIN 1–3, while significantly downregulated in cancer specimens compared to normal samples. In addition, the reduction of ANGPTL4 in tumors was also significantly different from CIN 1–3 samples (Fig. 1a). To further ascertain the downregulation of ANGPTL4 in CC cell lines, we quantified ANGPTL4 mRNA levels using real-time RT-PCR across a panel of CC cell lines and a primary cervical epithelial cell line (PCS-480-011) that served as the normal cells. Consistently, a significant decrease in ANGPTL4 expression was observed in all the CC cell lines examined (CaSki, C33A, ME180, HeLa, and SiHa) compared to PCS-480-011 (Fig. 1b). These results demonstrate the downregulation of ANGPTL4 in CC, which likely reflects its biological importance during CC progression.

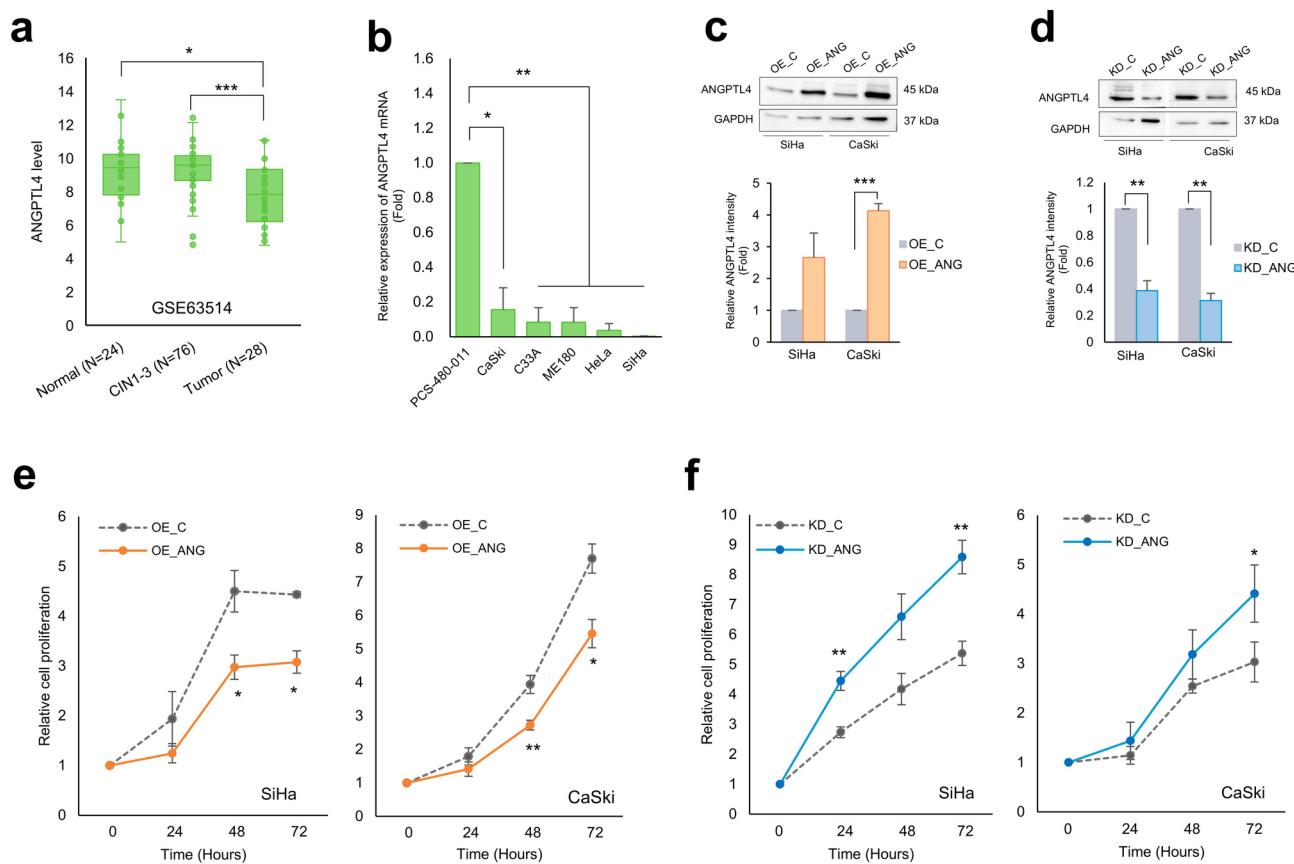


Fig. 1. Expression of ANGPTL4 and its effects on CC cell proliferation. ANGPTL4 mRNA levels in (a) GSE63514 and (b) CC cell lines. Levels of ANGPTL4 protein were assessed using western blot in SiHa and CaSki cells transfected with (c) ANGPTL4 plasmid (OE_ANG) or empty vector (OE_C), and (d) ANGPTL4 siRNA (KD_ANG) or control siRNA (KD_C). Original blots are available in Supplementary Fig. S1. Relative ANGPTL4 level was normalized to GAPDH. Relative cell proliferation was evaluated using MTS assays in (e) ANGPTL4-overexpressing cells and (f) ANGPTL4-knockdown cells. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

ANGPTL4 reduces proliferation and inhibits migration and invasion of CC cells

The decreased expression of ANGPTL4 in CC samples and cell lines led us to hypothesize that ANGPTL4 may play a key role in suppressing CC progression. To test this hypothesis, ANGPTL4 levels in SiHa and CaSki cells were modified using gene overexpression and RNA interference techniques. After introducing a plasmid encoding human ANGPTL4 or siRNA targeting ANGPTL4 into both cell lines, the level of ANGPTL4 was markedly increased in ANGPTL4-overexpressing cells (OE_ANG), while decreased in ANGPTL4-knockdown cells (KD_ANG) compared to their corresponding controls (Fig. 1c-d). These results indicate effective ANGPTL4 overexpression and silencing in the transfected SiHa and CaSki cells. Then, the impact of ANGPTL4 on CC cell proliferation was determined using MTS assay. We found that relative proliferation of OE_ANG cells, both SiHa and CaSki, were significantly reduced compared to their respective controls (OE_C) (Fig. 1e). In contrast, an enhancement of cell proliferation was observed in KD_ANG cells, especially in SiHa (Fig. 1f).

We subsequently examined the effect of ANGPTL4 on the migratory and invasive capabilities of CC cells using transwell migration and Matrigel invasion assays, respectively. It was observed that overexpression of ANGPTL4 resulted in a significant decrease in migration and invasion of SiHa and CaSki cells (Fig. 2a-b). In support of this finding, the number of migrating and invading cells was found to be increased following

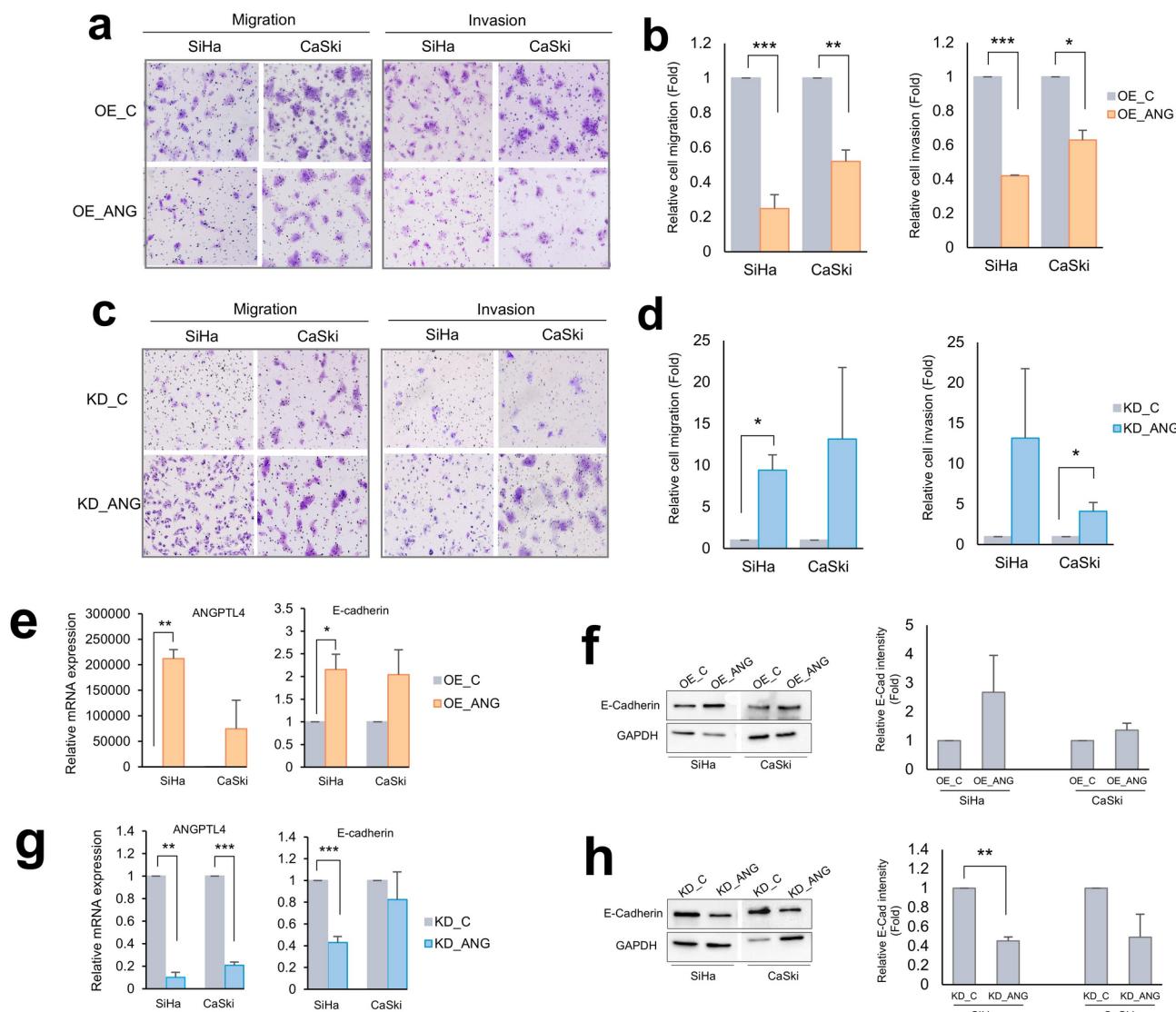


Fig. 2. Effects of ANGPTL4 on CC cell migration and invasion. **(a)** Representative images and **(b)** bar graphs illustrate the decreased migration and invasion capabilities of ANGPTL4-overexpressing cells compared to their control counterparts (OE_ANG vs. OE_C), as analyzed using transwell migration and Matrigel invasion assays. **(c)** Representative images and **(d)** bar graphs show the increased migration and invasion of ANGPTL4-knockdown cells in comparison to their respective controls (KD_ANG vs. KD_C). Expression levels of E-cadherin **(e)** mRNA and **(f)** protein were examined using real-time PCR and western blot, respectively in ANGPTL4-overexpressing cells. Levels of E-cadherin **(g)** mRNA and **(h)** protein in ANGPTL4-knockdown cells. (Supplementary Fig. S1). * $P<0.05$, ** $P<0.01$, and *** $P<0.001$.

silencing of ANGPTL4 (Fig. 2c-d). Since we observed the inhibitory properties of ANGPTL4 on cell migration and invasion, we proceeded to investigate the alteration of E-cadherin, a critical cell adhesion molecule and a marker for epithelial-mesenchymal transition²². As shown in Fig. 2e-f, E-cadherin was upregulated in SiHa and CaSki cells with ANGPTL4 overexpression while became inhibited in ANGPTL4-knockdown cells, especially in SiHa, where a significant decrease was observed (Fig. 2g-h). These results elucidate anti-proliferative, anti-migratory, and anti-invasive properties of ANGPTL4, thereby suggesting its suppressive role in CC progression.

ANGPTL4 sensitizes CC cells to cisplatin

We next evaluated the potential implication of ANGPTL4 for CC treatment. SiHa and CaSki with ANGPTL4 overexpression (OE_ANG) or silencing (KD_ANG) were cultured in the presence of cisplatin, which is the platinum-based compound widely used in combination therapy for advanced CC²³. Interestingly, OE_ANG cells, both SiHa and CaSki, exhibited a reduction in cell viability following cisplatin treatment at varying doses (10–40 μ M) and times (24 and 48 h) as shown in Fig. 3a-b. This observation was reversed in KD_ANG cells, which showed higher cell viability compared to that of controls, indicating reduced cisplatin sensitivity in ANGPTL4-knockdown cells (Fig. 3c-d). Moreover, the effect of ANGPTL4 on chemosensitivity of CC cells was also extended to the treatment with SAHA, a histone deacetylase inhibitor. Consistently, OE_ANG cells also showed a significant decrease in cell viability after exposing to different concentrations of SAHA (2.5–10 μ M) for 48 and 72 h, further confirming chemo-sensitizing effect of ANGPTL4 (Supplementary Fig. S2). Taken together, the results indicate that ANGPTL4 increases sensitivity of CC cells to chemotherapeutic agents, which highlights the potential of ANGPTL4 as a therapeutic target to be modulated for improving treatment outcome in CC.

ANGPTL4 increases apoptosis and caspase-3 activation in CC cells

Then, we detected apoptosis in SiHa and CaSki cells following modulation of ANGPTL4 expression, both alone and in combination with cisplatin treatment to elucidate whether chemo-sensitizing effects of ANGPTL4 resulted from induction of the programmed cell death. The Annexin V-FITC/PI double staining revealed that SiHa and CaSki cells overexpressing ANGPTL4 (OE_ANG) exhibited a significant increase in apoptosis compared to their controls (OE_C) (Fig. 4a-b). Conversely, ANGPTL4-knockdown cells (KD_ANG) demonstrated a marked reduction in apoptosis relative to their respective controls (Fig. 4c-d). These findings were consistently observed across both SiHa and CaSki under cisplatin treatment conditions.

We further investigated caspase-3 activation and PARP cleavage, which is a marker for apoptosis^{24,25}. Interestingly, SiHa and CaSki cells consistently demonstrated that ANGPTL4 overexpression markedly increased the levels of cleaved caspase-3 and cleaved PARP compared to their controls (OE_ANG vs. OE_C), even in the

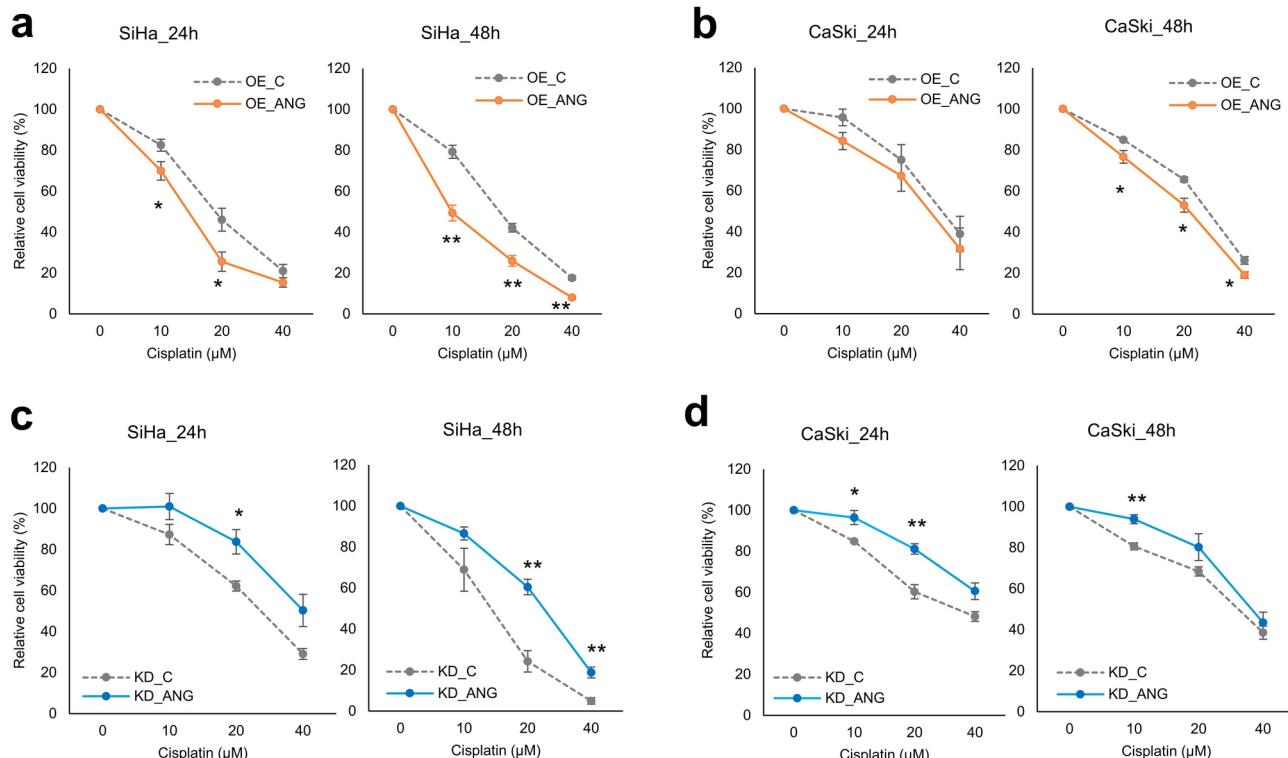


Fig. 3. ANGPTL4 enhances sensitivity of CC cells to cisplatin treatment. The viability of ANGPTL4-overexpressing cells was determined using MTS assay after cisplatin treatment for 24 and 48 h in (a) SiHa and (b) CaSki cells (OE_ANG vs. OE_C). The viability of ANGPTL4-knockdown cells was assessed following cisplatin exposure for 24 and 48 h in (c) SiHa and (d) CaSki cells (KD_ANG vs. KD_C). Cell viability was expressed as a percentage relative to the untreated controls. * $P < 0.05$ and ** $P < 0.01$.

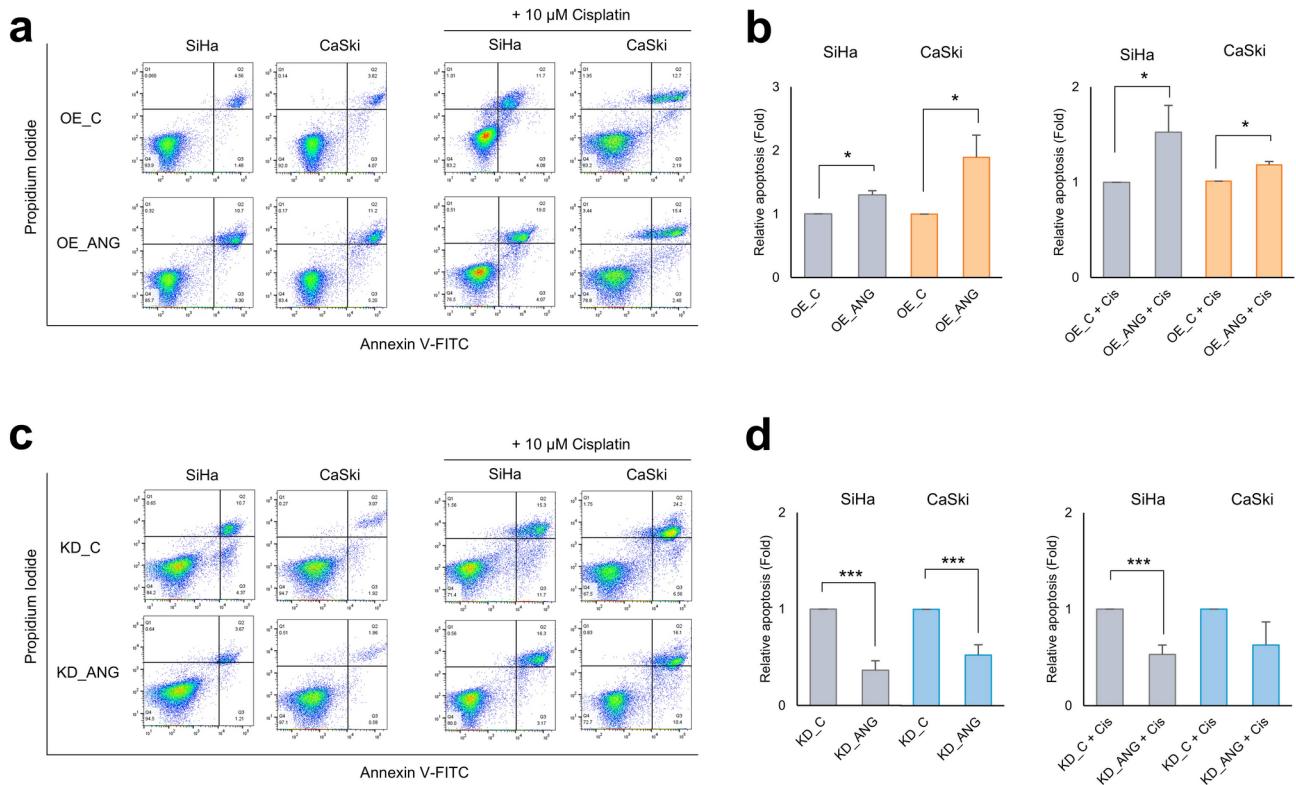


Fig. 4. ANGPTL4 influences CC cells apoptosis. **(a)** Representative FACS density plots and **(b)** relative apoptosis of SiHa and CaSki cells transfected with ANGPTL4 plasmid (OE_ANG) or empty vector (OE_C) with or without 10 μ M cisplatin treatment, as determined by Annexin V/PI staining. **(c)** Representative FACS density plots and **(d)** relative apoptosis of SiHa and CaSki cells transfected with ANGPTL4 siRNA (KD_ANG) or control siRNA (KD_C) in the absence or presence of 10 μ M cisplatin. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

absence of an apoptosis-inducing agent (Fig. 5a). Moreover, this effect was also found when OE_ANG cells from both SiHa and CaSki were exposed to 10 μ M cisplatin (Fig. 5b). To ascertain whether ANGPTL4 influenced caspase activation, we sought to determine caspase-3 and PARP in ANGPTL4-depleted cells (Fig. 5c and d). It was found that cleavage of caspase-3 and PARP appeared to be reduced in SiHa cells treated with cisplatin (Fig. 5d). Collectively, these results suggest that ANGPTL4 enhances apoptosis and caspase-3 activation, which is likely required for its chemosensitizing property (Fig. 5e).

Discussion

ANGPTL4 has attracted much attention in cancer research due to its potential involvement in progression of various tumors and association with patient prognosis⁹. The expression of ANGPTL4 is deregulated and varies across different cancer types. In this study, we found lower expression levels of ANGPTL4 mRNA in cervical cancer (CC) samples compared to normal cervical epitheliums. This decreased expression was also observed in CC cell lines. Previously, Nie and colleagues reported the upregulation of ANGPTL4 in CC samples, which was correlated with decreased survival in CC patients²⁰. The discrepancy between these results may indicate diverse expression patterns of ANGPTL4 in CC. Investigation of ANGPTL4 expressions in more independent samples could provide a better understanding of its pattern in CC.

Cancer development and progression is driven by a complex interplay of several hallmarks, including sustaining proliferative signaling, resisting cell death, and activating invasion and metastasis²⁶. The present study determined cell proliferation, migration, invasion, and apoptosis, which are essential biological capabilities to elucidate critical roles of ANGPTL4 in CC cell line model. Our results revealed the suppressive role of ANGPTL4, which delayed proliferative, migratory, and invasive abilities of CC cells that were modulated for ANGPTL4 overexpression. Several studies have previously shown tumor suppressive effects of ANGPTL4 through various cellular processes. For example, orthotopic xenograft model of hepatocellular carcinoma (HCC) showed that ANGPTL4 inhibited tumor growth, invasiveness, metastasis, and angiogenesis, whereas increased apoptosis²⁷. Mechanistically, ANGPTL4 decreased levels of Bcl2, VEGF, and phosphorylated Raf-MEK-Erk in HCC. Lin et al.¹² showed that ANGPTL4 knockdown promoted cell proliferation in osteosarcoma (OS). Silencing of ANGPTL4 led to mTOR activation through accumulated branched-chain amino acids (valine, leucine, and isoleucine). This tumor-inhibiting effect was validated in mouse tumor model. A study by Jin et al.¹¹ showed that loss of ANGPTL4 increased activity of lysosomal acid lipase (LAL), resulting in increased tumor growth and colony formation in renal cell carcinoma (RCC). In this study, our results provide insights into the biological

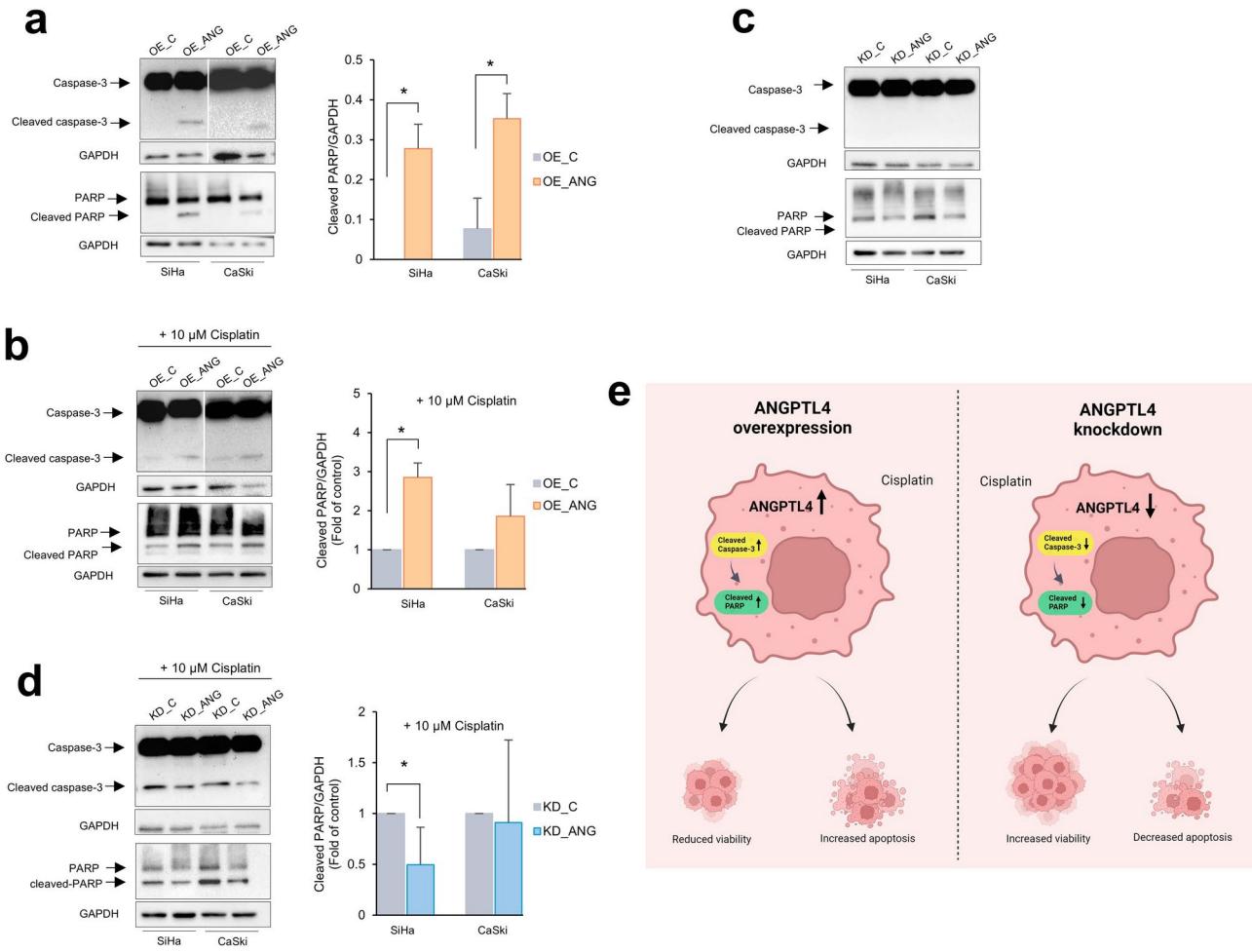


Fig. 5. ANGPTL4 increases cleaved caspase-3 and cleaved-PARP. Caspase-3, PARP, and their cleaved forms were analyzed using western blot in ANGPTL4-overexpressing cells in (a) the absence or (b) presence of cisplatin. These proteins were determined in ANGPTL4-knockdown cells (c) without or (d) with cisplatin treatment (Supplementary Fig.S1) Relative levels of cleaved PARP were normalized to GAPDH. (e) The illustration summarizes the effects of ANGPTL4 on caspase-3 activation and proposes that this activation, evidenced by increased levels of cleaved caspase-3 and cleaved PARP, enhances the sensitivity of cervical cancer cells to cisplatin, leading to reduced viability and increased apoptosis in cisplatin-treated cells. (Created with BioRender.com / Mahidol University). *P < 0.05, **P < 0.01, and ***P < 0.001.

roles of ANGPTL4, further supporting its tumor-suppressive function and aligning with several previous research findings.

Cisplatin is a cornerstone in the treatment of various malignancies including CC. Finding approaches to enhance its effectiveness is important for improving treatment outcomes of patients. Our study showed that ANGPTL4 overexpression sensitized CC cells to anti-cancer drugs, specifically cisplatin and SAHA, leading to reduced cell viability and increased apoptosis. Caspase-3 is an effector caspase that induces apoptosis in both extrinsic (death receptor) and intrinsic (mitochondrial) pathways²⁸. Caspase-3 activation results in cleavage of various substrates, including poly (ADP-ribose) polymerase (PARP), and cell apoptosis²⁹. Mechanistically, our results demonstrated that cleaved caspase-3 and cleaved PARP were increased following ANGPTL4 overexpression. Similarly, ANGPTL4 was found to promote apoptosis and increase caspase-7 and -9 activation in HCC cells²⁷.

Tumor-promoting effects of ANGPTL4 have also been evidenced to enhance proliferation and progression of several cancers, including ovarian cancer¹⁰, lung adenocarcinoma³⁰, thyroid cancer¹³, melanoma³¹, and gastric cancer¹⁵. A dual role, acting as both an oncogenic and suppressive factor, indicates its functional complexity and contextual dependence. The conflicting roles of ANGPTL4, even within the same types of tumors, emphasize its intricate nature. For example, Zhang et al.³² initially reported that ANGPTL4 was regulated by HIF1α under hypoxia and its overexpression was found to promote proliferation and migration of OS cells. The opposing role of ANGPTL4 was later shown to inhibit OS progression¹². In colorectal cancer (CRC), Zu et al.³³ showed that the miR-939-5p/hnRNPA1/ANGPTL4 axis underlies the inhibitory effect of linc02231 on CRC progression. In contrast, the TGF-β1/SMAD3/ANGPTL4 axis promotes peritoneal metastasis of CRC³⁴ and ANGPTL4 increases CRC proliferation under hypoxic conditions¹⁶.

Xie et al.³⁵ demonstrated that ANGPTL4 enhanced gastric cancer progression by increasing proliferation, angiogenesis, migration, invasion, and apoptosis invasion in SNU5 and MKN7 cells; however, these processes were suppressed by ANGPTL4 in AGS cells. Jin et al.¹¹ reported a tumor-suppressive function of ANGPTL4 in the CAK1-1 cell with wild-type *Von Hippel-Lindau* (VHL), but this suppressive phenotype was not observed in the 786O tumor model with mutant VHL. These findings suggest that the functional roles of ANGPTL4 cannot be confidently predicted without experimental validation, and specific conditions should be taken into account. Our results were validated using both overexpression and knockdown approaches to ascertain the effects of ANGPTL4 in SiHa and CaSki, both are HPV 16 positive cells. Although they vary in the copy number of integrated HPV (1–2 integrated copies in SiHa and 600 integrated copies in CaSki), both cell lines exhibited similar findings, albeit at different magnitudes.

This study primarily focused on the phenotypic changes resulting from ANGPTL4 modulations in cell lines. Addressing the following limitations would more thoroughly elucidate the significance of ANGPTL4 in CC. Firstly, detailed exploration of the molecular mechanisms driving its effects on CC proliferation, migration, invasion, and apoptosis is necessary to demonstrate how ANGPTL4 influences these fundamental processes. Investigation of reported signaling molecules, including Bcl2, STAT1, AKT, and ERK, can be considered. Secondly, future research in animal model for CC, which is currently lacking, is crucial and more biological relevant for validating the biological roles and potential therapeutic implications of ANGPTL4. Thirdly, our study demonstrated altered expression of ANGPTL4 in clinical samples included in the publicly available GSE63514 dataset. Extended examination for ANGPTL4 expressions in additional cohorts of clinical samples would provide deeper insights into its expression patterns.

In summary, this study demonstrates the suppressive roles of ANGPTL4 in CC cell progression, suggesting that ANGPTL4 could be a potential therapeutic target for CC treatment.

Materials and methods

Analysis of ANGPTL4 expression

Gene expression profile of CC based on microarray study was obtained from the Gene Expression Omnibus (GEO) database of the National Center for Biotechnology Information (NCBI). The GSE63514 dataset²¹ was included in the analysis of this study. This expression profile dataset contained normal cervical epitheliums ($N=24$), CIN 1–3 ($N=76$), and cervical squamous epithelial cancers ($N=28$) and was profiled based on the GPL570 platform using Affymetrix Human Genome U133 Plus 2.0 Array. The expression of ANGPTL4 (221009_s_at) in GSE63514 was analyzed by GEO2R tool (available from <https://www.ncbi.nlm.nih.gov/geo/>).

Cell lines

Human CC cell lines (SiHa, CaSki, ME-180, C33A, HeLa) and human primary cervical epithelial cells (PCS-480-011) were obtained from the American Type Culture Collection. CC cell lines were cultured in DMEM (Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific). The PCS-480-011 was cultured in cervical epithelial cell basal medium supplemented with cervical epithelial cell growth kit (ATCC, USA). All cell lines were maintained in a 5% CO_2 humidified atmosphere at 37 °C.

Cell transfection

Overexpression of ANGPTL4 was conducted using a plasmid encoding human ANGPTL4 (NM_139314.3) obtained from GenScript (Clone ID OHu25270) (Piscataway, NJ, USA). ANGPTL4 plasmid or empty vector (2.5 μg) were introduced into SiHa and CaSki using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. For ANGPTL4 knockdown, SiHa and CaSki were transfected with 50 pmol siRNA targeting ANGPTL4 (HSS181879; Thermo Fisher Scientific) or control DsiRNA (Integrated DNA Technologies, IL) using Lipofectamine RNAiMAX (Invitrogen). The transfected cells were collected after 24–48 h and efficiency of ANGPTL4 overexpression and knockdown was then examined by western blot.

Cell proliferation assay

Following transfection, cells were harvested and cultured in 96-well plates at 2.5×10^3 cells/well for 24, 48, and 72 h. At the end of incubation intervals, MTS reagent (Promega) was added to each well, followed by incubation for 2 h at 37 °C. Then, absorbance at 490 nm was determined using a microplate reader (Synergy/HTX multimode reader, BioTek, VT).

Drug treatment

The transfected cells were seeded in 96-well plates at 5×10^3 cells/well and incubated overnight. Next day, the cells were treated with 10, 20, and 40 μM cisplatin (Sigma-Aldrich, MO, USA) or 2.5, 5, and 10 μM suberoylanilide hydroxamic acid (SAHA or Vorinostat) (Sigma-Aldrich). PBS and DMSO were used as vehicle controls for cisplatin and SAHA, respectively. After incubation in a range of 24–72 h, viability of the treated cells was assessed by MTS (Promega), following the manufacturer's protocol.

Transwell migration and invasion assays

Migration assay was determined using transwell with 8.0 μm pore inserts for 24-well plates (Corning, MA, USA). Cell suspension (1×10^5 cells in 100 μL FBS-free medium) was loaded into the upper chamber, while 600 μL medium containing 30% FBS was filled in the lower compartment of the transwell. ANGPTL4-knockdown cells and ANGPTL4-overexpressing cells were allowed to migrate for 24–48 h, respectively. After removing cells in the upper chamber using cotton swabs, migrated cells were fixed and stained with crystal violet (0.5%). Invasion assays were performed using 24-well plates with 8.0 μm pore transwell inserts coated with Matrigel (BD Biosciences, NJ, USA). The protocol followed was similar to that of the migration assay.

Apoptosis detection

Cell apoptosis was measured using Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining (BioLegends, CA). Initially, cells were transfected with siRNA or plasmid to silence or overexpress ANGPTL4, respectively. The transfected cells were then harvested, resuspended in Annexin V binding buffer, and stained with Annexin V-FITC/PI. Finally, the cells were analyzed by flow cytometry using FACS Canto II and FlowJo software (both from BD Biosciences, CA). Apoptosis of cisplatin-treated cells was assessed after exposing the transfected cells to 10 μ M cisplatin for 24 h.

Real-time RT-PCR

Total RNA was extracted using PureLink RNA Mini Kit (Thermo Fisher Scientific, MA) and cDNA was synthesized using the iScript Reverse Transcription Supermix (Bio-Rad, CA), following the manufacturer's protocol. Expression of target genes was examined by real-time PCR using SsoFast EvaGreen Supermix (Biorad) along with primers specific to ANGPTL4, E-cadherin, and GAPDH³⁶. The amplification was conducted for forty cycles on the CFX96 detection system (Biorad). GAPDH was used as a reference gene and relative gene expression was analyzed using the $2^{-\Delta\Delta Ct}$ method.

Western blot

Cells were lysed with radioimmunoprecipitation assay (RIPA) lysis buffer (Cell Signaling Technology, MA, USA) in the presence of a proteinase and phosphatase inhibitors (Cell Signaling Technology). The concentration of protein samples was measured using the Bio-Rad protein assay and protein samples were separated in 10–15% SDS-PAGE before transferring onto nitrocellulose membrane. The membrane was blocked with 5% bovine serum albumin before sequentially incubating with primary antibodies specific to either ANGPTL4 (ab196746, Abcam), caspase 3, PARP, E-cadherin, or GAPDH (Cell Signaling) at 4 °C overnight. Horseradish peroxidase-conjugated secondary antibody (Cell Signaling) was used along with chemiluminescent HRP detection reagent (Merck Millipore, Germany). Signals were analyzed by ChemiDoc MP (Biorad Laboratories Inc., CA).

Statistical analysis

Data were expressed as mean \pm standard deviation obtained from at least three independent experiments. Statistical analyses were performed using PASW Statistics 18 software (SPSS Inc., IL). Comparison of ANGPTL4 expression levels in the GEO dataset was conducted using Kruskal-Wallis test. Two-group comparisons were analyzed using Student's t-test, while multiple group comparisons utilized one-way analysis of variance (ANOVA) with LSD post hoc testing. P -value < 0.05 was defined as a statistically significant difference.

Data availability

The dataset used in this work was retrieved from the publicly available NCBI's Gene Expression Omnibus database (GEO, <http://www.ncbi.nlm.nih.gov/geo/>). Dataset GSE63514 was generated by den Boon JA et al. and is available at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63514>.

Received: 29 September 2024; Accepted: 17 April 2025

Published online: 23 April 2025

References

1. Bray, F. et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* <https://doi.org/10.3322/caac.21834> (2024).
2. Crosbie, E. J., Einstein, M. H., Franceschi, S. & Kitchener, H. C. Human papillomavirus and cervical cancer. *Lancet.* **382**, 889–899. [https://doi.org/10.1016/S0140-6736\(13\)60022-7](https://doi.org/10.1016/S0140-6736(13)60022-7) (2013).
3. Johnson, C. A., James, D., Marzan, A. & Armao, M. Cervical cancer: an overview of pathophysiology and management. *Semin Oncol. Nurs.* **35**, 166–174. <https://doi.org/10.1016/j.soncn.2019.02.003> (2019).
4. Tewari, K. S. & Monk, B. J. Gynecologic oncology group trials of chemotherapy for metastatic and recurrent cervical cancer. *Curr. Oncol. Rep.* **7**, 419–434. <https://doi.org/10.1007/s11912-005-0007-z> (2005).
5. Marth, C. et al. Cervical cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **29**, iv262. <https://doi.org/10.1093/annonc/mdx220> (2018).
6. Koh, W. J. et al. Cervical cancer, version 3.2019, NCCN clinical practice guidelines in oncology. *J. Natl. Compr. Canc Netw.* **17**, 64–84. <https://doi.org/10.6004/jnccn.2019.0001> (2019).
7. Dijk, W. & Kersten, S. Regulation of lipid metabolism by angiopoietin-like proteins. *Curr. Opin. Lipidol.* **27**, 249–256. <https://doi.org/10.1097/MOL.0000000000000290> (2016).
8. Zuo, Y., He, Z., Chen, Y. & Dai, L. Dual role of ANGPTL4 in inflammation. *Inflamm. Res.* **72**, 1303–1313. <https://doi.org/10.1007/s00011-023-01753-9> (2023).
9. Liu, R. et al. Emerging roles of angiopoietin-like 4 in human tumors (Review). *Int. J. Oncol.* **66** <https://doi.org/10.3892/ijo.2024.5715> (2025).
10. Xu, J. et al. ANGPTL4 regulates ovarian cancer progression by activating the ERK1/2 pathway. *Cancer Cell. Int.* **24**, 54. <https://doi.org/10.1186/s12935-024-03246-z> (2024).
11. Jin, Z. et al. ANGPTL4 suppresses clear cell renal cell carcinoma via Inhibition of lysosomal acid lipase. *Cancer Res. Commun.* <https://doi.org/10.1158/2767-9764.CRC-24-0016> (2024).
12. Lin, S. et al. ANGPTL4 negatively regulates the progression of osteosarcoma by remodeling branched-chain amino acid metabolism. *Cell. Death Discov.* **8**, 225. <https://doi.org/10.1038/s41420-022-01029-x> (2022).
13. Yang, L. et al. ANGPTL4 promotes the proliferation of papillary thyroid Cancer via AKT pathway. *Onco Targets Ther.* **13**, 2299–2309. <https://doi.org/10.2147/OTT.S237751> (2020).
14. Cai, Y. C. et al. ANGPTL4 overexpression inhibits tumor cell adhesion and migration and predicts favorable prognosis of triple-negative breast cancer. *BMC Cancer.* **20**, 878. <https://doi.org/10.1186/s12885-020-07343-w> (2020).
15. Chen, J. W., Luo, Y. J., Yang, Z. F., Wen, L. Q. & Huang, L. Knockdown of angiopoietin-like 4 inhibits the development of human gastric cancer. *Oncol. Rep.* **39**, 1739–1746. <https://doi.org/10.3892/or.2018.6253> (2018).

16. Kim, S. H. et al. ANGPTL4 induction by prostaglandin E2 under hypoxic conditions promotes colorectal cancer progression. *Cancer Res.* **71**, 7010–7020. <https://doi.org/10.1158/0008-5472.CAN-11-1262> (2011).
17. Li, X. et al. Angiopoietin-like 4 enhances metastasis and inhibits apoptosis via inducing bone morphogenetic protein 7 in colorectal cancer cells. *Biochem. Biophys. Res. Commun.* **467**, 128–134. <https://doi.org/10.1016/j.bbrc.2015.09.104> (2015).
18. Gong, X. et al. Interaction of tumor cells and astrocytes promotes breast cancer brain metastases through TGF-beta2/ANGPTL4 axes. *NPJ Precis Oncol.* **3** <https://doi.org/10.1038/s41698-019-0094-1> (2019).
19. Qian, P. et al. LMX1A inhibits C-Myc expression through ANGPTL4 to exert tumor suppressive role in gastric cancer. *PLoS One.* **14**, e0221640. <https://doi.org/10.1371/journal.pone.0221640> (2019).
20. Nie, D., Zheng, Q., Liu, L., Mao, X. & Li, Z. Up-regulated of Angiopoietin-Like protein 4 predicts poor prognosis in cervical Cancer. *J. Cancer.* **10**, 1896–1901. <https://doi.org/10.7150/jca.29916> (2019).
21. den Boon, J. A. et al. Molecular transitions from papillomavirus infection to cervical precancer and cancer: role of stromal Estrogen receptor signaling. *Proc. Natl. Acad. Sci. U.S.A.* **112**, E3255–3264. <https://doi.org/10.1073/pnas.1509322112> (2015).
22. Kang, Y. & Massagué, J. Epithelial-mesenchymal transitions: twist in development and metastasis. *Cell* **118**, 277–279. <https://doi.org/10.1016/j.cell.2004.07.011> (2004).
23. Gadducci, A., Tana, R., Cosio, S. & Cionini, L. Treatment options in recurrent cervical cancer (Review). *Oncol. Lett.* **1**, 3–11. https://doi.org/10.3892/ol_00000001 (2010).
24. Tewari, M. et al. Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* **81**, 801–809. [https://doi.org/10.1016/0092-8674\(95\)90541-3](https://doi.org/10.1016/0092-8674(95)90541-3) (1995).
25. Kaufmann, S. H., Desnoyers, S., Ottaviano, Y., Davidson, N. E. & Poirier, G. G. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. *Cancer Res.* **53**, 3976–3985 (1993).
26. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674. <https://doi.org/10.1016/j.cell.2011.02.013> (2011).
27. Ng, K. T. et al. Clinical relevance and therapeutic potential of angiopoietin-like protein 4 in hepatocellular carcinoma. *Mol. Cancer.* **13**, 196. <https://doi.org/10.1186/1476-4598-13-196> (2014).
28. Asadi, M. et al. Caspase-3: structure, function, and biotechnological aspects. *Biotechnol. Appl. Biochem.* **69**, 1633–1645. <https://doi.org/10.1002/bab.2233> (2022).
29. Slee, E. A., Adrain, C. & Martin, S. J. Serial killers: ordering caspase activation events in apoptosis. *Cell. Death Differ.* **6**, 1067–1074. <https://doi.org/10.1038/sj.cdd.4400601> (1999).
30. Hu, Q. et al. ANGPTL4, a direct target of hsa-miR-133a-3p, accelerates lung adenocarcinoma lipid metabolism, proliferation and invasion. *Aging (Albany NY).* **16**, 8348–8360. <https://doi.org/10.18632/aging.205313> (2023).
31. Izraely, S. et al. ANGPTL4 promotes the progression of cutaneous melanoma to brain metastasis. *Oncotarget* **8**, 75778–75796. <https://doi.org/10.18632/oncotarget.19018> (2017).
32. Zhang, T., Kastrenopoulou, A., Larrouette, Q., Athanasou, N. A. & Knowles, H. J. Angiopoietin-like 4 promotes osteosarcoma cell proliferation and migration and stimulates osteoclastogenesis. *BMC Cancer.* **18**, 536. <https://doi.org/10.1186/s12885-018-4468-5> (2018).
33. Xu, S. et al. STAT2-induced linc02231 promotes tumorigenesis and angiogenesis through modulation of hnRNPA1/ANGPTL4 in colorectal cancer. *J. Gene Med.* **25**, e3506. <https://doi.org/10.1002/jgm.3506> (2023).
34. Zhu, C. et al. Adipose-derived stem cells promote Glycolysis and peritoneal metastasis via TGF-beta1/SMAD3/ANGPTL4 axis in colorectal cancer. *Cell. Mol. Life Sci.* **81**, 189. <https://doi.org/10.1007/s00018-024-05215-1> (2024).
35. Xie, J. et al. ANGPTL4 plays a Paradoxical role in gastric cancer through the LGALS7 and Hedgehog pathways. *Sci. Rep.* **14**, 23173. <https://doi.org/10.1038/s41598-024-71415-1> (2024).
36. San, T. T. et al. Curcumin enhances chemotherapeutic effects and suppresses ANGPTL4 in anoikis-resistant cholangiocarcinoma cells. *Helixon* **6**, e03255. <https://doi.org/10.1016/j.helixon.2020.e03255> (2020).

Acknowledgements

This research project was supported by Mahidol University (Fundamental Fund: fiscal year 2023 by National Science Research and Innovation Fund (NSRF)).

Author contributions

W.C. conceptualized and designed the study. W.C., M.T. and C.L. conducted the experiments. W.C., M.T. and A.C. performed the analyses and interpreted the results. W.C. drafted the manuscript. W.C., A.C., T.T., P.K. edited the manuscript. All authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-99136-z>.

Correspondence and requests for materials should be addressed to W.C.-o.

Reprints and permissions information is available at www.nature.com/reprints.

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

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025