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Dexmedetomidine inhibits inflammation and angiogenesis and alleviates esophageal cancer progression through ITGA6/PI3K/AKT pathway

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Esophageal cancer (EC) is an aggressive malignancy with high mortality and poor prognosis worldwide. Dexmedetomidine (DEX) shows anticancer potential but its effects on this disease are unknown. This study aims to investigate the role and mechanism of DEX in esophageal cancer through the ITGA6/ PI3K/AKT pathway. *In vitro* results indicated that DEX dose-dependently inhibited the proliferation, migration, and invasion of EC cells, while promoting apoptosis. DEX significantly reduced the secretion and expression of pro-inflammatory cytokines and downregulated the expression of angiogenesis-related factors. Mechanistic studies revealed that DEX significantly downregulated the expression of ITGA6 in EC cells and inhibited the phosphorylation activation of the PI3K/AKT pathway. Overexpression of ITGA6 partially reversed the inhibitory effects of DEX on the malignant progression, inflammatory response, and angiogenesis of EC cells, while inhibition of ITGA6 enhanced the antitumor effects of DEX. *In vivo* results were highly consistent with the *in vitro* findings, further confirming the antitumor effects of DEX. DEX inhibits the ITGA6/PI3K/AKT pathway, thereby suppressing the inflammatory response and angiogenesis, ultimately alleviating the progression of EC.

Keywords: Esophageal cancer progression, Pro-inflammatory factors, Invasion, Migration, Apoptosis, Cellular angiogenesis

Esophageal carcinoma (EC), a prevalent form of malignancy, is associated with significant morbidity and mortality rates globally[1]. It causes approximately 500,000 deaths annually worldwide, ranking sixth in cancer-related deaths[2]. Despite significant progress in therapeutic approaches including surgery, radiation, and systemic chemotherapy[3] the prognosis for EC patients remains poor, with fewer than 20% surviving beyond five years[4]. This dismal survival outcome is largely attributed to the disease's aggressive biological behaviour, challenges in detecting early-stage lesions, and the frequent development of therapeutic resistance[5]. Therefore, exploring new therapeutic targets and developing more effective anticancer drugs is an urgent research need. Esophageal carcinogenesis involves multiple pathological mechanisms, particularly chronic inflammatory responses, aberrant vascular proliferation, tumor microenvironment modulation, and signaling cascade disturbances[6]. This inflammatory milieu facilitates uncontrolled cellular growth, suppresses programmed cell death, and ultimately triggers malignant conversion through the upregulation of inflammatory mediators and activation of key regulatory molecules[7,8]. Additionally, tumor angiogenesis is another important mechanism of invasion and metastasis in EC. Elevated levels of angiogenic regulators, particularly vascular endothelial growth factor (VEGF), play a dual role in tumor progression by sustaining nutrient supply while simultaneously facilitating metastatic dissemination[9,10]. Therefore, targeting both inflammation and angiogenesis simultaneously may be an effective strategy to inhibit the progression of EC.

Integrin subunit alpha 6 (ITGA6), a key component of the integrin superfamily, mediates cellular processes including adhesion, motility, and intracellular signaling via binding to extracellular matrix components, particularly laminin[11]. Emerging evidence indicates that ITGA6 overexpression occurs frequently in multiple cancer types, with elevated

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Abbr: DEX; Dexmedetomidine, EC; Esophageal cancer, ESCC; Esophageal squamous cell carcinoma, EAC; Esophageal adenocarcinoma, PI3K; phosphatidylinositol 3-kinase, VEGFA;Vascular endothelial growth factor A, ITGA6; Integrin subunit alpha 6, MMP9; Matrix Metalloproteinase-9, MMP2; Matrix Metalloproteinase-2, FGF2; Fibroblast Growth Factor 2, CD31; Cluster of Differentiation 31.

expression levels demonstrating strong correlations with aggressive tumor behaviour, metastatic potential, and unfavourable clinical outcomes in EC[12]. ITGA6 hyperactivation has been shown to enhance neoplastic cell viability and growth by stimulating downstream effector cascades, particularly the phosphatidylinositol 3-kinase (PI3K)/AKT signaling network[13]. In EC, PI3K/AKT pathway activation drives disease progression by simultaneously enhancing inflammatory responses and angiogenic processes through transcriptional regulators[14,15]. The study findings highlight the therapeutic potential of targeting the ITGA6-mediated PI3K/AKT signaling pathway for EC treatment.

Dexmedetomidine (DEX), a selective α2-adrenoreceptor agonist, has become a mainstay in perioperative medicine and critical care settings[16]. Emerging preclinical evidence suggests this agent possesses multimodal biological activities, demonstrating immunomodulatory capabilities and significant anti-inflammatory/anti-angiogenic effects through pleiotropic mechanisms that may attenuate oncogenesis and tumor progression[17]. Research has found that DEX can inhibit the proliferation and invasion of esophageal cancer cells and exert an anti-tumor effect[18]. However, the role of DEX in esophageal cancer and its specific mechanisms remain incompletely elucidated. This study aims to explore the effects and mechanisms of DEX mediated through the ITGA6/PI3K/AKT pathway in esophageal cancer, further validating its therapeutic potential and providing new insights and strategies for future clinical treatment.

Materials and Methods

Cell culture

This investigation employed multiple cellular models, including: the normal human esophageal epithelial cell line Het-1A (GNHu51) and EC cell lines TE-1 (TCHu89) and KYSE-180 (TCHu265), obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China); additional EC cell lines KYSE-450 (ACC 387) and KYSE-410 (ACC 381) from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig). Along with human umbilical vein endothelial cells (HUVECs, PCS-100-010) acquired from the American Type Culture Collection (ATCC, Manassas, VA).

For cell culture maintenance, Het-1A cells were grown in complete medium (ZKCC808424, ZKCC) supplemented with 10% fetal bovine serum (FBS, A5670701, Gibco). TE-1, KYSE-450, KYSE-180, and KYSE-410 cells were maintained in RPMI-1640 medium (11875093, Gibco) containing 10% FBS and 1% penicillin-streptomycin, while HUVECs required cultivation in EGM-2 (CC-3162, Lonza)[19,20]. All cell lines were incubated at 37°C in a humidified 5% CO_2 environment. For subculture procedures, cellular dissociation was achieved using 0.25% trypsin (15050057, Gibco).

CCK-8

Cell viability was measured using the CCK-8 assay[21]. A total of 2×10^3 cells were seeded into each well of a 96-well plate, with 100 μL of complete culture medium added per well. Following 24-hour incubation to ensure cellular attachment, the medium was aspirated and replaced with DEX-containing medium at graded concentrations (0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 μM) for 48-hour exposure. Cellular metabolic activity was then evaluated by supplementing each well with 10 μL CCK-8 solution (A311-02, Vazyme), followed by 2.5-hour incubation under standard culture conditions (37°C, 5% CO_2; CB 160, Binder). The resulting chromogenic reaction was quantified by measuring optical density at 450 nm wavelength using a microplate spectrophotometer (iMark, Bio-Rad).

Cell experiment design

To investigate the regulatory effects of DEX on inflammatory and angiogenic processes mediated by the ITGA6/PI3K/AKT axis, we initially performed CCK-8 assays to determine optimal DEX concentrations for both normal esophageal epithelial cells (Het-1A) and malignant ESCC lines (TE-1 and KYSE-450). Based on these preliminary results, functional experiments were conducted by treating TE-1 and KYSE-450 cells with specified DEX doses (0.8, 1.6, and 3.2 μM) for a 48-hour incubation period.

Based on the above functional experimental results, 3.2 μM DEX showed the best effect and was thus selected for subsequent experiments. To further investigate the molecular mechanisms underlying DEX's anti-tumor activity via the ITGA6/PI3K/AKT signaling axis, we performed both gain-of-function and loss-of-function studies by genetically manipulating ITGA6 expression levels. Cells were treated with DEX (3.2 μM) for 48 hours, and six experimental groups were set: Control, DEX, DEX+oe-NC, DEX+oe-ITGA6, DEX+si-NC, and DEX+si-ITGA6. To manipulate ITGA6 expression levels, TE-1 and KYSE-

450 cells were transfected with either the ITGA6 overexpression construct (oe-ITGA6) or specific ITGA6-targeting siRNA (si-ITGA6), with respective negative controls (oe-NC and si-NC), using Lipofectamine 3000 transfection reagent (L3000150, Invitrogen)[22]. Following a 6-hour transfection period, the culture medium was refreshed, and cellular proteins were harvested after 48 hours for Western blot analysis to confirm transfection efficiency. All genetic constructs (oe-ITGA6, si-ITGA6, oe-NC, and si-NC) were commercially obtained from GenePharma (Shanghai, China).

Cell migration and invasion

To evaluate the metastatic potential of EC cells, we performed Transwell migration and invasion assays using polycarbonate membrane inserts (3464, Corning)[23]. Migration experiments were conducted by seeding 4×10^4 cells/well in serum-free medium into Transwell inserts (8 μm pore size), with 10% FBS in RPMI-1640 as the chemoattractant in the lower chamber. For invasion evaluation, chambers were coated with extracellular matrix (C0372, Beyotime) before cell plating. After appropriate incubation, membranes were processed by removing non-invading cells, fixing with PFA, and staining with crystal violet. Microscopic observation and quantitative assessment of cell migration were performed.

Angiogenesis assay

To investigate the anti-angiogenic properties of DEX, we conducted an *in vitro* tube formation assay[24]. Briefly, growth factor-reduced Matrigel (50 μL/well) was polymerised in pre-chilled 96-well plates at 37°C for 30 min. HUVECs (2×10^4 cells/well) were then plated onto the gel matrix and exposed to conditioned medium collected from DEX-treated TE-1 and KYSE-450 cells (24-hour treatment). Following 6-8 hours of incubation, capillary-like network formation was examined by phase-contrast microscopy. Quantitative analysis was performed by capturing five random microscopic fields per well and calculating branch points using ImageJ angiogenesis analysis modules.

Flow cytometry

To assess DEX-induced apoptotic responses in EC cells, we performed flow cytometric analysis using double-staining methodology[25]. Briefly, TE-1 and KYSE-450 cells were subjected to experimental treatments, harvested, and processed with the Annexin V-APC/PI apoptosis detection system (88-8007-74, Invitrogen) according to standardised protocols. Cellular suspensions were prepared in 1× binding buffer and sequentially stained with Annexin V-APC and PI, followed by 10-minute dark incubation at ambient temperature. Samples were then diluted with 400 μL binding buffer and immediately analysed on a BD FACSCanto II flow cytometer. Quantitative data interpretation was performed using FlowJo analytical software, and apoptotic populations were identified based on established fluorescence parameters.

To evaluate cellular proliferation dynamics, we employed carboxyfluorescein succinimidyl ester (CFSE) labeling[26]. Briefly, EC cells were incubated with 5 μM CFSE fluorescent dye (C34570, Invitrogen) under physiological conditions (37°C, protected from light) for 10 minutes. Following thorough washing with complete medium to remove unbound dye, cells were subjected to experimental treatments. At designated time points, harvested cells were analysed by flow cytometry to quantify CFSE fluorescence decay, where progressive reduction in fluorescent signal intensity directly correlates with successive cellular divisions.

Xenograft model

The experimental protocol was approved by the Animal Ethics Committee of The Second Affiliated Hospital of Fujian Medical University (Approval No. 2022-550). Thirty-six male BALB/c nude mice (4-6 weeks old, 16-20 g body weight) were subcutaneously inoculated with 1×10^6 TE-1 cells in the right axilla to generate subcutaneous EC xenograft models. Tumor development was monitored every 2-3 days by measuring tumor dimensions, with volume calculated as (length × width²)/2. Upon reaching a mean tumor volume of ~100 mm³, the animals were randomly assigned to six experimental groups (n=6): (1) sham control (saline, i.p.), (2) DEX treatment (25 μg/kg, i.p., daily), (3) DEX+oe-NC, (4) DEX+oe-ITGA6, (5) DEX+si-NC, and (6) DEX+si-ITGA6. The combination treatment groups received both DEX and genetically modified cells (overexpression or silencing of ITGA6) via intraperitoneal administration for a 28-day intervention period[27]. Following the experimental protocol, animals were humanely euthanized via sodium pentobarbital administration. Tumor tissues were then excised, precisely weighed, and prepared for subsequent experimental analyses.

Enzyme-Linked Immunosorbent Assay (ELISA)

The concentrations of inflammatory mediators in conditioned media and circulating factors in murine serum were quantified by enzyme-linked immunosorbent assay[28]. Following various experimental treatments, culture supernatants were harvested from EC cells, while blood samples were collected via retro-orbital bleeding from experimental animals. All biological specimens were immediately frozen and maintained at -80°C for subsequent analysis. Following the manufacturer's instructions, ELISA kits for human or mouse IL-1β (900-T95, Invitrogen), IL-6 (MBS2511815, MyBioSource), TNF-α (MBS396296; MBS494101, MyBioSource), and VEGFA (MBS452127, MyBioSource) were used for detection. Briefly, standards and samples were added to 96-well plates pre-coated with specific antibodies, incubated, and washed. Biotin-labeled detection antibodies were added, incubated, and washed. Next, HRP-labeled streptavidin was added, incubated, and washed. The enzymatic reaction was terminated by adding stop solution following a dark incubation period with substrate, after which optical density measurements were obtained at 450 nm wavelength using a microplate spectrophotometer.

TUNEL staining

To evaluate apoptotic activity in tumor specimens, a TUNEL assay was conducted[29]. Excised tumors were processed through fixation (4% PFA), paraffin embedding, and preparation of 4 μm histological sections. Following standard deparaffinization and rehydration procedures, tissue sections were subjected to enzymatic digestion using proteinase K to unmask nuclear DNA breaks. Apoptotic cells were detected by incubating sections with TUNEL reaction mixture (T2196, Solarbio) at 37°C for 2 hours in a humid environment. After thorough washing, cellular nuclei were visualised with DAPI counterstain (5 μg/mL, C1002, Beyotime). Fluorescence microscopy was employed to identify apoptotic cells, with quantitative analysis performed by calculating the ratio of TUNEL-positive cells.

Immunohistochemistry

Immunohistochemical analysis was performed on paraffin-embedded tumor sections through sequential processing steps[30]. Tissue sections underwent standard deparaffinization and rehydration procedures, followed by heat-mediated antigen retrieval. To eliminate endogenous peroxidase interference, sections were treated with 3% H_2O_2 for 10 min at RT. Non-specific antibody binding was prevented by incubation with 5% normal goat serum for 30 min. Primary antibody incubation was carried out at 4°C overnight using anti-Ki-67 (2 μg/mL, ab15580, Abcam) and anti-Cluster of Differentiation 31 (CD31, 1:2000, ab182981, Abcam). Following PBS washes, sections were exposed to HRP-conjugated secondary antibody (1:1000, ab6721, Abcam) for 30 min at ambient temperature. Chromogenic detection was achieved using DAB substrate, with quantitative evaluation performed by imaging five randomly selected high-power fields per section and analysing staining intensity using ImageJ software.

Western blot

TE-1 and KYSE-450 cells subjected to various treatments, along with tumor tissues from animal studies, were lysed using RIPA buffer (R0020, Solarbio) containing protease and phosphatase inhibitors[31]. Protein concentrations were measured with a BCA assay kit (PC0020, Solarbio), and equal protein quantities were separated by SDS-PAGE. The resolved proteins were then transferred onto PVDF membranes (05317, Millipore). To minimize nonspecific interactions, the membranes were blocked for 1 hour at room temperature (approximately 25°C) with 5% BSA in TBST, followed by overnight incubation at 4°C with the appropriate primary antibodies. The primary antibodies employed in this study were as follows: IL-1β (ab283818), IL-6 (ab233706), TNF-α (ab183218), Matrix Metalloproteinase-9 (MMP9, ab76003), Matrix Metalloproteinase-2 (MMP2, ab92536), VEGFA (1 μg/mL, ab46154), FGF2 (ab208687), ITGA6 (1:2000, ab181551) purchased from Abcam; p-PI3K (#4228), PI3K (#4292), p-AKT (#4060), AKT (#9272), and GAPDH (#2118) purchased from Cell Signaling Technology, unless indicated otherwise, antibodies were used at a 1:1000 dilution. Following overnight incubation, membranes were washed three times (10 min each) with TBST and then probed with an HRP-conjugated secondary antibody for 1 hour at room temperature. Protein bands were visualised using an ECL substrate (P0018S, Beyotime), and densitometric analysis was performed using ImageJ software.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0, with data expressed as

mean ± SD[32]. Student's *t*-test was used for two-group comparisons, while one-way ANOVA with Tukey's post-hoc test was applied for multi-group analyses. Statistical significance was set at $P < 0.05$.

Results

DEX inhibits the proliferation, invasion, and migration of EC cells, and promotes apoptosis

The CCK-8 assay was used to evaluate DEX effects on EC cells (TE-1, KYSE-450, KYSE-180, KYSE-410) and normal Het-1A cells. Results demonstrated that DEX treatment (0.2, 0.4, 0.8, 1.6, 3.2 μM, 48h) showed no significant effect on Het-1A cell viability (Fig. 1A). In cancer cells, concentrations of 0.8, 1.6, and 3.2 μM induced dose-dependent growth inhibition (Fig. 1B). Subsequent studies employed 0.8, 1.6, and 3.2 μM DEX concentrations based on their selective anti-proliferative effects against malignant versus normal cells.

Cell migration is an early indicator of tumor cell dissemination, while invasiveness is a core aspect of tumor metastasis[33]. Transwell assays demonstrated a

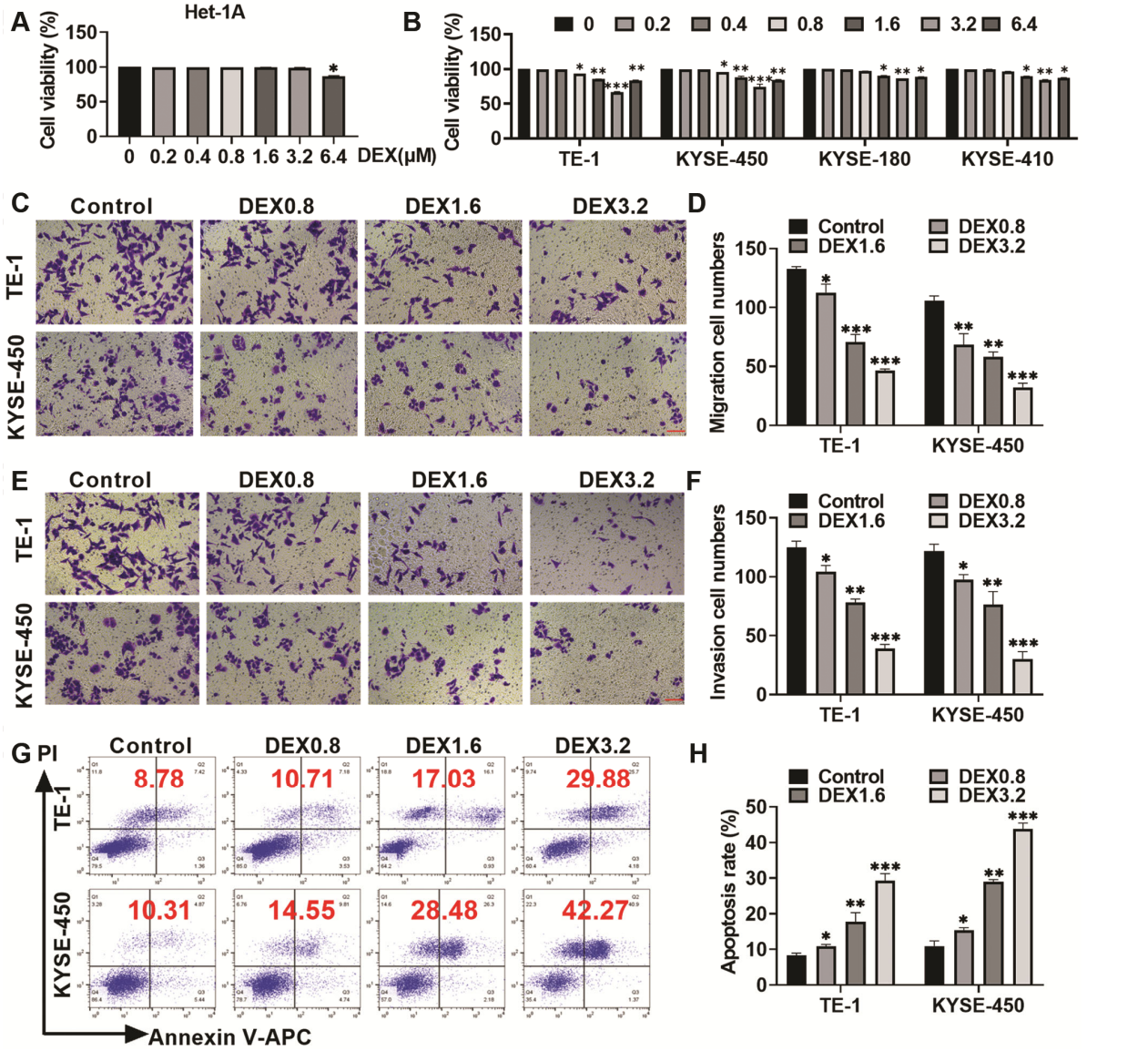

Fig. 1 — DEX inhibits the proliferation, invasion, and migration of EC cells, and promotes apoptosis. (A) CCK-8 assay showing viability of Het-1A cells treated with DEX (0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 μM) for 48h. (B) CCK-8 assay showing viability of EC cell lines (TE-1, KYSE-450, KYSE-180, KYSE-410) treated with DEX for 48h. The concentrations of DEX for subsequent experiments were selected as 0.8, 1.6, and 3.2 μM. (C-D) Transwell migration assay of EC cells treated with DEX. Scale bar 100 μm. (E-F) Transwell invasion assay of EC cells treated with DEX. Scale bar 100 μm. (G-H) Flow cytometric analysis of apoptosis in EC cells treated with DEX for 48 h using Annexin V-APC/PI staining. n=3. * P<0.05, ** P<0.01, *** P<0.001 vs Control.

dose-dependent reduction in migratory and invasive capacities of both TE-1 and KYSE-450 cells following DEX exposure (Fig. 1C-F), revealing its suppressive effect on EC cell metastasis. Flow cytometric analysis further revealed DEX-induced apoptosis in these cell lines (Fig. 1G-H). Collectively, these findings demonstrate DEX's multifaceted anti-tumor activity, including proliferation inhibition, metastasis suppression, and apoptosis induction in EC cells.

DEX inhibits EC inflammation and angiogenesis

To investigate DEX's anti-inflammatory properties in EC, we analysed inflammatory mediators in EC cells. ELISA quantification demonstrated a dose-dependent reduction in IL-1β, IL-6, and TNF-α secretion following DEX treatment (Fig. 2A-C). This anti-inflammatory activity was further validated at the protein level, as western blot analysis showed corresponding decreases in cytokine expression (Fig. 2D-G). These findings collectively establish

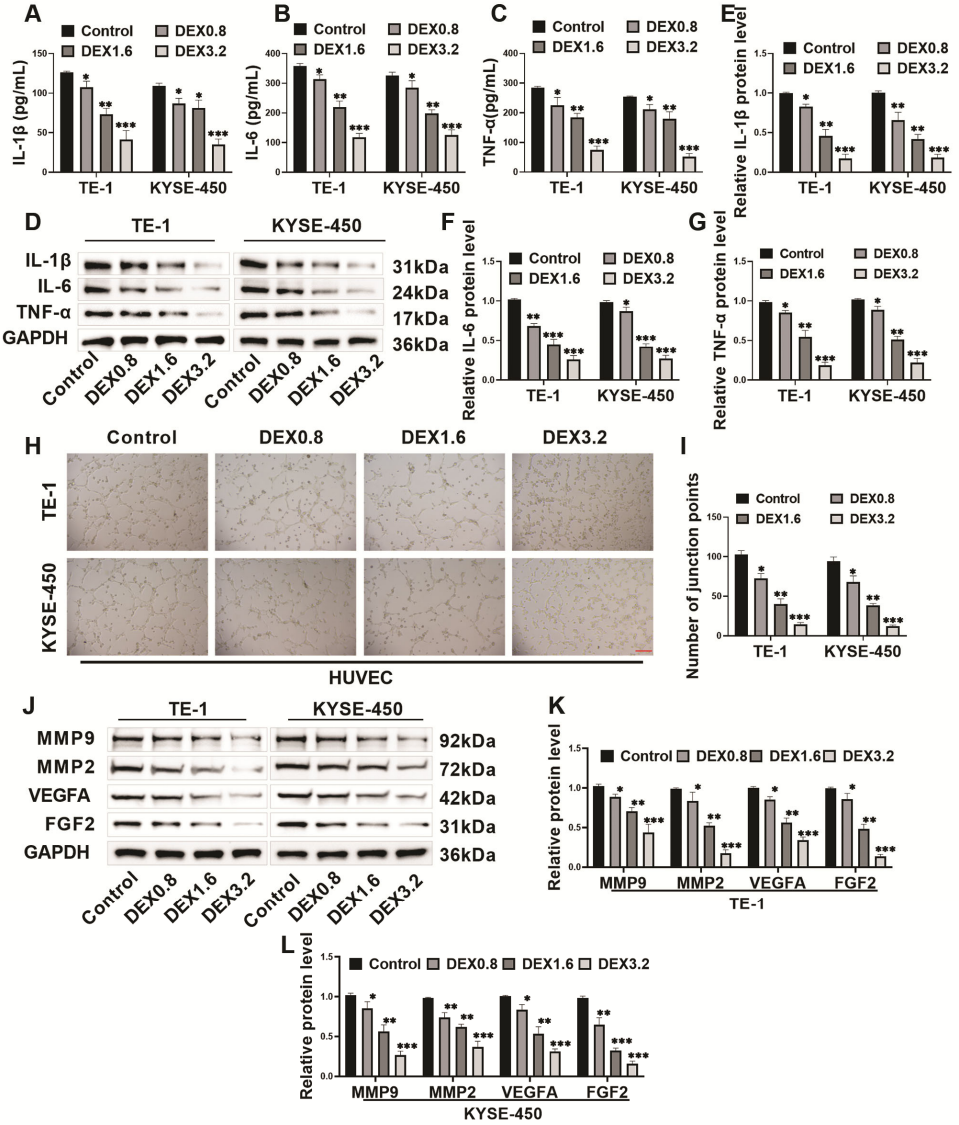

Fig. 2 — DEX inhibits EC inflammation and angiogenesis. (A-C) ELISA was used to measure the secretion levels of inflammatory cytokines IL-1β, IL-6, and TNF-α in the culture supernatants of TE-1 and KYSE-450 cells treated with DEX, evaluating the inhibitory effect of DEX on the inflammatory response of EC cells. (D-G) Western blot analysis assessed the protein expression levels of IL-1β, IL-6, and TNF-α in DEX-treated TE-1 and KYSE-450 cells, examining the regulatory effects of DEX on inflammation-related proteins. (H-I) Tube formation assays evaluated the impact of co-culturing DEX-pretreated TE-1 and KYSE-450 cells with HUVECs on angiogenic capacity, investigating the inhibitory role of DEX on tumor angiogenesis. (J-L) Western blot analysis measured the protein expression levels of MMP9, MMP2, VEGFA, and FGF2 in DEX-treated TE-1 and KYSE-450 cells, exploring the regulatory mechanisms of DEX on angiogenesis-related factors. n=3. * P<0.05, ** P<0.01, *** P<0.001 vs Control.

DEX's capacity to modulate inflammatory pathways in EC cells.

The tube formation assay results showed that the conditioned medium from DEX-treated ECs significantly inhibited the formation of tubular structures by HUVECs (Fig. 2H-I) suggesting that DEX can block tumor angiogenesis. To investigate the underlying mechanism, we analysed key angiogenesis-related proteins: matrix metalloproteinases (MMP9, MMP2) and vascular growth factors (VEGFA, FGF2). Western blot analysis revealed dose-dependent downregulation of these factors (Fig. 2J-L) suggesting DEX suppresses angiogenesis through coordinated inhibition of extracellular matrix degradation and growth factor signaling pathways. These experimental results confirm that DEX not only inhibits the inflammatory response in EC cells but also effectively suppresses tumor angiogenesis by regulating the expression of MMPs and angiogenesis-related factors (VEGFA, FGF2), thereby exerting potential anti-tumor effects.

DEX inhibits ITGA6/PI3K/AKT signaling pathway

To elucidate DEX's mechanism of action, we examined its effects on the ITGA6/PI3K/AKT pathway in EC cells (TE-1 and KYSE-450). Western blot analysis demonstrated that DEX treatment markedly reduced ITGA6 expression and suppressed PI3K and AKT phosphorylation (p-PI3K/PI3K and p-AKT/AKT ratios) (Fig. 3A-D). To establish ITGA6's functional role in this pathway, we generated isogenic cell models with either ITGA6 overexpression (oe-ITGA6) or silencing (si-ITGA6) (Fig. 3E-H). Western blot analysis revealed comparable expression patterns of ITGA6, p-PI3K, PI3K, p-AKT, and AKT in both DEX-treated and DEX+NC groups (Fig. 3I-L). Genetic silencing of ITGA6 effectively suppressed both ITGA6 expression and PI3K/AKT phosphorylation, whereas ITGA6 overexpression attenuated DEX-mediated inhibition of this signaling axis. These findings establish ITGA6 as the primary molecular target through which DEX exerts its anti-tumor activity.

DEX inhibits the malignant progression of EC through ITGA6/PI3K/AKT signaling pathway

To investigate the molecular mechanism underlying DEX-mediated regulation of EC progression via the ITGA6/PI3K/AKT axis, we initially employed CFSE-based flow cytometry to examine cellular proliferation. Our data revealed that DEX treatment markedly suppressed the proliferative capacity of both TE-1 and KYSE-450 cell lines (Fig. 4A-B). Notably, genetic overexpression of ITGA6 substantially attenuated this growth-inhibitory effect, whereas ITGA6 silencing potentiated DEX's anti-proliferative activity, confirming the critical involvement of ITGA6-mediated PI3K/AKT signaling in this process.

Transwell assays revealed DEX's potent inhibition of both migration and invasion in TE-1 and KYSE-450 (EC) cells (Fig. 4C-F). The anti-metastatic effects were modulated by ITGA6 expression levels: overexpression partially rescued cellular motility, while silencing synergistically enhanced DEX's inhibitory action. These results demonstrate that DEX impairs metastatic potential primarily through ITGA6-mediated regulation of PI3K/AKT signaling. Flow cytometric analysis demonstrated that DEX treatment significantly enhanced apoptosis in both TE-1 and KYSE-450 cell lines (Fig. 4G-H). The pro-apoptotic effect was modulated by ITGA6 expression levels, with overexpression attenuating and genetic silencing potentiating DEX-induced cell death. These findings provide direct experimental evidence that DEX triggers apoptosis through specific inhibition of the ITGA6/PI3K/AKT signaling cascade.

This study establishes that DEX exerts comprehensive anti-tumor activity in EC cells, simultaneously suppressing proliferation, inhibiting metastatic potential (migration and invasion), and inducing apoptotic cell death. All these therapeutic effects are mediated primarily through targeted disruption of ITGA6-dependent PI3K/AKT signaling.

DEX inhibits EC inflammation and angiogenesis through ITGA6/PI3K/AKT signaling pathway

Building upon our demonstration of DEX-mediated ITGA6/PI3K/AKT pathway inhibition, we investigated its impact on the tumor microenvironment. ELISA quantification revealed significant reductions in IL-1β, IL-6, and TNF-α secretion from DEX-treated TE-1 and KYSE-450 cells (Fig. 5A-C). Corresponding decreases in these inflammatory mediators were confirmed at the protein level by western blot analysis (Fig. 5D-G). The anti-inflammatory effects were mechanistically linked to ITGA6 signaling, as evidenced by partial reversal upon ITGA6 overexpression and enhancement following ITGA6 silencing. These results establish that DEX-mediated suppression of inflammatory

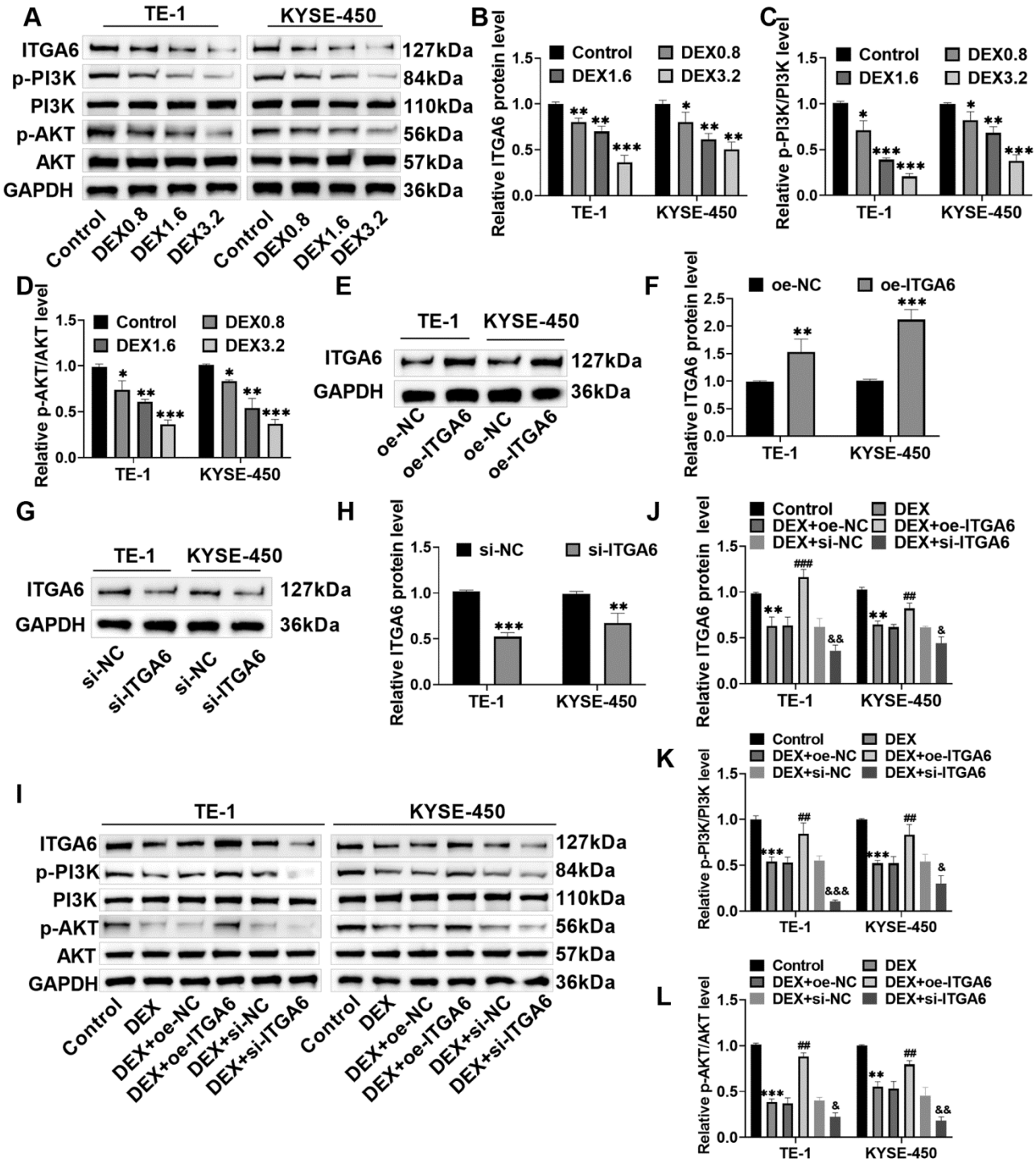

Fig. 3 — DEX inhibits the ITGA6/PI3K/AKT signaling pathway. (A-D) Western blot was used to detect the protein expression levels of ITGA6, p-PI3K, PI3K, p-AKT, and AKT in TE-1 and KYSE-450 cells treated with DEX, analyzing the inhibitory effect of DEX on the ITGA6/PI3K/AKT signaling pathway. n=3. * P<0.05, ** P<0.01, *** P<0.001 vs Control. (E-H) Validation of ITGA6 silencing and overexpression efficiency in EC cells by western blot. n=3. ** P<0.01, *** P<0.001 vs NC. (I-L) Western blot evaluation of ITGA6/PI3K/AKT pathway components in Control, DEX, DEX+oe-NC, DEX+oe-ITGA6, DEX+si-NC, and DEX+si-ITGA6 treatment groups. n=3. ** P<0.01, *** P<0.001 vs Control; ## P<0.01, ### P<0.001 vs DEX+oe-NC; & P<0.05, && P<0.01, &&& P<0.001 vs DEX+si-NC.

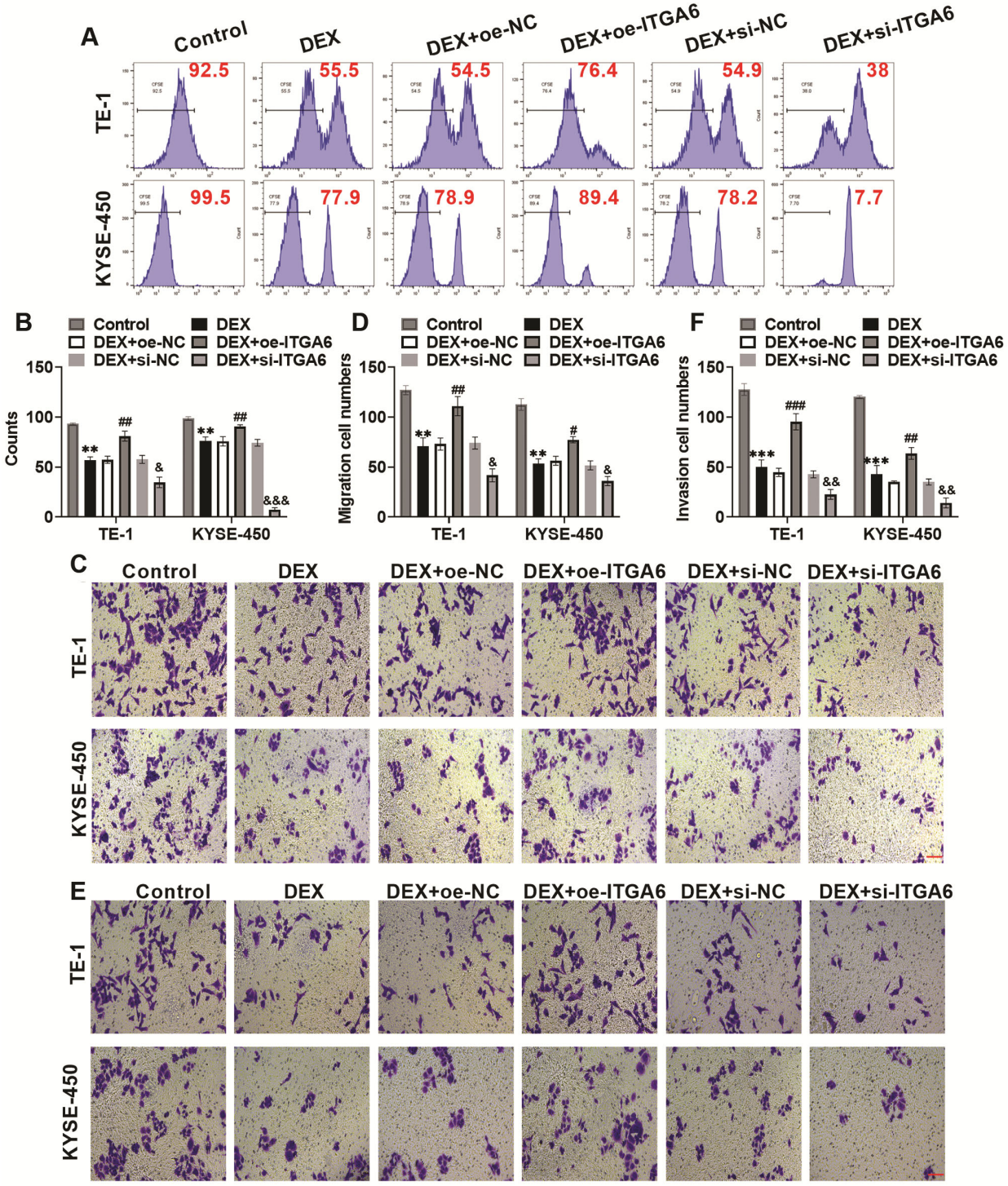

Fig. 4 — DEX inhibits the malignant progression of EC via the ITGA6/PI3K/AKT signaling pathway. (A-B) CFSE flow cytometry was used to assess the proliferative capacity of TE-1 and KYSE-450 cells in each group, verifying the inhibitory effect of DEX on cell proliferation via the ITGA6/PI3K/AKT pathway. (C-D) Transwell migration assays were performed to assess the migratory abilities of TE-1 and KYSE-450 cells in each group, investigating the regulatory effect of DEX on cell migration via the ITGA6/PI3K/AKT pathway. Scale bar 100 μm. (E-F) Transwell invasion assays detected the invasive capabilities of TE-1 and KYSE-450 cells in each group, analysing the inhibitory effect of DEX on cell invasion through the ITGA6/PI3K/AKT pathway. Scale bar 100 μm. (G-H) Annexin V-FITC/PI double staining flow cytometry was used to evaluate apoptosis in TE-1 and KYSE-450 cells across groups. n=3. ** P<0.01, *** P<0.001 vs Control; # P<0.05, ## P<0.01, ### P<0.001 vs DEX+oe-NC; & P<0.05, && P<0.01, &&& P<0.001 vs DEX+si-NC.

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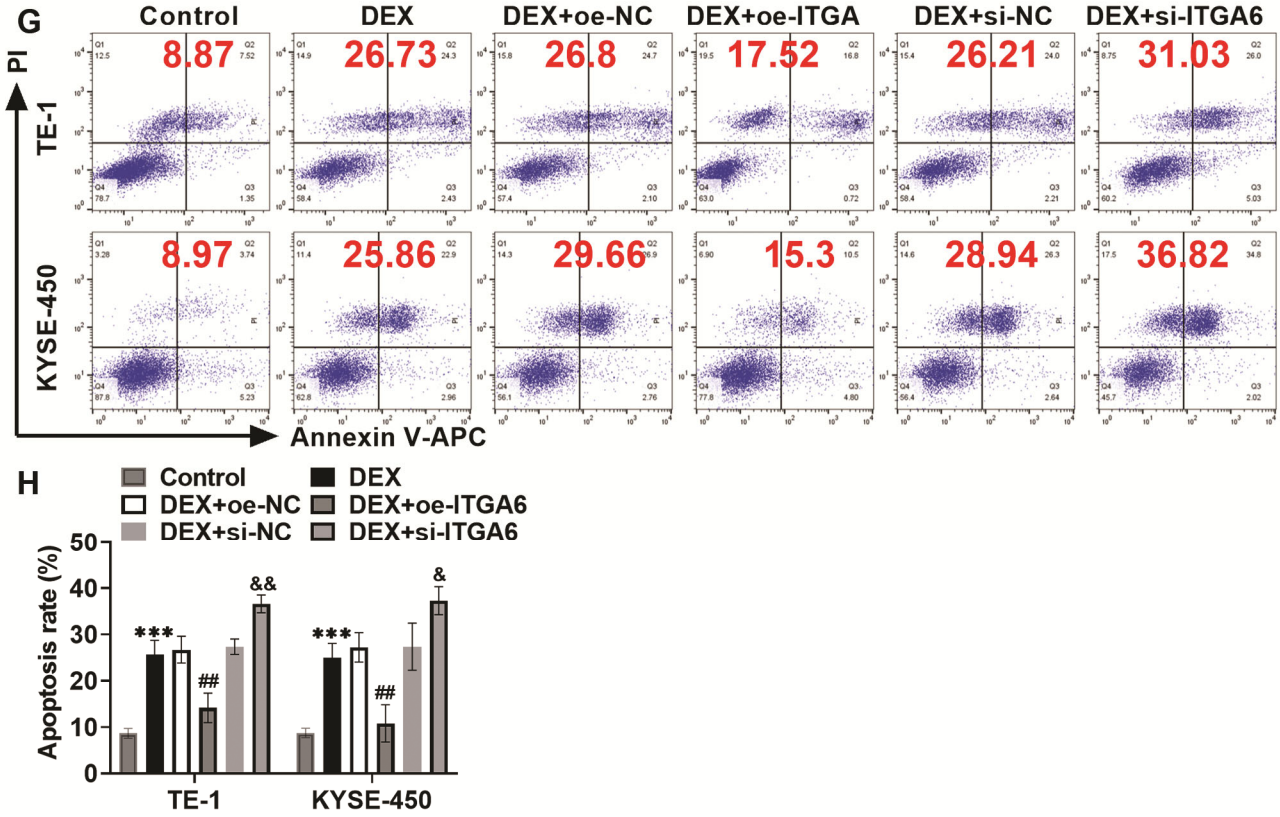

Fig. 4 — DEX inhibits the malignant progression of EC via the ITGA6/PI3K/AKT signaling pathway. (A-B) CFSE flow cytometry was used to assess the proliferative capacity of TE-1 and KYSE-450 cells in each group, verifying the inhibitory effect of DEX on cell proliferation via the ITGA6/PI3K/AKT pathway. (C-D) Transwell migration assays were performed to assess the migratory abilities of TE-1 and KYSE-450 cells in each group, investigating the regulatory effect of DEX on cell migration via the ITGA6/PI3K/AKT pathway. Scale bar 100 μm. (E-F) Transwell invasion assays detected the invasive capabilities of TE-1 and KYSE-450 cells in each group, analysing the inhibitory effect of DEX on cell invasion through the ITGA6/PI3K/AKT pathway. Scale bar 100 μm. (G-H) Annexin V-FITC/PI double staining flow cytometry was used to evaluate apoptosis in TE-1 and KYSE-450 cells across groups. n=3. ** P<0.01, *** P<0.001 vs Control; # P<0.05, ## P<0.01, ### P<0.001 vs DEX+oe-NC; & P<0.05, && P<0.01, &&& P<0.001 vs DEX+si-NC.

cytokine production occurs through modulation of the ITGA6/PI3K/AKT axis.

DEX exhibited potent anti-angiogenic effects by significantly suppressing HUVEC tube formation in co-culture systems with TE-1 and KYSE-450 cells (Fig. 5H-I). Molecular analysis demonstrated marked downregulation of angiogenic factors MMP9, MMP2, VEGFA, and bFGF following DEX treatment (Fig. 5J-L). Notably, these inhibitory effects were modulated by ITGA6 expression levels overexpression partially reversed while silencing enhanced DEX's anti-angiogenic activity, confirming ITGA6's pivotal role in mediating these effects. These results not only confirm that DEX regulates inflammation and angiogenesis in the tumor microenvironment via the ITGA6/PI3K/AKT pathway, thereby exerting anti-tumor effects, but also highlight that ITGA6 is a key regulatory factor mediating the anti-tumor effects of DEX.

DEX inhibits tumor progression *in vivo* through ITGA6/PI3K/AKT signaling pathway

To investigate DEX's anti-tumor efficacy *in vivo*, a xenograft model was established by subcutaneous inoculation of TE-1 cells in nude mice. Upon reaching 100-mm³ tumor volume, animals were randomized into six treatment groups for a four-week intervention. DEX administration demonstrated potent tumor-suppressive activity, as manifested by marked decreases in both tumor volume and mass compared to control groups (Fig. 6A-C). Notably, ITGA6 overexpression significantly weakened the anti-tumor effect of DEX, while ITGA6 silencing enhanced this effect.

Ki-67, a well-established nuclear proliferation marker[34], serves as a reliable indicator of tumor growth kinetics based on its immunohistochemical staining pattern. CD31 (PECAM-1), an endothelial junctional glycoprotein with critical functions in

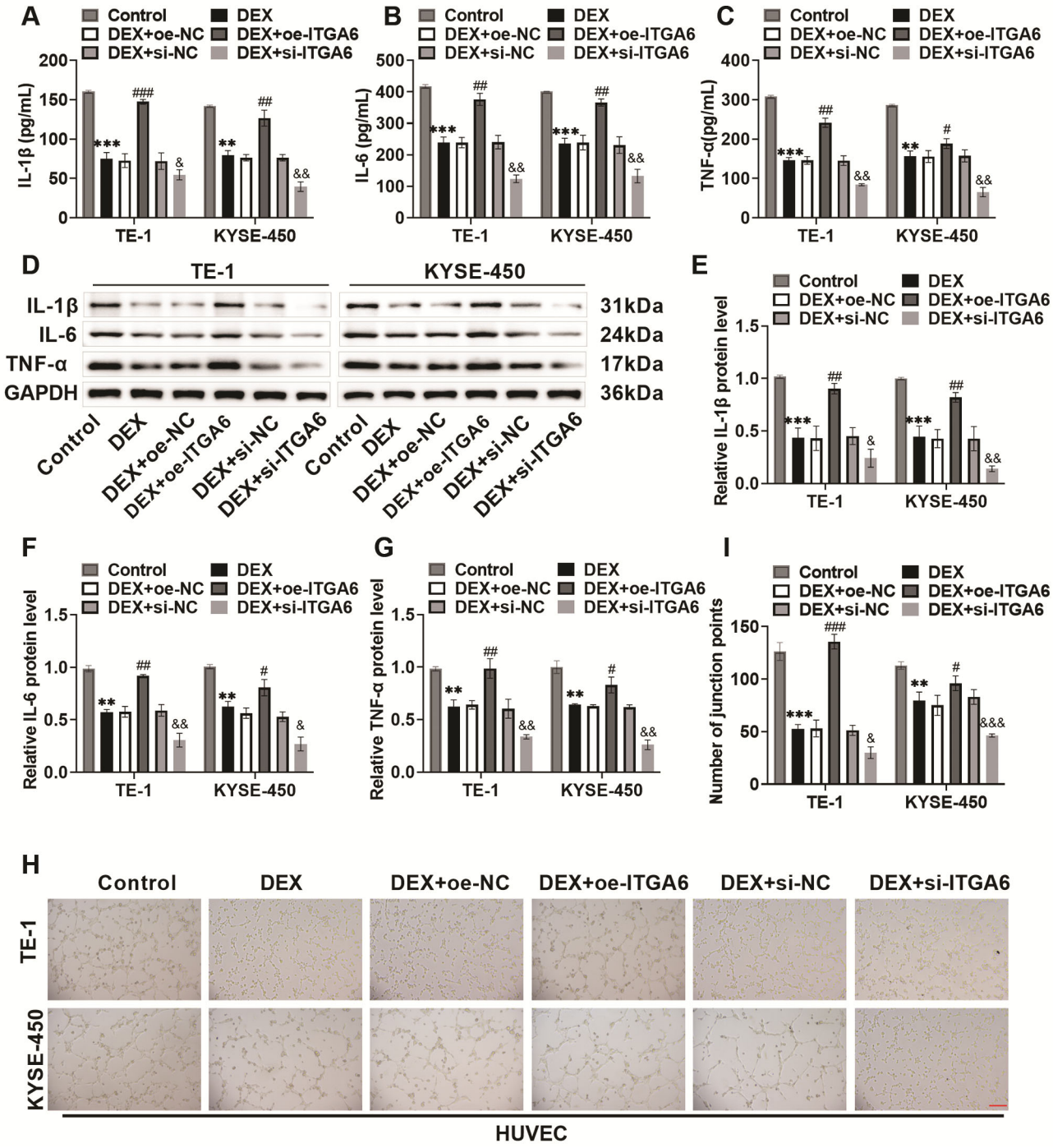

Fig. 5 — DEX inhibits EC inflammation and angiogenesis through the ITGA6/PI3K/AKT signaling pathway. (A-C) ELISA was used to measure the secretion levels of inflammatory cytokines IL-1β, IL-6, and TNF-α in the culture supernatants of TE-1 and KYSE-450 cells from each group, verifying the inhibitory effect of DEX on the inflammatory response via the ITGA6/PI3K/AKT pathway. (D-G) Western blot analysis assessed the protein expression levels of IL-1β, IL-6, and TNF-α in TE-1 and KYSE-450 cells from each group, elucidating the molecular mechanism by which DEX regulates inflammation-related factors through the ITGA6/PI3K/AKT pathway. (H-I) HUVEC tube formation assay using EC cell-conditioned medium. Scale bar 200 μm. (J-L) Western blot analysis measured the protein expression levels of MMP9, MMP2, VEGFA, and FGF2 in TE-1 and KYSE-450 cells from each group, investigating the influence of DEX on angiogenesis-related factors via the ITGA6/PI3K/AKT pathway. n=3. ** P<0.01, *** P<0.001 vs Control; # P<0.05, ## P<0.01, ### P<0.001 vs DEX+oe-NC; & P<0.05, && P<0.01, &&& P<0.001 vs DEX+si-NC.

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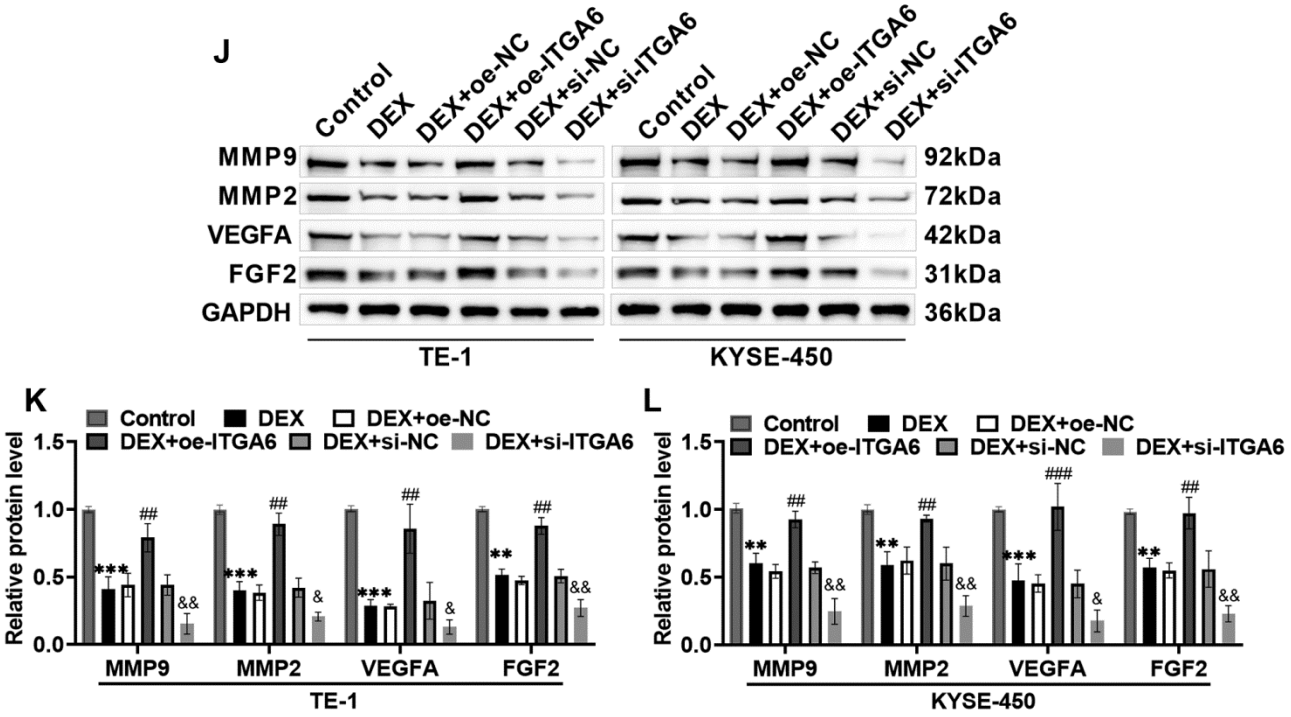

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angiogenesis and leukocyte trafficking[35], provides quantitative assessment of tumor vascularization through microvessel density measurements. Immunohistochemical analysis demonstrated that DEX treatment significantly decreased both the Ki-67 proliferation index and CD31-based microvascular density in tumor tissues (Fig. 6D-G) indicating potent inhibition of tumor cell proliferation and angiogenesis. Furthermore, TUNEL assays revealed a substantial increase in apoptotic cells following DEX treatment (Fig. 6H-I). These findings collectively demonstrate that DEX exerts comprehensive anti-tumor effects *in vivo* through simultaneous modulation of proliferation, apoptosis, and angiogenesis within the tumor microenvironment. Serological analysis revealed DEX-mediated suppression of both TNF-α (inflammatory mediator) and VEGFA (angiogenic factor) levels (Fig. 6J-K), corroborating its dual anti-inflammatory and anti-angiogenic properties. Tumor tissue immunoblotting demonstrated concurrent downregulation of ITGA6 expression and PI3K/AKT phosphorylation following DEX treatment (Fig. 6L-O). Genetic modulation experiments established ITGA6's central role, where its overexpression attenuated while silencing potentiated DEX's therapeutic outcomes across multiple tumor parameters. These *in vivo* findings mechanistically validate ITGA6/PI3K/AKT pathway inhibition as the primary mode of DEX's anti-tumor action.

Discussion

EC remains a global health challenge characterised by aggressive progression, frequent late-stage diagnosis, and limited therapeutic efficacy, contributing to its poor clinical outcomes[36,37]. These limitations underscore the urgent need for mechanistic investigations and novel treatment approaches. Our findings reveal DEX's potent and selective anti-tumor activity against EC cells, as evidenced by

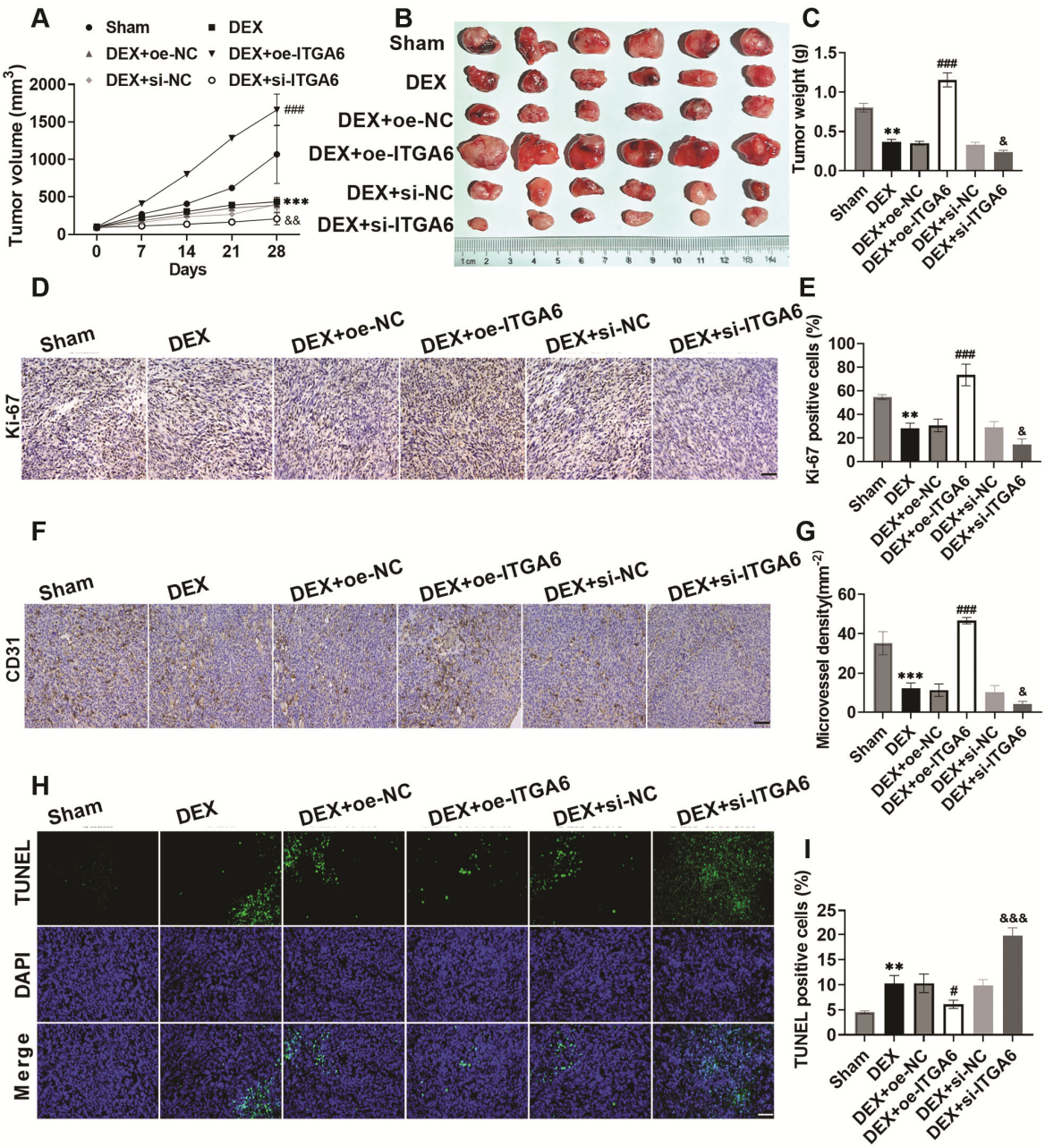

Fig. 6 — DEX inhibits tumor progression *in vivo* through the ITGA6/PI3K/AKT signaling pathway. (A) Establishment of EC xenograft model and experimental design: TE-1 cells were subcutaneously injected into the right axilla of nude mice. When the tumor volume reached 100 mm³, mice were randomly divided into six groups (n=6): Sham, DEX, DEX+oe-NC, DEX+oe-ITGA6, DEX+si-NC, and DEX+si-ITGA6. Treatments were administered continuously for 4 weeks, with tumor volumes measured weekly. (B-C) Gross tumor observation and tumor weight comparison after 28 days of treatment to evaluate the inhibitory effect of DEX on tumor growth. (D-E) Immunohistochemistry detection of Ki-67 expression in tumor tissues to analyze the impact of DEX on tumor cell proliferation. Scale bar 50 μm. (F-G) Immunohistochemistry detection of CD31 expression in tumor tissues to assess the inhibitory effect of DEX on tumor angiogenesis. Scale bar 50 μm. (H-I) TUNEL assay to detect the number of apoptotic cells in tumor tissues, confirming the pro-apoptotic effect of DEX on tumor cells. Scale bar 50 μm. (J-K) ELISA measurement of TNF-α and VEGFA levels in mouse serum to evaluate the influence of DEX on systemic inflammation and angiogenic factors. (L-O) Western blot analysis of protein expression levels of ITGA6, p-PI3K/PI3K, and p-AKT/AKT in tumor tissues to elucidate the molecular mechanism by which DEX exerts its antitumor effects via the ITGA6/PI3K/AKT pathway. n=3. ** P<0.01, *** P<0.001 vs Control; # P<0.05, ## P<0.01, ### P<0.001 vs DEX+oe-NC; & P<0.05, &&& P<0.001 vs DEX+si-NC.

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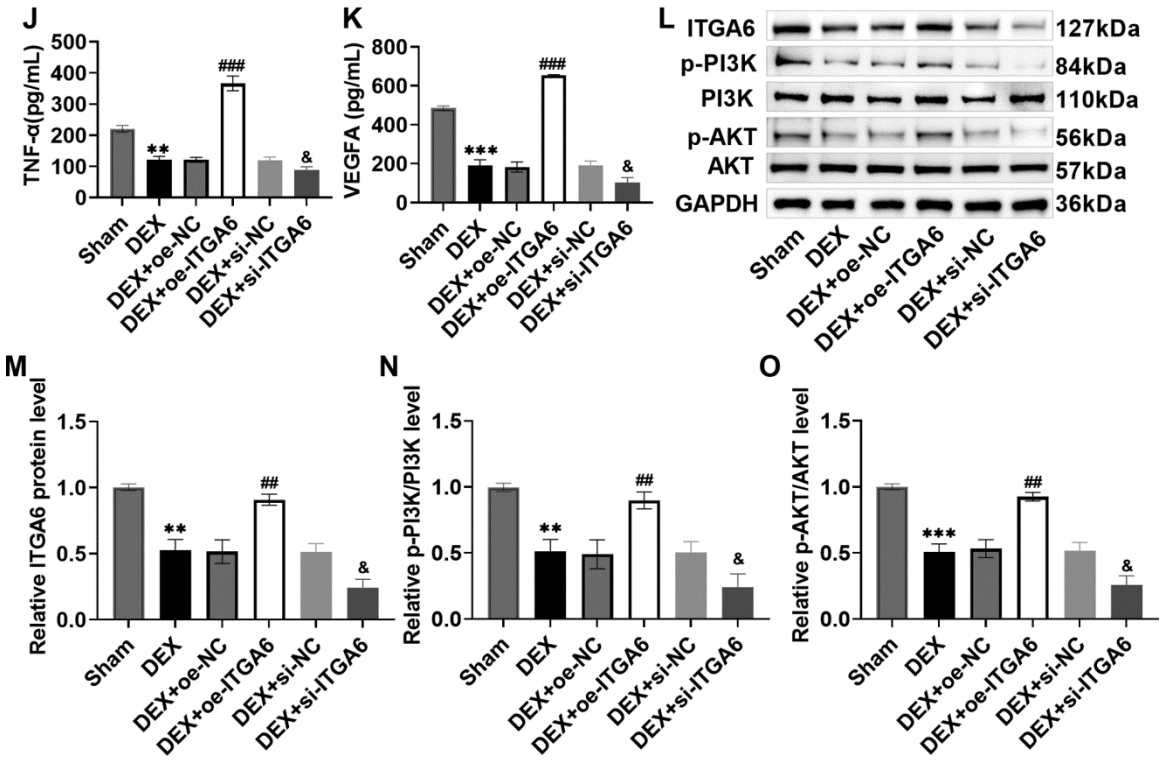

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dose-dependent proliferation inhibition in TE-1 and KYSE-450 cell lines, while exhibiting significantly reduced cytotoxicity in normal Het-1A epithelial cells. This cancer-selective profile suggests DEX's potential as a targeted therapeutic agent. Quantitative colony formation analysis revealed DEX's significant attenuation of EC cell clonogenicity, reflecting impaired tumor cell replication capacity. The Transwell assay demonstrated that DEX markedly reduced the migratory and invasive capabilities of endometrial carcinoma cells, indicating its potential to prevent cancer spread by interfering with cellular movement. Additionally, flow cytometric examination showed that DEX treatment increased apoptotic cell death in these tumor cells, potentially representing another key pathway through which it exerts its anticancer effects. These experimental observations align with existing literature documenting DEX's inhibitory actions in various malignancies. For instance, prior research has reported similar growth-restricting and motility-suppressing effects of DEX in colorectal adenocarcinoma cell lines[38]. To validate these laboratory findings in a living organism, the current investigation employed a BALB/c nude mouse model implanted with endometrial cancer xenografts. The animal experiments yielded results that closely mirrored the cellular studies, demonstrating that DEX administration effectively restrained tumor development through measurable decreases in both tumor dimensions and mass. Histopathological examination revealed markedly diminished Ki-67

immunoreactivity a well-established proliferation indicator in DEX-treated tumor specimens. Concurrently, TUNEL analysis detected elevated apoptotic activity within these treated tumors, thereby confirming the pro-apoptotic effects observed in cell culture experiments. These collective findings suggest DEX exerts its anticancer activity through multiple pathways, potentially involving both cell cycle modulation to impede neoplastic cell division and programmed cell death induction to eliminate malignant cells. Mechanistically, DEX may impair tumor cell motility and invasiveness through modulation of key cellular processes, including cytoskeletal reorganization, intercellular adhesion dynamics, and extracellular matrix degradation enzymes[39]. Furthermore, as a selective α2-adrenergic receptor agonist, DEX potentially influences tumor energy metabolism pathways - particularly glycolysis and mitochondrial oxidative phosphorylation which may contribute to its observed anti-proliferative effects[40,41]. DEX appears to induce programmed cell death through multiple apoptotic mechanisms, potentially involving both mitochondrial-mediated (intrinsic) and death receptor-mediated (extrinsic) pathways. This pro-apoptotic activity may stem from its ability to modulate critical apoptotic regulators, including enhancing the Bax/Bcl-2 protein ratio and triggering sequential caspase activation[42,43]. Further investigation is warranted to fully elucidate these molecular mechanisms and uncover the complete spectrum of DEX's anticancer properties.

The tumor microenvironment in EC is characterised by persistent inflammatory responses and dysregulated vascular formation, both of which significantly contribute to disease progression[44,45]. Our experimental data demonstrate that DEX administration markedly decreased both the secretion and intracellular expression of pro-inflammatory mediators in EC cells. This anti-inflammatory property of DEX is particularly relevant since chronic inflammation facilitates neoplastic development through multiple pathways: stimulating uncontrolled cellular proliferation, enhancing invasive potential, and promoting metastatic dissemination via cytokine-mediated mechanisms[46]. The therapeutic significance of this finding is supported by existing literature documenting DEX's capacity to mitigate surgical stress-induced inflammation and suppress pro-inflammatory cytokine production[47], with similar observations reported in malignancies of the digestive tract and pulmonary system[48,49]. The suppression of inflammatory processes exerts dual antitumor effects: directly impairing cancer cell viability and proliferation, while simultaneously modifying the tumor niche to create unfavourable conditions for neoplastic expansion and dissemination[50,51]. Subsequent investigations should focus on elucidating DEX's immunomodulatory effects within the EC microenvironment, particularly its influence on tumor-infiltrating immune populations and its potential to modulate critical inflammatory signaling cascades at the molecular level.

Pathological angiogenesis enables malignant advancement by generating tumor-nourishing capillaries that promote proliferation, invasive capacity, and distant colonization. Once a tumor reaches a critical size (typically 1–2 mm^3), its further expansion becomes dependent on neovascularization to ensure adequate oxygen and nutrient supply (e.g., glucose and amino acids) while facilitating the clearance of metabolic byproducts[52,53]. Consequently, targeting angiogenic processes has emerged as a key therapeutic approach in oncology. *In vitro* experiments using a tube formation assay revealed that DEX substantially suppresses the vascular network-forming capacity of HUVECs. Notably, this anti-angiogenic effect was significantly enhanced in co-culture systems with EC cells, suggesting a potential tumor microenvironment-mediated mechanism. This suggests that DEX can directly or indirectly interfere with tumor-induced angiogenesis. MMPs, particularly MMP-9 and MMP-2, are critical mediators of tumor progression, facilitating both invasive behaviour and metastatic spread through extracellular matrix degradation, while also contributing to angiogenic processes[54]. Similarly, VEGF-A and FGF-2 represent two of the most potent angiogenic regulators, whose overexpression correlates strongly with enhanced tumor vascularization and unfavourable clinical outcomes[55,56]. Our experimental data demonstrated that DEX administration markedly reduced the protein expression of these key angiogenic mediators (MMP-9, MMP-2, VEGF-A, and FGF-2) in endometrial carcinoma cells, as evidenced by western blot analysis. This downregulation of pro-angiogenic factors was associated with significant inhibition of neovascularization. Consistent with these *in vitro* findings, *in vivo* studies revealed a substantial decrease in CD31 immunoreactivity (a specific

endothelial cell marker) within DEX-treated tumors, further confirming the compound's anti-angiogenic efficacy in a physiological context. ELISA testing of serum TNF-α and VEGFA levels in mice further confirmed that DEX could reduce systemic inflammation and angiogenesis. It is further confirmed that the anti-angiogenic effect of DEX may also synergize with its anti-inflammatory effect, collectively inhibiting tumor growth and metastasis.

ITGA6, a prominent member of the integrin superfamily, demonstrates elevated expression across multiple malignancies and has been strongly implicated in tumor aggressiveness, metastatic dissemination, and clinical outcomes[57]. Concurrently, the PI3K/AKT signaling cascade represents a fundamental cell survival pathway whose dysregulation significantly contributes to oncogenesis and disease progression[58]. In endometrial carcinoma research, the activation status of PI3K/AKT/mTOR pathway components (including mTOR and phosphorylated AKT) shows significant correlation with both tumor aggressiveness and patient prognosis. This critical pathway not only modulates malignant phenotypes such as invasive potential and chemoresistance but also functionally interacts with regulatory molecules like RNF2, thereby emerging as both a promising prognostic indicator and therapeutic target[59]. Western blot analysis in the current investigation revealed that DEX treatment substantially decreased protein expression of ITGA6, p-PI3K, and p-AKT in endometrial carcinoma cells, whereas total PI3K and AKT levels remained unaltered. These findings indicate that DEX not only suppresses ITGA6 expression but also attenuates PI3K/AKT pathway activation through inhibition of phosphorylation events. Given ITGA6's role as a cell surface adhesion receptor, its downregulation likely impairs tumor cell-extracellular matrix interactions, consequently inhibiting critical oncogenic processes including cellular adhesion, motility, and invasive capacity[60]. The reduced expression of ITGA6 appears to attenuate cellular sensitivity to both growth factor stimulation and extracellular matrix signaling, consequently suppressing downstream PI3K/AKT pathway activation[61]. This pathway blockade subsequently modulates multiple downstream effectors involved in critical cellular processes including proliferation, programmed cell death, motility, and blood vessel formation, ultimately generating comprehensive antitumor activity[62].To further elucidate the functional role of ITGA6 in DEX-mediated tumor suppression, we performed both gain- and loss-of-function experiments. Notably, forced expression of ITGA6 (oe-ITGA6) not only reversed DEX-induced downregulation of ITGA6, p-PI3K, and p-AKT but also attenuated DEX's inhibitory effects on endometrial cancer cell proliferation, migration, invasion, and apoptosis. Furthermore, oe-ITGA6 partially restored the expression of inflammatory cytokines and angiogenesis-related proteins that were suppressed by DEX treatment. Conversely, genetic silencing of ITGA6 (si-ITGA6) synergistically enhanced DEX's ability to suppress these molecular markers and potentiated its antitumor efficacy. These complementary genetic approaches provide compelling evidence that ITGA6 serves as a critical molecular target of DEX, through which it exerts its therapeutic effects by modulating the ITGA6/PI3K/AKT signaling axis to coordinately inhibit malignant progression, inflammatory responses, and angiogenic processes in endometrial carcinoma. Although we have confirmed the regulatory effects of DEX on the ITGA6/PI3K/AKT pathway, how DEX directly or indirectly affects the expression of ITGA6, and whether there are other potential bypass mechanisms, still require further in-depth investigation.

Conclusion

This study is the first to comprehensively reveal that DEX inhibits the proliferation, migration, and invasion of EC cells, promotes apoptosis, and simultaneously reduces inflammation and angiogenesis by suppressing the ITGA6/PI3K/AKT signaling pathway. These findings not only provide a solid theoretical foundation and experimental evidence for the application of DEX in EC treatment but also offer new targets and strategies for EC therapy.

Ethic statement

This study was approved by The Second Affiliated Hospital of Fujian Medical University Committee (Approval No.: 2022-550).

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Conflicts of interest

The authors declare that they have no financial conflicts of interest.

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