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Original Article

GDF15 predisposes aortic dissection through regulation of vascular smooth muscle cell proliferation and phenotypic change via the ERK/Akt pathway

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Abstract: Objective: Aortic dissection (AD) represents a life-threatening vascular disorder characterized by abnormal vascular smooth muscle cell (VSMC) behavior and extracellular matrix remodeling. Growth differentiation factor 15 (GDF15) is a stress-related cytokine associated with the pathogenesis of cardiovascular diseases, but its exact role in AD remains to be elucidated. Methods: Human aortic smooth muscle cells (HASMCs) were incubated with angiotensin II (Ang II) to simulate an in vitro pathological tension environment. Gain- and loss-of-function experiments were carried out to investigate the functional role of GDF15 in regulating cell growth, migration, phenotypic transformation, and inflammatory reaction. The involvement of the ERK/protein kinase B (Akt) pathway was studied using a ERK inhibitor. Clinical significance was analyzed by detecting the plasma levels of GDF15 and matrix metalloproteinases (MMPs) in AD patients and healthy controls. Results: Ang II upregulated GDF15 expression in HASMCs in a dose- and time-dependent manner. GDF15 overexpression promoted HASMC proliferation, migration, and phenotypic switching from a contractile to a synthetic state, accompanied by increased expression of MMP-2 and MMP-9 and pro-inflammatory cytokines (TNF-α, IL-6, and MCP-1). GDF15 knockdown produced opposite effects. Mechanistically, GDF15 activated both ERK and Akt pathways; however, pharmacological inhibition experiments suggested that the pro-remodeling effects of GDF15 were primarily mediated through ERK signaling. Clinically, plasma concentrations of GDF15, MMP-2, and MMP-9 were significantly higher in AD patients compared with healthy controls. Conclusions: GDF15 promotes aberrant remodeling of HASMCs by enhancing proliferation, migration, phenotypic transformation, and inflammatory responses via ERK-dependent signaling. Elevated plasma GDF15 levels in AD patients suggest that GDF15 can serve as a potential diagnostic biomarker and therapeutic target for AD.

Keywords: Aortic dissection, GDF15, vascular smooth muscle cells, phenotypic switching, ERK/Akt signaling, angiotensin II

Introduction

Aortic dissection (AD) is a life-threatening vascular disease characterized by a tear in the intima that allows blood to flow into the media and form a false lumen, thereby weakening the aortic structure [1]. Despite advances in surgical techniques and endovascular therapies, the mortality rate of AD is still high, largely due to limited understanding of its underlying molecular mechanisms [2, 3].

Vascular smooth muscle cells (VSMCs), which constitute most of the aortic media, play a critical role in the development of AD [4]. Under physiological conditions, VSMCs maintain the contractile state necessary for regulation of arterial pressure [5, 6]. However, in response to disease-associated stimuli such as inflammation, oxidation, or mechanical injury, VSMCs undergo phenotypic switching to a synthetic state. This transition is characterized by enhanced proliferation and migration, increased extracellular matrix (ECM) remodeling, and upregulated expression of matrix metalloproteinase (MMP) [7, 8]. By destroying the architectural structure of the aortic layer, such cellular remodeling constitutes a key pathogenic factor in AD formation [9, 10]. As a result, identification of molecular mediators that regulate VSMC

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dynamics may provide potential biomarkers for early detection and targets for clinical treatment of AD [11].

Growth differentiation factor 15 (GDF15), an atypical member in the transforming growth factor - β (TGF-β) family, functions as a stress-induced regulator associated with inflammation, apoptosis, and endothelial remodeling in cardiovascular disorders [12, 13]. Elevated plasma GDF15 levels are related to various cardiovascular conditions, including heart failure, atherosclerosis, and hypertension [14]. However, the precise role of GDF15 in AD remains unclear [10, 15]. Previous studies suggested that GDF15 may influence the behavior of VSMCs, but its molecular mechanisms and functional contributions in the AD process have not been fully elucidated [12, 16].

Serving as a major effector of the renin-angiotensin system, Angiotensin II (Ang II) is a well-established inducer of vascular remodeling in both *in vitro* and *in vivo* experimental models [17, 18]. Ang II induces oxidative stress, inflammation, and phenotypic changes in VSMCs, thereby recapitulating the pathological microenvironment observed in AD [19]. Ang II-stimulated human aortic smooth muscle cells (HASMCs) represent an appropriate cell model for investigating VSMC behavior under AD-like conditions [5, 20].

In this study, we hypothesized that GDF15 promotes AD development by regulating VSMC phenotypic transformation. To test this, GDF15 expression in Ang II-stimulated HASMCs and clinical samples was assessed. Furthermore, the effects of GDF15 overexpression (GDF15-OE) or knockdown (GDF15-KD) on VSMC proliferation, migration, phenotypic transformation, and inflammatory responses, as well as the involvement of ERK and Akt signaling pathways were analyzed [21]. These findings may provide novel insights into the pathophysiological role of GDF15 in AD and identify potential molecular targets for therapeutic intervention.

Materials and methods

Study population and serum analysis

AD patients were sequentially recruited from the Xiangxi Autonomous Prefecture People's Hospital. The diagnosis was confirmed by computed tomography angiography (CTA). Age- and sex-matched asymptomatic individuals without a history of cardiovascular disease, hypertension, or diabetes who underwent routine health examinations at the same hospital during same time period served as the control group.

Exclusion criteria: 1) a history of malignancy, chronic inflammatory disorders, severe hepatic or renal dysfunction; 2) acute infection within the previous month; 3) use of immunosuppressive or anti-inflammatory medications.

Peripheral venous blood samples were collected from AD patients within 24 h of admission before surgery, and from controls during routine medical examinations. Blood was collected in serum separator tubes and allowed to coagulate at room temperature for 30 minutes, and then centrifuged at 3,000×g for 15 minutes at 4°C. The resulting serum was aliquoted and stored at -80°C till further analysis. Plasma concentrations of GDF15, MMP-2, and MMP-9 were measured using commercial ELISA kit according to manufacturer's protocols. This study was approved by the Ethic Committee of Xiangxi Autonomous Prefecture People's Hospital, and written informed consent was obtained from each subject.

Cell culture

HASMCs (Cerebrox Biological Products, Hong Kong) were cultured in SmGM-2 medium (Lonza) with 10% FBS, 1% penicillin-streptomycin, and 1% L-glutamine at 37°C in a humidified atmosphere containing 5% CO_2. Cells between passages 3 and 8 were used for further study [22].

Ang II stimulation

HASMCs were cultured to 70-80% confluence and subjected to serum starvation for 12 h to induce a quiescent state. Cells were subsequently incubated with Ang II (Sigma-Aldrich, USA) at different concentrations (10, 50, 100, or 200 nM) for 24 h to evaluate dose-dependent effects. For time-course studies, cells were incubated with Ang II (100 nM) for 1, 6, 12 and 24 h. Cells cultured in the standard growth medium without Ang II served as controls.

Gene overexpression and knockdown

The GDF15 overexpression vector (GDF15-OE) and the shRNAs targeting GDF15 for knock-

down (GDF15-KD) were obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences. The specific sequence used was: shGDF15, 5'-GGA AGA TCT GCC ACC ATG CCC GGG CAA GAA C-3'. Transfections were performed using Lipofectamine 2000 reagent (Invitrogen, USA) according to the manufacturer's instructions. HASMCs were seeded in six-well plates and transfected with 2 μg/well of GDF15-OE vectors or 100 nM of GDF15 shRNAs in serum-free conditions for 6 hours. The transfection medium was then replaced with complete growth medium, and cells were incubated for an additional 24 hours prior to subsequent experiments [23].

Pharmacological inhibition of the ERK signaling pathway

To study the involvement of the ERK signaling pathway, HASMCs were pretreated with the selective ERK inhibitor PD98059 (Beijing Tongren Institute of Chemistry, China) at a final concentration of 20 μM for 1 h before Ang II stimulation. Cells were subsequently used for downstream experiments.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from HASMCs using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. RNA concentration was determined using NanoDrop2000 Spectrophotometer (Thermo Fisher Scientific, USA). RNA (1 μg) was reverse transcribed into complementary DNA (cDNA) using a commercial kit (Takara, Japan). Quantitative PCR was carried out on a QuantStudio 6 Flex instrument (Applied Biosystems, USA) using SYBR Green Master Mix (Takara, Japan). Thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a housekeeping gene, and relative mRNA level was calculated according to the $2^{(-\Delta\Delta Ct)}$ method [22]. Primer sequences of GDF15 were as follows: forward, 5'-CAA CCA GAG CTG GGA AGA TTC G-3'; reverse, 5'-CCC GAG AGA TAC GCA GGT CCA-3'.

Western blot analysis

Cytoplasmic proteins were extracted from HASMCs using RIPA lysis solution supplemented with protease and phosphatase inhibitors (Beyotime, China). Protein concentrations were determined using a BCA Protein Assay kit (Beiyoutime, China). Equal amounts of protein (20-30 μg) were separated by Sodium dodecyl sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, USA). The membranes were then blocked with 5% skimmed milk in TBST at room temperature for 1 h before being incubated overnight at 4°C with primary antibodies against GDF15, PCNA, CDK2, p21, α-smooth muscle actin (α-SMA), SM22α, MMP-2, MMP-9, phosphorylated ERK1/2 (p-ER K1/2), phosphorylated protein kinase B (p-Akt), and GAPDH. After extensive washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h. Immunoreactive signals were observed using enhanced chemiluminescence substrate (Thermo Fisher Scientific, USA) and band densities were quantified using Image J software [24].

Cell proliferation assay

HASMCs were placed in 96-well plates at a density of about 5×10^4 cells/well, then subjected to the designated treatments. After incubation, 10 μL of Cell Counting kit-8 (CCK-8) reagent was added into each well, and cells were incubated at 37°C for another two hours. Optical density at 450 nm was measured using a microplate reader (BioTek, USA).

5-ethynyl-2'-deoxyuridine (EdU) assay

Cell viability was further evaluated using EdU incorporation assay. After treatment, HASMCs were incubated with 10 μM EdU for 2 hours, fixed, permeabilized, and stained according to the protocol for Click-it EdU kit. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). EdU-positive cells were visualized using a fluorescent microscope (Olympus, Japan), and the proliferation index was calculated as the ratio of EdU-positive nuclei to total nuclei using ImageJ software.

Wound healing assay

HASMCs were seeded in six-well plates and cultured until they reached about 90% cell confluency. A linear scratch was made across the cell monolayer using a sterile 200 μL pipette tip. Detached cells were removed by washing with

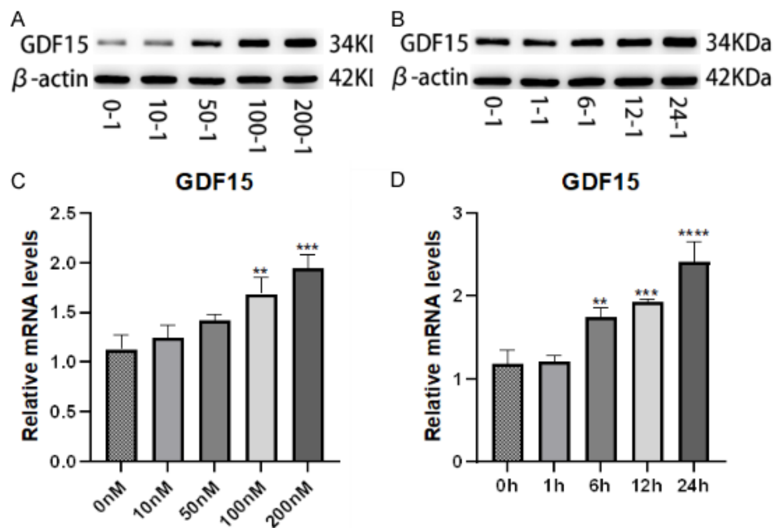

Figure 1. Ang II upregulated GDF15 expression in HASMCs in a dose- and time-dependent manner. A. Western blot analysis of GDF15 protein expression in HASMCs treated with Ang II at various concentration (0, 10, 50, 100, 200 nM) for 24 h. B. Western blot analysis of GDF15 protein expression in HASMCs treated with 100 nM Ang II for 1, 6, 12, and 24 h. C. RT-q PCR analysis of GDF15 mRNA expression in HASMCs treated with different concentrations of Ang II (0, 10, 50, 100, 200 nM) for 24 h. D. RT-qPCR analysis of GDF15 mRNA expression in HASMCs treated with 100 nM Ang II for, 6, 12, and 24 h. Notes: Ang II, Angiotensin II; GDF15, Growth differentiation factor 15; HASMCs, human aortic smooth muscle cells. **P < 0.01; ***P < 0.001; ****P < 0.0001, vs.0 nM or 0 hour.

PBS, and the remaining cells were cultured in serum-free medium under the designated experimental conditions. Images of the wound region were captured using an Olympus phase-contrast microscope at 0, 24 and 48 hours. Wound closure was quantified using Image J software, and cell migration was calculated by comparing the wound area at each time point with the initial gap.

Enzyme-linked immunosorbent assay

Venous blood samples were collected from 6 verified AD cases and 6 age- and gender-matched healthy controls following approval from the Institutional Ethics Committee of Xiangxi Autonomous Prefecture People's Hospital. Blood samples were centrifuged at 3,000 rpm for 10 minutes, and the resulting serum was aliquoted and stored at -80°C until further analysis. Plasma concentrations of GDF15, MMP-2 and MMP-9 were determined using commercial ELISA Kits (MultiSciences, China) per the manufacturer's instructions. Absorbance at 450 nm was measured using a microplate reader, and protein concentrations were determined based on standard curves.

Immunofluorescence staining

The distribution of contractile proteins SM22α and α-SMA in HASMCs was assessed by immunofluorescence. The HASMCs on the slides after incubation were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and then blocked with 5% BSA. Slides were then incubated overnight at 4°C with primary antibodies against SM22α and α-SMA (1:200). After washing, cells were incubated with fluorophore-conjugated secondary antibodies (1:500) at room temperature. Nuclei were counterstained with DAPI. Fluorescent images were obtained using a fluorescence microscope, and fluorescence intensity was quantified using ImageJ software.

Statistical analysis

All data were expressed as mean ± standard deviation (SD) from at least three independent replicates. Statistical analyses were performed using Graphpad prism 10.0 (Graphpad Software, USA). Differences between two groups were assessed using unpaired two-tailed Student's t-tests, while comparisons among multiple groups were determined using One-way ANOVAs followed by Tukey's post hoc test. Statistical significance was defined as follows: *P < .05, **P< 0.01, ***P < 0.001, and ****P < 0.0001.

Results

Ang II induced GDF15 expression in HASMCs in a dose- and time-dependent manner

To determine whether GDF15 is a downstream target of Ang II, HASMCs were cultured with different concentration of Ang II for 24 h. Western blot showed that GDF15 protein expression was up-regulated in a concentration-dependent manner, reaching maximum expression at 100 nM (**Figure 1A**). RT-qPCR results confirmed the dose-dependent increase in GDF15mRNA content under these experimental conditions

(**Figure 1C**). To further characterize the temporal response, HASMCs were treated with 100 nM Ang II for 1, 6, 12, and 24 hours. Both protein and mRNA levels of GDF15 increased gradually over time and peaked at 24 h (**Figure 1B**, **1D**). These findings suggest that Ang II profoundly enhances GDF15 expression in HASMCs in a dose- and time-dependent manner.

GDF15 promoted HASMCs proliferation via the ERK pathway under Ang II stimulation

To study the biological function of GDF15 in AD development, an in vitro Ang II-induced HASMC model was established for gain- and loss-of-function experiments (**Figure 2A**). CCK-8 assays showed that GDF15 overexpression significantly increased cell proliferation compared to the Ang II group, whereas GDF15 knockdown inhibited cell proliferation (**Figure 2B**). However, ERK blockage completely abrogated GDF15 overexpression-enhanced proliferation. Western Blot and RT-qPCR results showed that GDF15 overexpression upregulated proliferation-related factors (e.g., PCNA, CDK2) and downregulated cell cycle inhibitor p21. Conversely, GDF15 silencing decreased PCNA and CDK2 expression but increased p21 expression. The regulatory effects of GDF15 overexpression were completely reversed upon ERK suppression (**Figure 2C**, **2D**). Similarly, EdU incorporation assays showed that GDF15 overexpression increased EdU positive cells, whereas GDF15 knockdown exerted the opposite effects (**Figure 2E**, **2F**). Similarly, GDF15 overexpression-induced increase in EdU-positive cells was significantly suppressed after ERK blockade. These results show that GDF15 promotes HASMC proliferation in response to Ang II stimulation via the ERK signaling pathway.

GDF15 promoted phenotypic switching of HASMCs via the ERK pathway under Ang II stimulation

To determine whether GDF15 regulates the phenotypical transition of VSMCs under pathophysiological conditions, markers for contractile and synthetic phenotype were evaluated after Ang II treatment. As expected, Ang II stimulation decreased the levels of contractile proteins (SM22α and α-SMA) and increased matrix-remodeling enzymes (MMP-2, MMP-9) (**Figure 3A**, **3B**). Notably, such effects were significantly enhanced by GDF15 overexpression, and GDF15 knockdown reversed the Ang II-mediated changes. Crucially, pharmacological blockade of ERK completely eliminated the promoting effects of GDF15 overexpression on phenotypic switching. Immunofluorescence studies confirmed that Ang II reduced the fluorescence intensity of SM22α and α-SMA (**Figure 3C-F**), which was further enhanced by GDF15 overexpression and reversed by GDF15 knockdown. However, ERK inhibition completely restored the effects of GDF15 overexpression on SM22α and α-SMA. These results collectively demonstrate that GDF15 promotes Ang II-induced phenotypic switch of HASMCs from a contractile to a synthetic state primarily through the ERK pathway. This shift may enhance the proliferative and matrix-remodeling capacity of VSMCs under a pathological state.

GDF15 enhanced the inflammatory response in HASMCs through the ERK pathway under Ang II stimulation

A hallmark of the phenotypic switch from contractile to synthetic VSMCs is increased secretion of inflammatory mediators. To explore if GDF15 modulates the inflammatory environment under Ang II-mediated pathological change, the expression of proinflammatory cytokines (TNF-α, IL-6) and chemokine MCP-1 was analyzed. RT-qPCR results indicated that Ang II treatment notably upregulated the mRNA concentrations of TNF-α, IL-6, and MCP-1 in HASMCs (**Figure 4A-C**). Notably, GDF15 overexpression further enhanced this Ang II-Induced transcriptional elevation, whereas GDF15 knockdown significantly attenuated it. In addition, ELISA showed that Ang II stimulation increased the secretion of TNF-α, IL-6 and MCP-1 (**Figure 4D-F**). GDF15 overexpression further promoted the protein secretion of these factors, whereas GDF15 knockdown inhibited the secretion of these factors. Moreover, the promotive effects of GDF15 overexpression on both mRNA and protein levels were completely abolished by ERK inhibition. These results indicate that GDF15 enhances the inflammatory activity of Ang II in HASMCs via the ERK signaling pathway, concomitantly promoting proliferation and phenotypic transformation, thereby potentially exacerbating vascular injury.

GDF15 promoted HASMCs migration via the ERK/Akt signaling pathway under Ang II stimulation

To investigate whether GDF15 influences VSMC migration under pathophysiological states,

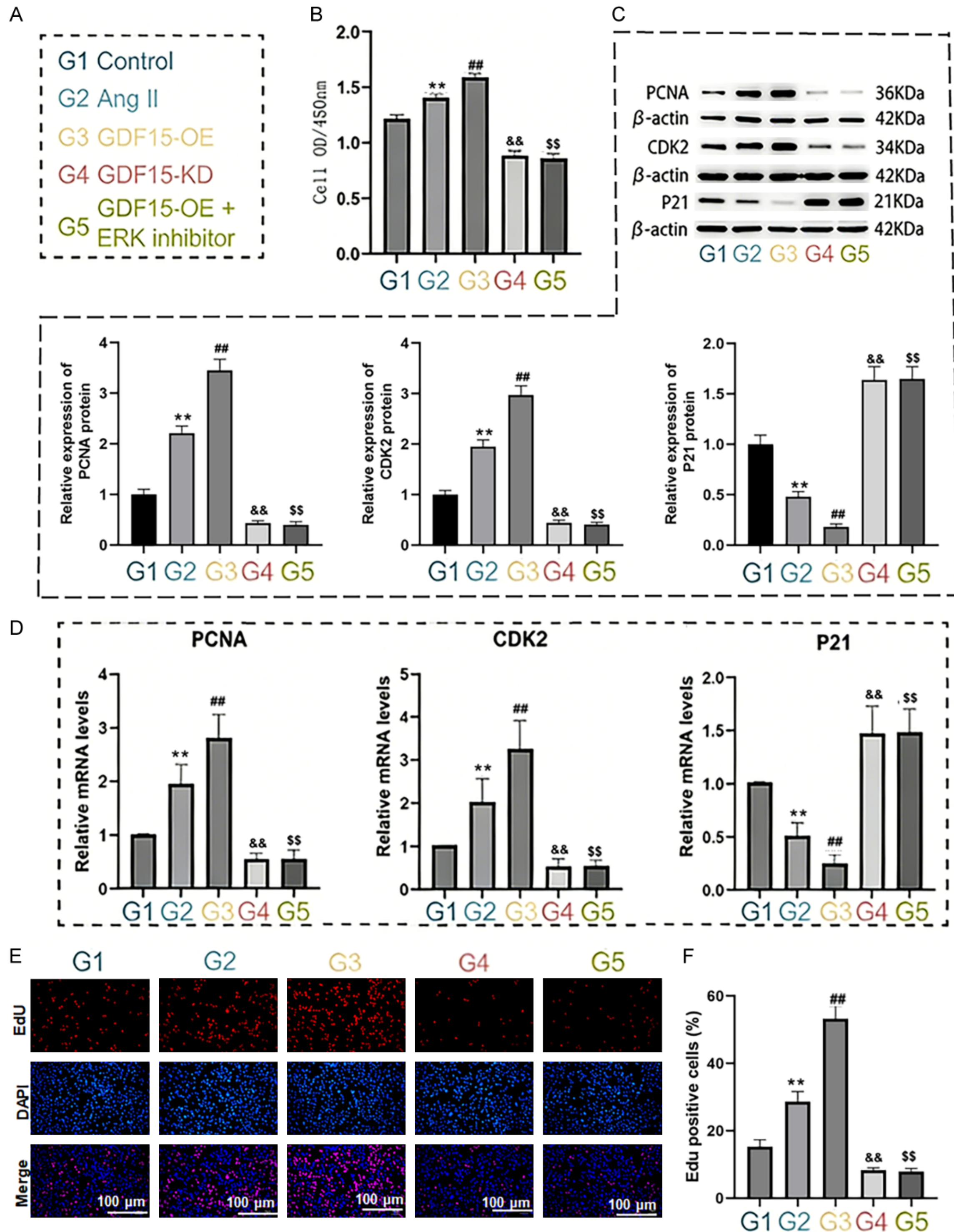

Figure 2. GDF15 regulated HASMC proliferation via the ERK signaling pathway under Ang II stimulation. A. Experimental group design: G1 (control), G2 (Ang II), G3 (Ang II + GDF15 overexpression), G4 (Ang II + GDF15 knockdown), and G5 (Ang II + GDF15 overexpression + ERK inhibitor). B. Cell proliferation was assessed using the CCK-8 assay. C. Western blot analysis of proliferation-related proteins (PCNA, CDK2, and p21) in each group. D. qPCR analysis of PCNA, CDK2, and p21 in each group. E, F. EdU incorporation assays. Notes: Ang II, Angiotensin II; GDF15, Growth differentiation factor 15; HASMCs, human aortic smooth muscle cells; ERK, extracellular signal-regulated kinase. **P < 0.01, G2 vs. G1; ##P < 0.01, G3 vs. G2; &&P < 0.01, G4 vs. G2; $$P < 0.01, G5 vs. G3.

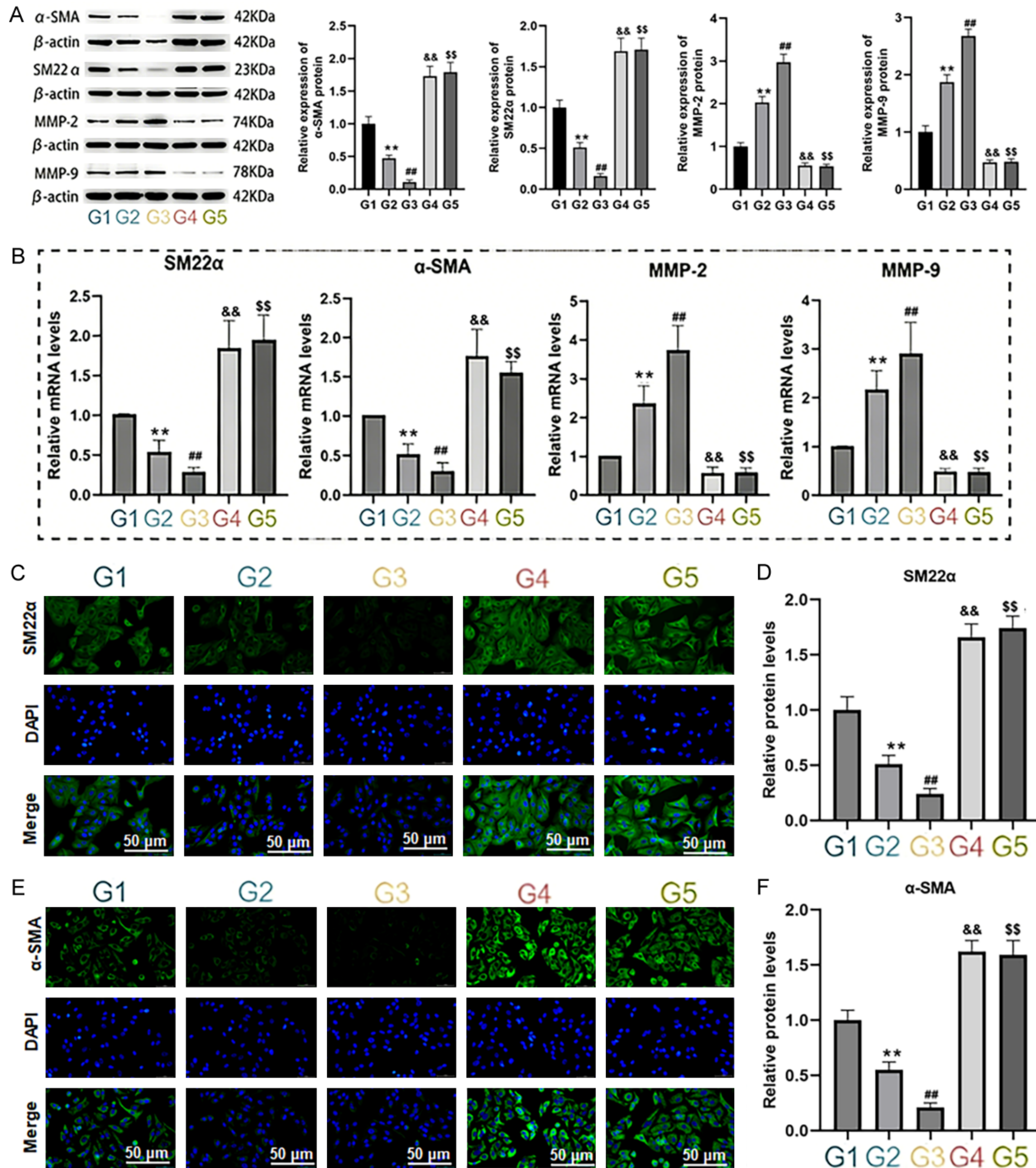

Figure 3. GDF15 promoted phenotypic switching of HASMCs via the ERK signaling pathway under Ang II stimulation. A, B. Western blot and qPCR analyses of contractile markers (α-SMA and SM22α) and matrix remodeling proteins (MMP-2 and MMP-9) in HASMCs. C-F. Immunofluorescence analysis of α-SMA and SM22α expression in HASMCs. Notes: G1 (control), G2 (Ang II), G3 (Ang II + GDF15 overexpression), G4 (Ang II + GDF15 knockdown), and G5 (Ang II + GDF15 overexpression + ERK inhibitor). Ang II, Angiotensin II; GDF15, Growth differentiation factor 15; HASMCs, human aortic smooth muscle cells; ERK, extracellular signal-regulated kinase. **P < 0.01, G2 vs. G1; ##P < 0.01, G3 vs. G2; &&P < 0.01, G4 vs. G2; $$P < 0.01, G5 vs. G3.

wound healing assays were performed. Ang II stimulation promoted wound healing at 24 h and 48 h (**Figure 5A**, **5B**). GDF15 overexpression significantly accelerated wound closure compared with the Ang II group, whereas GDF15 knockdown hindered wound healing, indicating a pro-migratory role of GDF15.

Mechanistically, Western blot analysis demonstrated that GDF15 overexpression elevated

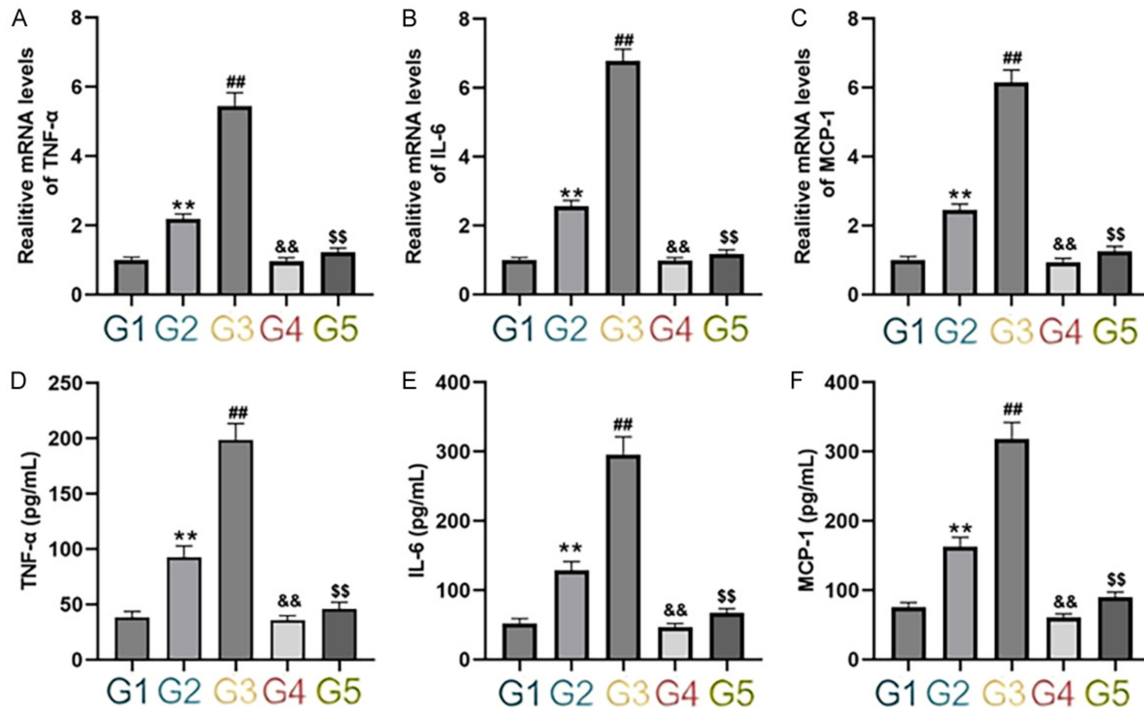

Figure 4. GDF15 enhanced inflammatory responses in HASMCs via the ERK pathway under Ang II stimulation. A-C. RT-qPCR analysis of mRNA expression levels of proinflammatory cytokines (TNF-α, IL-6) and chemokine MCP-1 in HASMCs. D-F. ELISA analysis of TNF-α, IL-6, and MCP-1 levels in the culture supernatant of HASMCs. Notes: G1 (control), G2 (Ang II), G3 (Ang II + GDF15 overexpression), G4 (Ang II + GDF15 knockdown), and G5 (Ang II + GDF15 overexpression + ERK inhibitor); Ang II, Angiotensin II; GDF15, Growth differentiation factor 15; HASMCs, human aortic smooth muscle cells; ERK, extracellular signal-regulated kinase. **$P < 0.01$, G2 vs. G1; ##$P < 0.01$, G3 vs. G2; &&$P < 0.01$, G4 vs. G2; $$P < 0.01$, G5 vs. G3.

phosphorylation of ERK1/2 and Akt, suggesting activation of the ERK/Akt signaling cascade (**Figure 5C**). ERK inhibitor reversed the GDF15 overexpression-induced phosphorylation of both ERK1/2 and Akt. Collectively, these data demonstrate that GDF15 enhances migration and phenotypic transition of HASMCs via activation of the ERK/Akt pathway. Collectively, GDF15 promotes proliferation, migration, and phenotypic switching of HASMCs under Ang II stimulation via the ERK/Akt pathway (**Figure 5D**).

Serum GDF15 and MMPs levels are upregulated in patients with AD

To explore the clinical relevance of GDF15, plasma samples were collected from AD patients and age-matched healthy controls. ELISA results demonstrated that serum levels of GDF15 were significantly higher in the AD group compared with the control group (**Figure 6A**). Likewise, plasma levels of MMP-2 and MMP-9 were substantially elevated in AD patients (**Figure 6B**, **6C**). These clinical observations are consistent with *in vitro* findings and suggest that GDF15 may contribute to pathological extracellular matrix remodeling in AD.

Discussion

In this study, GDF15 expression in HASMCs was significantly upregulated by Ang II in a dose- and time-dependent manner [25]. Functional assays showed that GDF15 promoted cell proliferation, migration, and phenotypic switching from a contractile to a synthetic status, reflected by reduced α-SMA and SM22α expression, and increased MMP-2/9 levels [26]. Mechanistically, GDF15 activated the ERK and Akt signal pathways, and pharmacological inhibition of ERK effectively abrogates its proliferative and migratory effects [24]. Clinical assessment also indicated that plasma levels of GDF15, MMP-2 and MMP-9 were significantly elevated in patients with AD, suggesting their potential diagnostic utility.

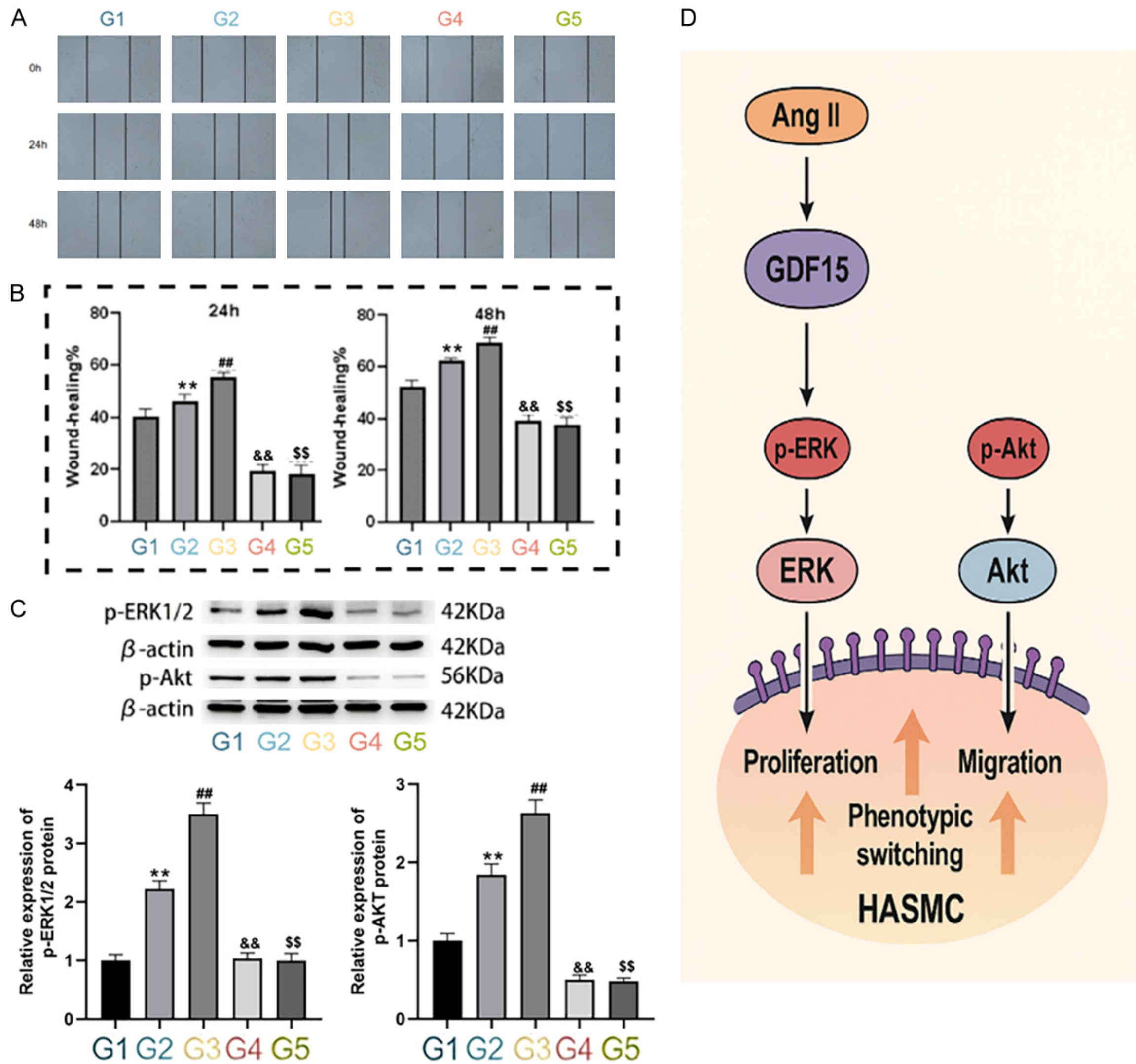

Figure 5. GDF15 promoted HASMC migration via activation of the ERK/Akt signaling pathway under Ang II stimulation. A. Representative images of wound healing assays at 0, 24, and 48 hours. B. Quantitative analysis of wound closure. C. Western blot analysis of phosphorylated ERK1/2 (p-ERK1/2) and Akt (p-Akt). D. Schematic illustration of the proposed mechanism by which GDF15 promotes VSMC phenotypic switching via activation of ERK and Akt signaling pathways. Notes: G1 (control), G2 (Ang II), G3 (Ang II + GDF15 overexpression), G4 (Ang II + GDF15 knockdown), and G5 (Ang II + GDF15 overexpression + ERK inhibitor). Ang II, Angiotensin II; GDF15, Growth differentiation factor 15; HASMCs, human aortic smooth muscle cells; ERK, extracellular signal-regulated kinase; AKT, protein kinase B. $^{**}P < 0.01$, G2 vs. G1; $^{\#\#}P < 0.01$, G3 vs. G2; $^{\&\&}P < 0.01$, G4 vs. G2; $^{\$\$}P < 0.01$, G5 vs. G3.

Previous studies have highlighted the critical role of VSMC dysfunction in the course of AD development [27]. Excessive proliferation and phenotypic switching of VSMCs lead to maladaptive vascular re-arrangement, ECM degradation, and overall weakening of the aortic structure [28, 29]. Various stimuli, including Ang II, TGF-β1, and proinflammatory cytokines, can induce VSMC phenotypic change and ECM remodeling through the ERK signal path [30, 31]. Our findings support these observations and extend them by identifying GDF15 as a novel modulator that amplifies ERK/Akt-mediated pathological remodeling. While ERK and Akt are often considered independent signaling cascades, increasing evidence suggests substantial crosstalk between these pathways [32, 33]. Traditionally, PI3K-generated phosphatidylinositol (3,4,5)-trisphosphate (PIP3) recruits and activates Akt [34]. Remarkably, ERK signaling has been reported to regulate the PI3K-Akt pathway at multiple levels, including

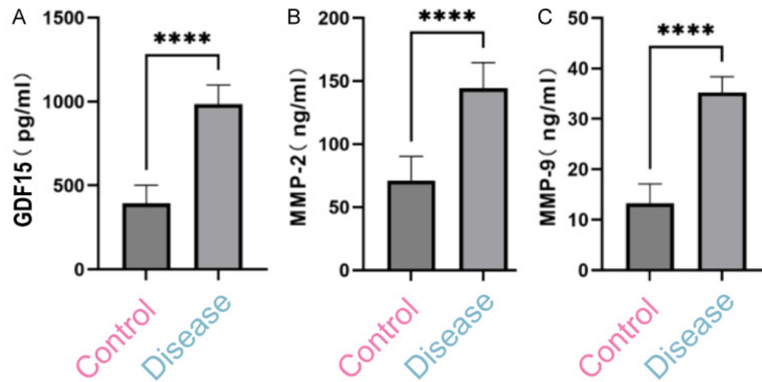

Figure 6. Serum levels of GDF15 and MMPs were elevated in AD patients. A. ELISA analysis of serum GDF15 levels in AD patients and healthy controls. B. ELISA analysis of serum MMP-2 levels in AD patients and healthy controls. C. ELISA analysis of serum MMP-9 levels in AD patients and healthy controls. Notes: GDF15, Growth differentiation factor 15; MMP, matrix metalloproteinases; AD, Aortic dissection. ****P < 0.0001, vs. the Control group.

direct phosphorylation of upstream regulators and a feedback loop [35]. In our research, we observed that ERK inhibition using PD98059 not only blocked ERK phosphorylation but also significantly reduced Akt phosphorylation, suggesting that maximal Akt activation may require the participation of ERK. This finding is consistent with previous reports that ERK could influence Akt signaling through mTOR complex regulation or direct interactions with PI3K subunits [36]. Further studies using specific Akt inhibitors or genetic approaches are warranted to fully elucidate the complex relationship among these two pathways in GDF15-induced malfunction of VSMCs. Notably, GDF15 has been previously characterized as a stress-induced cytokine in cardiovascular diseases, including heart failure and atherosclerosis, and is associated with maladaptive vascular remodeling and inflammation [20, 37]. Our work shows that similar processes occur in AD, linking GDF15 to aberrant VSMC behavior. By promoting phenotypic change and enhancing MMP secretion through the ERK/Akt pathway, GDF15 contributes to the pathological remodeling of the aortic wall, which provide novel insights into the molecular mechanisms underlying AD and highlight GDF15 as a potential therapeutic target.

MMP-2 and MMP-9 are also elevated in both tissues and pathological specimens, supporting the involvement of GDF15 in ECM degradation [38]. MMPs are recognized as key mediators of aortic wall degradation and dissection formation [39]. Previous studies have demonstrated that Ang II-induced MMP production via the ERK signaling pathway contributes to elastin disintegration and increased vulnerability of vessels [40]. Our observations are consistent with these findings, suggesting that GDF15 may act as a critical upstream regulator of MMP activation during disease states, therefore facilitating the matrix remodeling process essential for aortic dissection progressions.

The clinical significance of our findings is highlighted by the elevated levels of GDF15 in AD subjects. Although prior clinical studies have focused mainly on inflammatory cytokines and genetic factors, our results suggest that GDF15 could serve as a circulating biomarker reflecting continuous vascular reconstruction and ECM degradation [41, 42]. This is consistent with other studies linking increased circulating GDF15 with vascular pathology and adverse outcomes, underscoring its potential utility as a diagnostic or prognostic marker in AD.

Despite these insights, some limitations should be recognized. First, our study was limited to *in vitro* experiments and cross-sectional clinical observation. Further validation using appropriate in vivo models is required to establish a causal relationship between GDF15 and AD. Second, although the ERK/Akt pathways were identified as key mediators of GDF15-induced effects, other downstream effectors may be involved, which warrants further exploration. Third, while we demonstrated that GDF15 activates both ERK and Akt and that ERK inhibition blocks GDF15-induced effects, no specific AKT inhibitors were used to dissect the role of AKT. Subsequent research using Akt-targeted pharmacological agents or siRNA-mediated silencing is needed to clarify the specific effects of Akt signaling on GDF15-induced proliferation, migration, and phenotypic changes. Finally, larger and more diverse clinical cohorts should be included to further assess the diagnostic and prognostic value of plasma GDF15 levels, as well as to investigate its correlation with disease stage, severity, and treatment response.

Conclusions

GDF15 acts as a key regulator of VSMC phenotype by promoting proliferation, migration, and phenotypic switching through ERK/Akt signaling. Through these mechanisms, GDF15 promotes ECM remodeling and mechanical instability of the aortic wall, thereby facilitating the development of aortic dissection. These findings not only provide insights into the molecular mechanisms underlying AD but also suggest that GDF15 may serve as a circulating biomarker and a potential therapeutic target for AD.

Disclosure of conflict of interest

None.

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