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Hypoxia reprograms VEGF signaling to differentially control ADAMTS2 and ADAMTS3 expression in endothelial cells

Candan Altuntaş [a], Meltem Alper [b], Yasemin Kalfa [c], Feyza Nur Sav [c], Feray Kockar [c,*]

[a] Kocaeli University, Türkiye
[b] Dokuz Eylul University, Türkiye
[c] Balikesir University, Türkiye

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ABSTRACT

ADAMTS2/-3, key metalloproteinases involved in collagen processing and extracellular matrix dynamics, remain insufficiently characterized in terms of their transcriptional regulation under hypoxic and pro-angiogenic conditions. In this study, we demonstrate that $VEGF_{165}$ robustly enhances ADAMTS2/-3 expression in endothelial cells, with hypoxia providing a striking amplification of this response. Bioinformatic analyses revealed that hypoxia and VEGF induced HIF-mediated and time-varying expression responses in ADAMTS2/-3. Using HUVECs exposed to $CoCl_2$-induced hypoxia, VEGF stimulation led to substantial increases in ADAMTS2 (approximately 19-fold at 3 h) and ADAMTS3 (approximately 46-fold at 3 h) mRNA levels, accompanied by concordant protein upregulation. Promoter–reporter assays revealed strong VEGF responsiveness in defined ADAMTS2 (–658/+112) and ADAMTS3 (–131/+40; –1340/+40) promoter fragments, particularly under hypoxic conditions. Pharmacological inhibition showed that JNK, MAPK/ERK, p38, and PI3K pathways each contributed partially to VEGF-mediated transcription, indicating multi-pathway convergence rather than single-pathway dependency. This finding is consistent with RNA-seq analyses showing that VEGF-related signaling is extensively re-regulated under hypoxic conditions. Extension of these analyses to MG-63 and SAOS-2 cell lines revealed modest but consistent VEGF-induced upregulation, supporting a tissue-independent regulatory axis. Collectively, these findings position ADAMTS2/-3 as potent hypoxia- and VEGF-responsive genes, uncovering their integration into HIF-1α–dependent transcriptional networks and VEGF-activated signaling cascades. This work highlights the relevance of ADAMTS2/-3 in angiogenesis-associated extracellular matrix remodeling and identifies them as promising biomarkers and potential therapeutic targets in hypoxia-driven vascular pathology.

1. Introduction

Endothelial cells (ECs), the fundamental structural components of the vascular system, are critically important for maintaining vascular integrity and regulating vascular function. In adulthood, ECs are largely quiescent; however, under injury or pathological stimuli, they can rapidly activate and transition to a proliferative and angiogenic phenotype. Angiogenesis, the process of new vessel formation, is primarily triggered by hypoxia (Carmeliet, 2003; Zhou et al., 2025). HIF−1α, which stabilizes under hypoxic conditions, is the key transcriptional regulator that directs angiogenic and wound healing programs, particularly via VEGF expression (Bakleh and Al Haj Zen, 2025; Bogadi et al., 2025). The VEGF–VEGFR interaction plays a central role in vascular morphogenesis, the organization of vascular architecture, and the maturation stages of angiogenesis (Lee et al., 2025a; Shah et al., 2025).

This multi-step process begins with the activation of VEGFR−2 on the endothelial cell surface; it then initiates and sustains angiogenesis by triggering the PI3K/AKT and MAPK/ERK pathways, which coordinate endothelial proliferation, migration, and survival (Shah et al., 2025). VEGF also increases vascular permeability and shapes the angiogenic microenvironment by modulating the expression of enzymes involved in

Abbreviations: **ADAMTS2,**, A Disintegrin And Metalloproteinase with Thrombospondin Motifs,2; **VEGF**, Vascular Endothelial Growth Factor; **HUVEC,**, Human umbilical vein endothelial cells; **HIF,**, Hypoxia-Inducible Factor.

* Corresponding author.
E-mail addresses: altuntascandan@gmail.com (C. Altuntaş), meltem.alper@deu.edu.tr (M. Alper), yaseminkls06@gmail.com (Y. Kalfa), savnurfeyza@gmail.com (F.N. Sav), fkockar@balikesir.edu.tr (F. Kockar).

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extracellular matrix (ECM) remodelling (Zabihi, 2025). The ADAMTS (A Disintegrin And Metalloproteinase with Thrombospondin Motifs family is defined as a group of potential modulator proteins that can function in the processes of extracellular matrix remodeling and angiogenesis regulation (Lambert et al., 2020; Lee et al., 2012). Specifically, ADAMTS2/−3 have been shown to play roles in collagen maturation (Joannes et al., 2025) and VEGF-C activation (Janssen et al., 2016), respectively. Additionally, it has been demonstrated that ADAMTS1 sequesters $VEGF_{165}$ by physically binding to it, particularly in breast cancer, thereby attenuating VEGFR2 signalling (Freitas et al., 2013). ADAMTS9 has been reported to limit tumour angiogenesis and tumourigenicity in oesophageal and nasopharyngeal cancers by suppressing VEGFA expression at the transcriptional level (Lo et al., 2010). Similarly, an inverse relationship between ADAMTS8 expression and VEGFA levels has been reported in lung cancer, with increased ADAMTS8 suppressing tumour-derived vessel formation by reducing VEGFA mRNA and protein levels (Zhang et al., 2022).

Despite a thorough review of the existing literature, the precise molecular mechanisms linking VEGF signaling to the regulation of ADAMTS2/−3 in endothelial cells remain largely undefined. Of particular interest is understanding how these genes respond to hypoxic and normoxic conditions, which significantly shape the angiogenic program. It is imperative to address this knowledge gap, as elucidating the VEGF-mediated regulation of ADAMTS family members could offer novel insights into vascular homeostasis and identify potential therapeutic targets in hypoxia-induced pathologies. Therefore, this study aimed to systematically characterize the hypoxia-dependent and VEGF-mediated transcriptional regulation of ADAMTS2/−3 in endothelial cells by integrating qRT-PCR, Western blotting, promoter–reporter assays, pathway-specific inhibitor analyses, and HIF−1α–associated regulatory studies. Our findings reveal that both genes are robustly induced by hypoxia, with ADAMTS3 showing an exceptionally strong early response (up to ~46-fold), and that VEGF further amplifies transcriptional activation through specific promoter regions. We also demonstrate that this regulation is partially dependent on HIF−1α and MAPK/JNK/p38 signaling pathways and is conserved—although attenuated—in osteosarcoma (MG−63, SAOS−2) cell lines, indicating a tissue-independent regulatory axis. Collectively, these data identify ADAMTS2/−3 as previously unrecognized hypoxia- and VEGF-responsive genes and establish a mechanistic framework linking endothelial stress signaling to ADAMTS transcriptional control.

2. Material and methods

2.1. RNA-seq data processing and pathway analysis

To evaluate the transcriptional responses of HUVEC cells to hypoxia and VEGF, three independent RNA-seq datasets (GSE186474, GSE190240, and GSE71967) were analysed in the R environment; the tidyverse, dplyr, and tidyr packages were used for data processing, and the ggplot2 and ggh4x packages were used for visualisation.

In the GSE186474 dataset, normalised expression values (log_2[FPKM]) were calculated as the average per gene across three biological replicates, and the hypoxia response was defined as log_2FC = (Hypoxia – Normoxia).

In the GSE190240 dataset, groups stabilised with DMOG (proline hydroxylase inhibitor) were compared with groups silenced with HIF1A and HIF1/2 A siRNA; Log_2(FPKM+1) values for all ADAMTS genes were visualised with panel graphs.

In the GSE71967 dataset, time-dependent effects of VEGF were examined under normoxia (30 min–18 h) and hypoxia (1–24 h) conditions; log_2(VEGF/Control) ratios were calculated to evaluate changes in VEGFA, HIF1A, ADAMTS2, and ADAMTS3.

Changes in the VEGF signalling pathway under hypoxia were mapped using PathView by matching the GSE186474 differential expression results to the KEGG hsa04370 (VEGF signalling pathway) map, converting gene symbols to Entrez ID using org. Hs.eg.db, and displaying log_2FC values with a colour scale.

Group comparisons were performed using an independent two-sample *t*-test ($p < 0.05$).

2.2. Cell culture assay

Before the experiments, HUVEC (Human Umbilical Vein Endothelial Cells) were cultured in high-glucose DMEM supplemented with 10% heat-inactivated fetal calf serum (FCS) and 1% antibiotic–antimycotic solution and maintained in a humidified incubator at 37 °C with 5% CO_2. Chemical hypoxia was induced by applying 150 μM $CoCl_2$ to the cells. In all experiments, 2×10^6 cells were seeded into 25 cm^2 culture flasks and allowed to adhere overnight; serum starvation was then induced using a medium containing 0.1% BSA to reduce basal signalling. Recombinant human $VEGF_{165}$ (20 ng/mL) was applied, and samples were collected at 1, 3, 6, 24, 48, and 72 h.

MG−63 and Saos−2 osteosarcoma cells were processed in parallel under the same culture and treatment conditions.

To assess the contribution of individual signalling pathways, HUVECs were pre-treated with pathway-specific inhibitors one hour before VEGF stimulation: Wortmannin (1 μM; PI3K), PD98059 (10 μM; MEK1), PD169316 (12.5 μM; p38 MAPK), and SP600125 (20 μM; JNK). After a 6-hour incubation, cells were harvested and pelletised for RNA or protein isolation. All experiments were performed with at least three independent biological replicates (Alper et al., 2025a, 2025b).

2.3. Transient-transfection and reporter assays

The VEGF-dependent transcriptional activation of ADAMTS2/−3 promoters was assessed using a transient transfection-based luminescence reporter assay. As described in our group's previous work, the ADAMTS2/−3 proximal promoters were cloned into the pMetLuc vector (Alper and Kockar, 2014; Aydemir et al., 2018). HUVECs were seeded into 12-well plates and transfected using the calcium phosphate method; each well received 0.5 μg of promoter-reporter construct and 0.5 μg of SEAP−2 internal control vector.

Forty-eight hours after transfection, culture supernatants were collected, and luciferase and SEAP activities were measured as part of a dual reporter assay (Ready-To-Glow™, Takara). All experiments were performed in triplicate.

2.4. RNA extraction and qRT-PCR analysis

Total RNA was isolated from HUVEC cells using the GeneJET™ RNA Purification Kit (Thermo Scientific), and 1 μg of RNA was converted to cDNA using RevertAid Reverse Transcriptase (200 U, Thermo Scientific) (Hacıoğlu et al., 2021). qRT-PCR was performed with gene-specific primers (Supp. Table 1) on a LightCycler® 480 (Roche) instrument in technical triplicates. Relative gene expression was calculated using the $2^{-}\Delta\Delta Ct$ method, normalised against the h-ß-2M reference gene (Livak and Schmittgen, 2001).

2.5. Western blotting assay

Total protein was isolated from HUVEC cells using RIPA lysis buffer supplemented with a protease inhibitor cocktail. Approximately 2×10^6 cells were lysed in 200 μL RIPA buffer, incubated on ice for 45 min, and then clarified by centrifugation at 14,000 rpm for 15 min at 4 °C; supernatants were stored at −80 °C. Protein concentration was determined by a colorimetric method (Alper et al., 2021).

Equal amounts of protein were separated by 10% SDS-PAGE and transferred to 0.45 μm PVDF membranes using the Bio-Rad Trans-Blot system (4 °C, overnight, 15 V). After blocking, membranes were incubated with ADAMTS2 (ab125226, Abcam; 1 μg/mL), ADAMTS3 (Y058016, Abm; 1:500), and HIF−1α (Thermo Scientific; 1:500)

antibodies; followed by application of HRP-conjugated goat anti-rabbit IgG (ab97069, Abcam; 1:5000). β-Actin (ab8227, Abcam) was used as a loading control. Signals were detected using ECL and recorded with a UVP imaging system (Tokay et al., 2021).

2.6. Statistics analysis

The statistical analysis of the data was performed using GraphPad Prism 8.0.0 (GraphPad Software, USA). The One-Way ANOVA test was preferred for evaluating differences between groups; the validity of the results was supported by Minitab 14 software. A $p \leq 0.05$ value was set as the statistical significance threshold.

3. Results

3.1. Bioinformatic analysis of VEGF-induced oxygen-sensitive gene expression patterns

To evaluate the effect of hypoxia on the ADAMTS gene family, GSE186474 RNA-seq data (log_2[FPKM]) derived from HUVECs were analysed. The normoxia–hypoxia comparison revealed a slight hypoxia-dependent increase in ADAMTS2/−3 transcript levels; however, these changes were not statistically significant ($p > 0.05$) (Fig. 1a). In contrast, our previous experimental study demonstrated that hypoxia significantly increased both mRNA and protein levels of ADAMTS2/−3 in HUVEC cells (Altuntaş et al., 2023).

To evaluate the HIF-dependent regulation of ADAMTS genes under hypoxic conditions, normalised RNA-seq data (log_2[FPKM+1]) from HUVEC cells in the GSE190240 dataset were analysed. In this dataset, DMOG (proline−4-hydroxylase inhibitor) was used as a positive control for HIF stabilisation, while the HIF1A and HIF1/2 A siRNA groups represented HIF-suppressed conditions. Compared to the DMOG control, HIF1A silencing caused a marked decrease in ADAMTS2/−3 expression; dual silencing of HIF1/2 A produced the strongest decrease and demonstrated that both genes are highly dependent on HIF activity (Fig. 1b).

In the GSE71967 time series analysis, VEGF application was observed to induce different mRNA expression responses in HUVEC cells, depending on normal and low oxygen levels, in the expression of VEGFA, HIF1a, ADAMTS2, and ADAMTS3 mRNA (Fig. 1c). Under normoxic conditions, the VEGFA response to VEGF application showed changes at different levels throughout the time series. A slight increase in expression was observed at 30 min and 8 h. However, under hypoxic conditions, VEGFA expression exhibited positive log_2 fold change values at the 2nd, 4th, 8th, and 24th hours.

The HIF1A response to VEGF administration in both normoxic and hypoxic environments produced a fluctuating expression profile over time. HIF1A exhibited a small increase in expression was observed at early time points.

The ADAMTS2 response to VEGF administration under normoxic conditions showed a marked time-dependent change. At 2 h, expression reached a positive log_2 fold change, indicating the greatest increase; at 4 h, it decreased slightly again. At later time points, expression resumed an upward trend; a second positive change was observed at the 8th hour, and a low-level but positive expression persisted at the 18th hour. Under hypoxic conditions, ADAMTS2 expression exhibited negative log_2 fold changes at early hours; however, it shifted to the positive region at 8 h and reached its highest increase level at 24 h.

The ADAMTS3 response to VEGF administration under normoxic conditions showed a marked increase in the early period. While no significant change was recorded at 30 min, expression reached its highest positive log_2 fold change level at 1 h and 8 h. Under hypoxic conditions, ADAMTS3 expression showed positive log_2 fold changes at 1, 2, and 4 h; at 8 h, expression shifted markedly into the negative region; and at 24 h, this decrease continued at a lower intensity.

3.2. Regulation of ADAMTS2/−3 gene expression associated with hypoxia

The relationship between the ADAMTS2/−3 genes and VEGF165 in endothelial cells has not yet been fully elucidated. Therefore, total RNA and protein were isolated from HUVEC cells treated with VEGF165 under normoxic and hypoxic conditions; hypoxia was chemically mimicked using the hydroxylase inhibitor $CoCl_2$. cDNA was synthesized from the isolated RNA via reverse transcription, and gene expression was analysed by qRT-PCR using ADAMTS2/−3-specific primers; the hβ−2 reference gene was used for normalization. Our findings indicate that 20 ng/mL VEGF165 treatment significantly increased ADAMTS2/−3 expression under both normoxic and hypoxic conditions.

It was determined that the increase in gene expression was more pronounced and higher under hypoxic conditions than under normoxic conditions. It has been determined that ADAMTS2 gene expression increases over time under normoxic conditions. Under normoxic conditions, cells showed a marked increase in ADAMTS2 gene expression; this increase was 1.6-fold at 3 h and 1.7-fold at 24 h compared with the control (Fig. 2a).

In contrast, under hypoxic conditions, a significant increase of approximately 46-fold at 3 h and 16-fold at 6 h was observed (Fig. 2c). To evaluate the response of HUVEC cells to VEGF165, the cells were cultured in a medium stimulated with 20 ng/mL VEGF, and changes in protein levels were examined by western blot analysis. Time-dependent analyses revealed that under normoxic conditions, ADAMTS2 protein levels peaked at the 3rd hour, increasing by approximately 1.7-fold (Fig. 2b). It was determined that ADAMTS2 protein expression was induced in a time-dependent manner under hypoxic conditions, reaching its maximum level at the 3rd hour and increasing approximately 1.9-fold (Fig. 2d). Under normoxic conditions, ADAMTS3 expression increased 1.4-fold at 3 h and 1.7-fold at 6 h compared to the control group (Fig. 2e), while it increased approximately 19-fold at 3 h and 8-fold at 6 h in hypoxic conditions (Fig. 2g). ADAMTS3 protein levels were examined under normoxic conditions to 1.5-fold at the 3-hour maximum level (Fig. 2f) and under hypoxic conditions to 2.3-fold at the 3-hour maximum level (Fig. 2h). In addition to these analyses, the relationship between the ADAMTS2/−3 genes and 20 ng/mL VEGF165 was also examined in SAOS−2 and MG−63 cell lines. To determine whether the VEGF–ADAMTS regulatory axis exhibits a similar or distinct transcriptional response across tissue origins, these osteosarcoma cell lines were included as additional models in the study. VEGF165 stimulation at 20 ng/mL in MG−63 cells does not induce an upregulation of both genes, with maximum expression levels reaching approximately 1.1-fold for ADAMTS2 (Suppl. Fig. 1a) and 1.2-fold for ADAMTS3 (Suppl. Fig. 1b). Following VEGF165 stimulation in SAOS−2 cells, ADAMTS2 expression increased approximately 1.2-fold at 24 h (Suppl. Fig. 1c), whereas ADAMTS3 reached a maximum 1.5-fold induction at the same time point (Suppl. Fig.1d).

3.3. The role of VEGF stimulation on ADAMTS2/−3 promoter activity under normoxic and hypoxic conditions

To delineate the VEGF-responsive regions within the ADAMTS2 promoter and to assess the impact of oxygen availability on this regulation, four promoter fragments (−180/+112, −324/+112, −530/+112, and −658/+112) were analyzed using luciferase reporter assays under normoxic and hypoxic conditions, in the presence or absence of VEGF stimulation (Fig. 3a–b). Under normoxic conditions, $VEGF_{165}$ selectively and robustly increased the transcriptional activity of the −658/ + 112 promoter fragment, which exhibited the strongest VEGF-induced luciferase activity among all constructs (Fig. 3a).

In contrast, shorter promoter fragments failed to respond significantly to VEGF stimulation. These findings indicate that critical VEGF-responsive elements are localized within the −658 to −530 bp region of the ADAMTS2 promoter, a region that is dispensable for basal

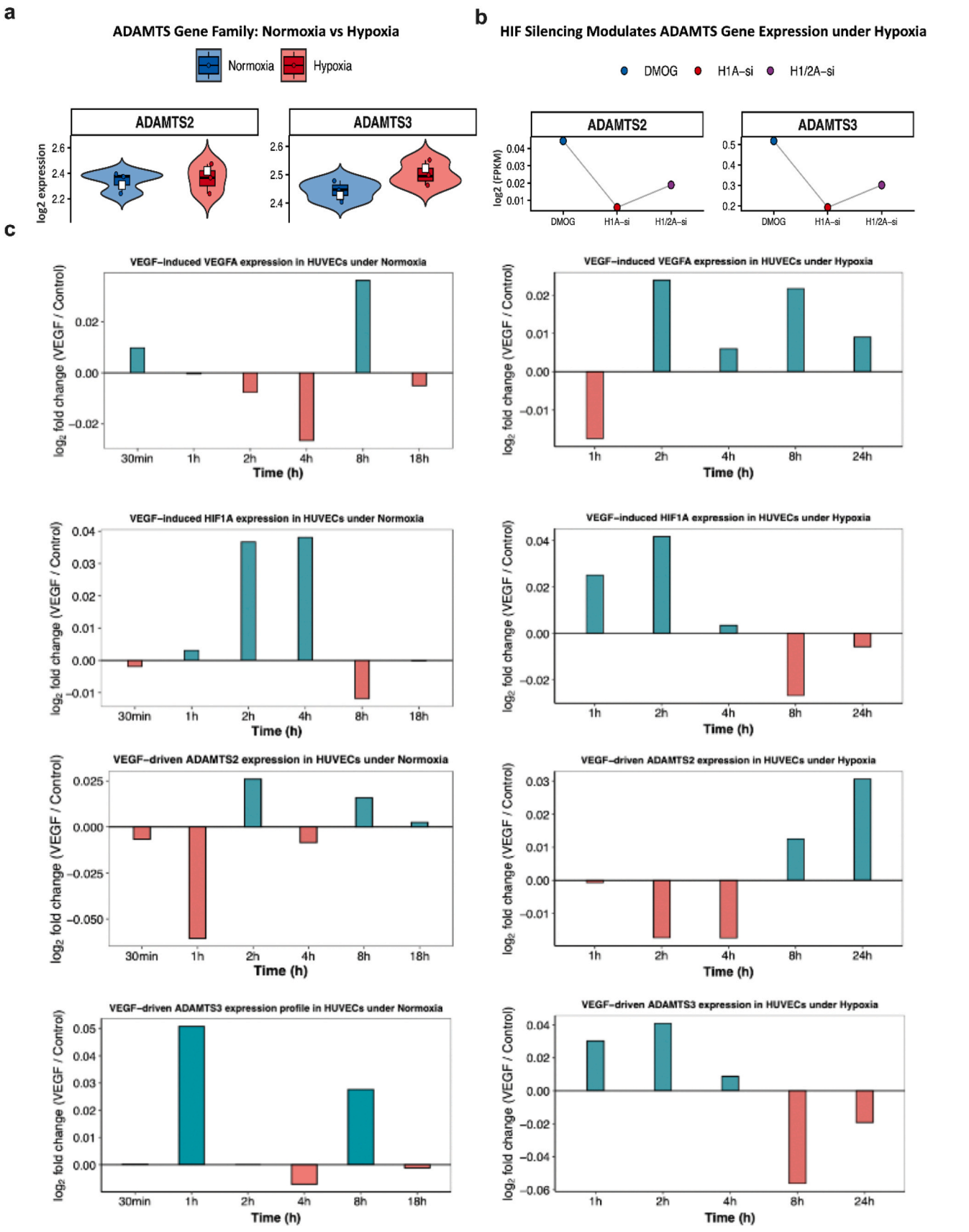

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Fig. 1. The hypoxic response of ADAMTS2/−3 in HUVEC cells and the dependence of this response on HIF activity. a) Comparative expression analysis of the ADAMTS gene family in HUVEC cells under normoxic and hypoxic conditions. The graphs show the normalized log_2(FPKM) values obtained from three biological replicates. A slight increase in ADAMTS2/−3 gene expression was observed under hypoxic conditions. Statistical analysis was performed using an independent two-sample *t*-test, with $p < 0.05$ considered statistically significant. b) Changes in the expression of the ADAMTS gene family associated with HIF silencing under hypoxic conditions. Each panel shows the normalized log_2(FPKM+1) expression values for the indicated ADAMTS gene derived from the GSE190240 RNA-seq dataset. Compared to the control group, where HIF was stabilized with DMOG, siRNA silencing of HIF1A and HIF1/2 A led to a marked decrease in expression, particularly in ADAMTS2/−3. This suggests that both genes are part of the HIF-mediated transcriptional activation pathway. c) Time-dependent changes in the expression of VEGFA, HIF1A, ADAMTS2, and ADAMTS3 genes in HUVEC cells following VEGF application under normoxic and hypoxic conditions (GSE71967). Each panel shows the log_2(VEGF/Control) ratios of the relevant gene in response to VEGF stimulation under different oxygen conditions. Green bars represent upregulation, while red bars represent downregulation. A marked increase in VEGFA and HIF1A expression was observed in the early stages under hypoxia, while a significant induction of ADAMTS2/−3 genes was observed under normoxic conditions, particularly at 2 and 8 h. These data indicate that VEGF signaling induces different transcriptional responses depending on oxygen levels. The stars in the figures indicate the following levels of significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

transcription but essential for VEGF-mediated activation under normal oxygen tension. Under hypoxic conditions, VEGF stimulation significantly increased the activity of the −180/ + 112 promoter fragment ($p < 0.05$), whereas the more distal promoter fragments failed to exhibit a comparable VEGF-induced response. Notably, the −658/ + 112 fragment retained moderate basal and VEGF-induced activity under hypoxia, suggesting the presence of hypoxia-responsive regulatory elements within the distal promoter region that may partially compensate for reduced proximal promoter activity (Fig. 3b).

To identify VEGF-responsive regions within the ADAMTS3 promoter and to assess oxygen-dependent regulation, four promoter constructs spanning progressive 5′-flanking regions (−131/+40, −576/+40, −879/+40, and −1340/+40) were transiently transfected into HUVECs and analyzed under normoxic and hypoxic conditions in the presence or absence of VEGF stimulation (Figs. 3c–3d). Under normoxic conditions, basal promoter activity varied across constructs, indicating that intrinsic transcriptional capacity is length-dependent. VEGF stimulation resulted in a significant induction of promoter activity predominantly in the minimal −131/ + 40 construct ($p < 0.05$), whereas longer promoter fragments (−576/+40, −879/+40, and −1340/+40) showed either modest or no additional VEGF responsiveness. In contrast, under hypoxic conditions, overall basal promoter activity was reduced; however, VEGF-induced activation was markedly enhanced and extended to multiple promoter fragments. Notably, both the minimal −131/ + 40 and the distal −1340/ + 40 constructs exhibited significant VEGF-dependent upregulation, with the −131/ + 40 fragment showing the most robust response ($*p < 0.01$). These findings suggest that hypoxia unmasks or potentiates VEGF-responsive cis-regulatory elements within both proximal and distal regions of the ADAMTS3 promoter.

3.4. Oxygen-dependent regulation of ADAMTS2 expression by VEGF signaling

To investigate the regulation of ADAMTS2 expression by VEGF under normoxic conditions, ADAMTS2 mRNA and protein levels were analyzed following VEGF stimulation in the presence or absence of pathway-specific inhibitors. Under normoxia, basal ADAMTS2 expression was detectable at both the transcript and protein levels (Figs. 4a-4b). VEGF treatment resulted in a significant induction of ADAMTS2 mRNA expression, which was accompanied by a marked increase in ADAMTS2 protein abundance compared with untreated controls.

Pharmacological inhibition of PI3K signaling with wortmannin markedly attenuated VEGF-induced ADAMTS2 expression at both the mRNA and protein levels, reducing expression toward basal values. Similarly, inhibition of SP600125 (SP6) substantially suppressed VEGF-mediated ADAMTS2 protein accumulation and was associated with reduced mRNA expression. In contrast, inhibition of MEK/ERK signaling with PD98059 (PD9) resulted in a differential response, characterized by enhanced ADAMTS2 mRNA levels but only partial suppression of ADAMTS2 protein expression. PD169316 (PD1) treatment led to a moderate reduction in both ADAMTS2 transcript and protein levels. β-actin expression remained unchanged across all experimental conditions, confirming equal protein loading.

To evaluate the impact of VEGF signaling on ADAMTS2 transcription under hypoxic conditions, ADAMTS2 mRNA levels were quantified following VEGF stimulation in the presence or absence of pathway-specific inhibitors (Fig. 4c). Under hypoxia alone, ADAMTS2 mRNA expression remained at low basal levels. VEGF treatment under hypoxic conditions resulted in a significant induction of ADAMTS2 mRNA compared with untreated hypoxic controls ($p < 0.05$). Pharmacological inhibition of MEK/ERK signaling using PD9 markedly enhanced VEGF-induced ADAMTS2 mRNA expression, leading to a robust increase compared with VEGF treatment alone ($***p < 0.0001$). In contrast, inhibition of PI3K signaling with wortmannin substantially reduced VEGF-mediated ADAMTS2 induction, restoring expression levels toward baseline. Treatment with SP6 or PD1 moderately attenuated VEGF-induced ADAMTS2 mRNA expression without fully abolishing the response. Collectively, these findings demonstrate that VEGF-dependent ADAMTS2 transcription under hypoxic conditions is positively regulated by PI3K signaling, while MEK/ERK signaling exerts a restraining effect on ADAMTS2 mRNA induction.

To investigate the regulation of ADAMTS2 expression by VEGF under hypoxic conditions, ADAMTS2 mRNA and protein levels were analyzed following VEGF stimulation in the presence or absence of pathway-specific inhibitors (Figs. 4c-4d). Under hypoxia alone, ADAMTS2 expression remained at low basal levels at both the mRNA and protein levels. VEGF treatment significantly increased ADAMTS2 mRNA expression compared with untreated hypoxic controls ($p < 0.05$), which was paralleled by an approximately 3.1-fold increase in ADAMTS2 protein abundance. Pharmacological inhibition of MEK/ERK signaling with PD9 markedly enhanced VEGF-induced ADAMTS2 mRNA expression ($***p < 0.0001$), while partially attenuating ADAMTS2 protein accumulation. In contrast, inhibition of PI3K signaling with wortmannin strongly suppressed VEGF-induced ADAMTS2 expression at both the mRNA and protein levels, reducing expression toward basal values. Similarly, inhibition of SP6 significantly decreased VEGF-induced ADAMTS2 protein levels and was associated with reduced ADAMTS2 mRNA expression. PD1 treatment resulted in a modest reduction in ADAMTS2 expression compared with VEGF alone. β-actin expression remained unchanged across all conditions, confirming equal protein loading.

To examine the transcriptional regulation of ADAMTS2 by VEGF, the −658/ + 110 promoter fragment was cloned upstream of a luciferase reporter and transiently transfected into cells. Following transfection, cells were exposed to VEGF under normoxic or hypoxic conditions in the presence or absence of pathway-specific inhibitors, and promoter activity was quantified using a dual-luciferase assay (Figs. 4e-4f). Under both oxygen conditions, basal ADAMTS2 promoter activity was detectable, and VEGF stimulation resulted in a significant increase in luciferase activity compared with untreated controls. Inhibition of MEK/ERK signaling with PD9 significantly suppressed VEGF-induced promoter activity under both normoxia and hypoxia, indicating a requirement for ERK signaling in ADAMTS2 transcriptional activation. Similarly, SP6 treatment reduced VEGF-driven promoter activity, with a more

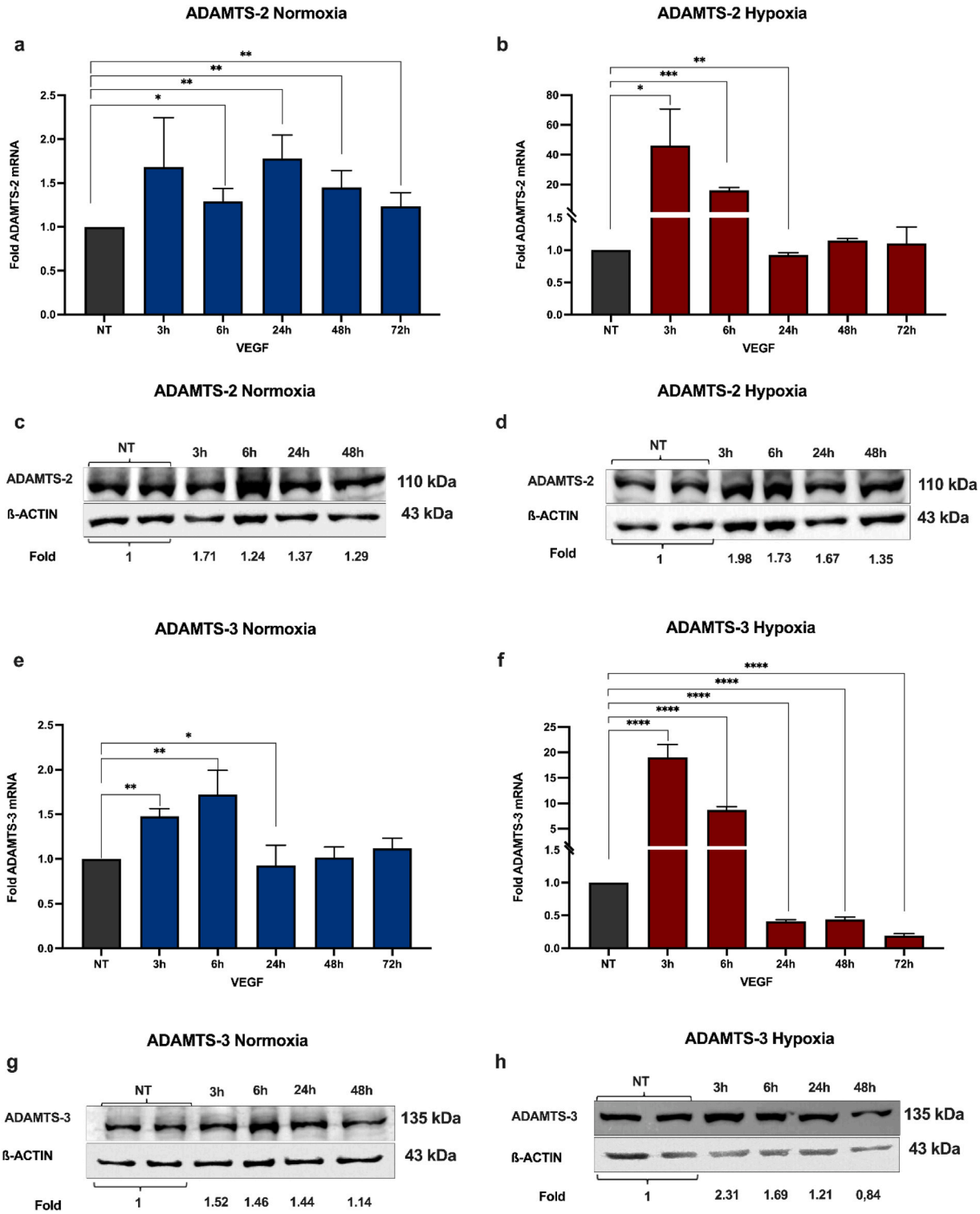

Fig. 2. VEGF application regulates the mRNA and protein expression of ADAMTS2/−3 under different oxygen conditions (normoxia and hypoxia). Under hypoxic and normoxic conditions, VEGF increases the expression of the ADAMTS2/−3 genes in HUVEC cells. The cells were incubated overnight, then treated with 20 ng/mL VEGF in FCS-free medium. 150 mM CoCl2 was applied to induce hypoxic conditions. No cytokine or VEGF was administered to the control group. mRNA levels were calculated using qRT-PCR. Results were evaluated using the Livak Method, with standard deviations calculated based on cT values. The housekeeping gene hβ−2 was used for normalization. All statistical analyses were conducted using the One-Way ANOVA approach, with a significance threshold of *$p \leq 0.05$. Under normoxic (a) and hypoxic conditions (c), changes in ADAMTS2 mRNA and protein expression (b and d). Protein samples were isolated with RİPA buffer. The expression levels of the target proteins were quantified by Western blot analysis. Actin-β was used as the normalized and as a loading control. Densiometric analyses of the bands obtained with the ImageJ program were performed. The mRNA levels of ADAMTS3 under normoxic (e) and hypoxic (g) conditions and the corresponding protein expressions (f and h) are shown. The stars in the figures indicate the following levels of significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

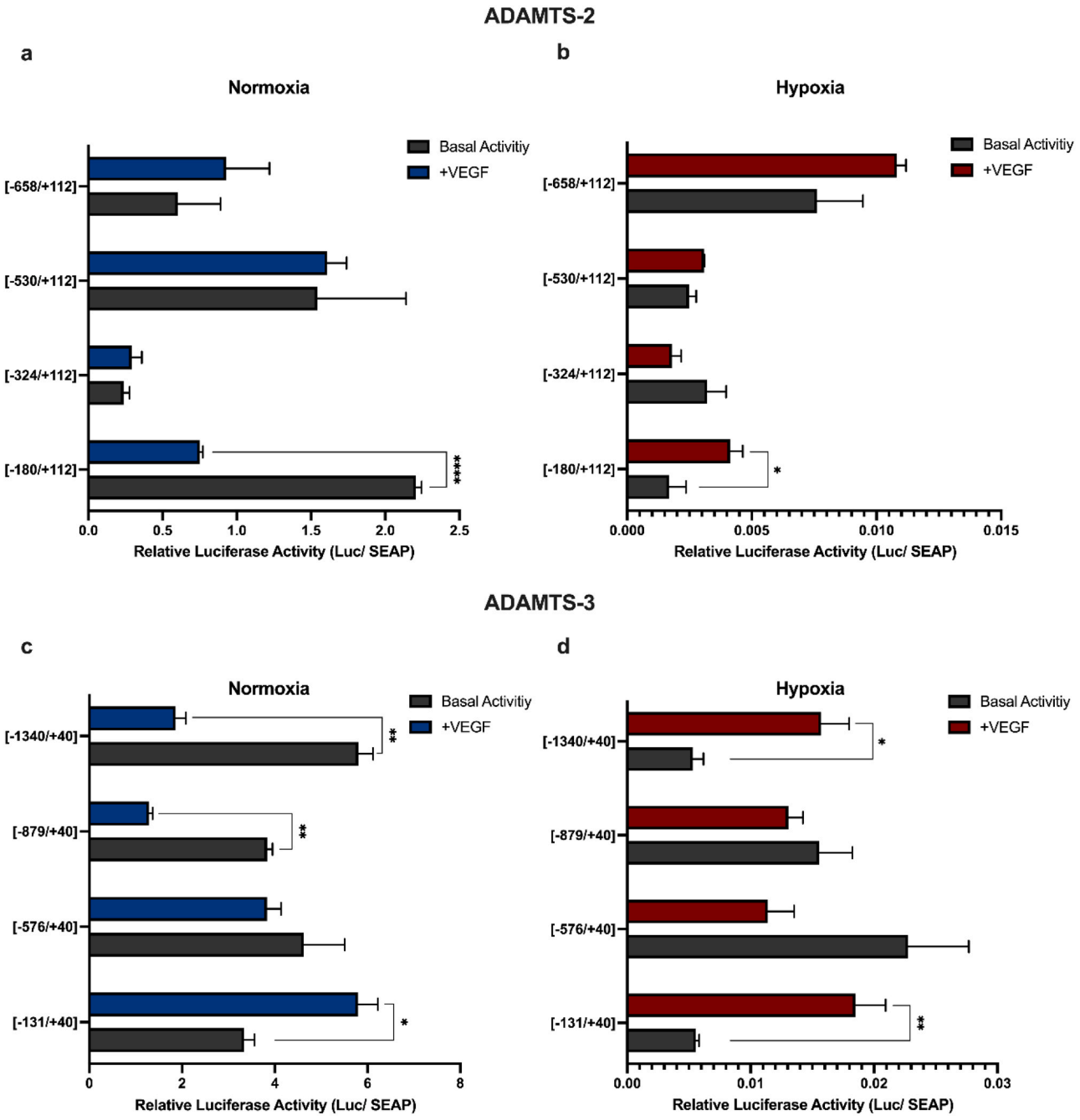

Fig. 3. The regulatory effect of VEGF stimulation on the promoter activity of ADAMTS2 (a–b) and ADAMTS3 (c–d) under normoxic and hypoxic conditions. Four truncated ADAMTS2 and ADAMTS3 promoter constructs were evaluated in transient transfection experiments using the HUVEC cell model. The cells were incubated overnight, then treated with 20 ng/mL VEGF in FCS-free medium. Following transient transfection, supernatant samples were collected at 48 h, and luciferase and SEAP activities were determined by quantitative analysis. Experiments were repeated three times. Analyses were performed using One-Way ANOVA; $p \leq 0.05$ was considered the threshold for statistical significance. The stars in the figures indicate the following levels of significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

pronounced inhibitory effect observed under hypoxic conditions. In contrast, inhibition of PI3K signaling with wortmannin enhanced VEGF-induced ADAMTS2 promoter activity under both oxygen conditions, with the strongest effect detected under hypoxia. PD1 treatment consistently reduced promoter activity irrespective of oxygen tension, lowering transcriptional output toward basal levels. Collectively, these findings demonstrate that VEGF regulates ADAMTS2 transcription through its proximal promoter region in an oxygen-dependent manner, and that the contribution of individual signaling pathways to promoter activation is differentially modulated by normoxic and hypoxic conditions.

Collectively, the combined mRNA, protein, and promoter-reporter analyses demonstrate that VEGF is a potent regulator of ADAMTS2 expression under both normoxic and hypoxic conditions, while the magnitude and signaling dependency of this regulation are strongly influenced by oxygen availability. Under normoxia, VEGF-induced ADAMTS2 upregulation at the mRNA level is largely mirrored at the protein level and is supported by increased promoter activity, indicating coordinated transcriptional and translational regulation. In contrast, under hypoxia, VEGF-driven ADAMTS2 expression is more pronounced

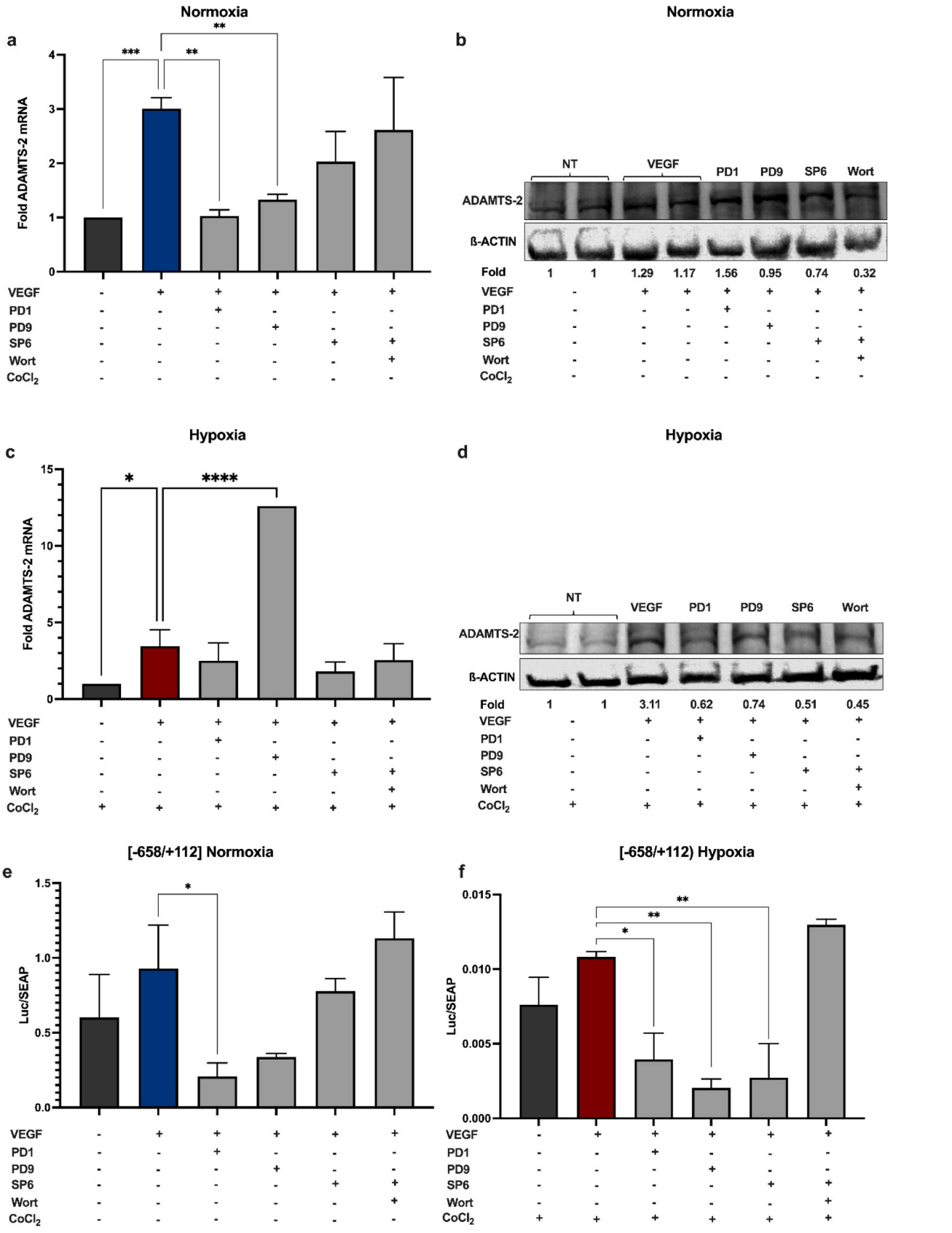

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Fig. 4. Regulatory effects of VEGF stimulation and related inhibitor treatment on ADAMTS2 gene expression and promoter activity in HUVEC cells under normoxic and hypoxic conditions. ADAMTS2's response to VEGF induction is regulated via the JNK, MAPK, PI3K, and p38 signalling pathways. The relevant effects are presented at the mRNA (a) and protein (b) levels under normoxic conditions and at the mRNA (c) and protein (d) levels under hypoxic conditions. HUVECs were incubated overnight. Prior to VEGF induction, cells were pre-treated with selective inhibitors to suppress target signalling pathways. mRNA samples obtained after 6 h of treatment and analysed by qRT-PCR; expression data were normalised to the hβ−2 gene. No cytokine or inhibitors were administered for the control group. Normoxic conditions (−658/+112) promoter truncated (e) under hypoxic conditions (−658/+112) promoter truncated (f) for ADAMTS2 transiently transfected in HUVECs. The cells were incubated overnight, then treated with 20 ng/mL VEGF in FCS-free medium. Luciferase and SEAP activities were determined in the culture medium collected 48 h after transfection. Experiments were repeated three times. All statistical analyses were performed using the one-way ANOVA test; $p \leq 0.05$ values were considered significant. The stars in the figures indicate the following levels of significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

at the transcriptional level, with mRNA induction exceeding protein accumulation and exhibiting heightened sensitivity to pathway modulation. Notably, PI3K signaling is required for VEGF-mediated ADAMTS2 expression at both mRNA and protein levels under both oxygen conditions, whereas MEK/ERK signaling displays a context-dependent role, restraining ADAMTS2 transcription under hypoxia while supporting promoter activity under normoxia. Together, these findings reveal an oxygen-dependent rewiring of VEGF signaling that differentially governs ADAMTS2 transcriptional output and protein expression.

3.5. VEGF regulates ADAMTS3 transcription and protein expression through oxygen-dependent signaling pathways

To investigate the mRNA expression of ADAMTS3 by VEGF under normoxic conditions, HUVECs were pretreated with pathway-specific inhibitors for 45 min, followed by stimulation with VEGF for 6 h, and ADAMTS3 mRNA levels were quantified by qRT-PCR (Fig. 5a). The mRNA and protein analyses demonstrate that VEGF robustly induces ADAMTS3 expression in HUVECs under normoxic conditions, and that this induction is strictly dependent on multiple intracellular signaling pathways (Fig. 5a–b). At the transcriptional level, VEGF stimulation resulted in a marked upregulation of ADAMTS3 mRNA, which was significantly attenuated by pharmacological inhibition of JNK, MEK/ERK, PI3K, and p38 MAPK signaling, with p38 inhibition exerting the most pronounced suppressive effect, nearly restoring ADAMTS3 transcript levels to basal values (Fig. 5a). Consistent with these transcriptional findings, VEGF-induced ADAMTS3 protein accumulation closely mirrored the mRNA response. VEGF treatment increased ADAMTS3 protein abundance, whereas inhibition of the same signaling pathways markedly reduced protein levels. Among these, PI3K inhibition produced the strongest reduction in ADAMTS3 protein expression, followed by p38 MAPK, MEK/ERK, and JNK inhibition (Fig. 5b). The concordance between mRNA and protein data indicates that VEGF regulates ADAMTS3 expression predominantly through signaling-dependent transcriptional control, which is effectively translated into protein expression in endothelial cells.

Under hypoxic conditions, basal ADAMTS3 expression was maintained at low but detectable levels, while VEGF stimulation elicited a significant induction of ADAMTS3 expression at both the mRNA and protein levels, indicating that VEGF remains functionally active toward ADAMTS3 in hypoxic endothelial cells. At the transcriptional level, VEGF significantly increased ADAMTS3 mRNA abundance compared with untreated hypoxic controls (**$p < 0.01$) Notably, hypoxia profoundly altered the signaling dependency of this response: inhibition of the MEK/ERK pathway with PD9 resulted in a marked accumulation of ADAMTS3 mRNA, yielding the highest transcript levels among all experimental groups (****$p < 0.0001$), whereas inhibition of p38 MAPK with PD1 significantly suppressed ADAMTS3 mRNA expression, restoring transcript levels toward basal hypoxic values (Fig. 5c) ($p < 0.05$).

Consistent with the transcriptional findings, VEGF stimulation under hypoxia led to a robust accumulation of ADAMTS3 protein, reaching approximately 1.79-fold relative to non-treated hypoxic cells. In contrast, pharmacological inhibition of VEGF-associated signaling pathways markedly attenuated ADAMTS3 protein expression. Inhibition of p38 MAPK, MEK/ERK, JNK, or PI3K signaling significantly reduced ADAMTS3 protein abundance, with the strongest suppressive effects observed upon JNK and PI3K inhibition (both ~0.24-fold) (Fig. 5d). Taken together, these data demonstrate that hypoxia not only potentiates VEGF-induced ADAMTS3 expression but also reprograms the relative contribution of downstream signaling pathways, resulting in distinct regulatory profiles at the mRNA and protein levels.

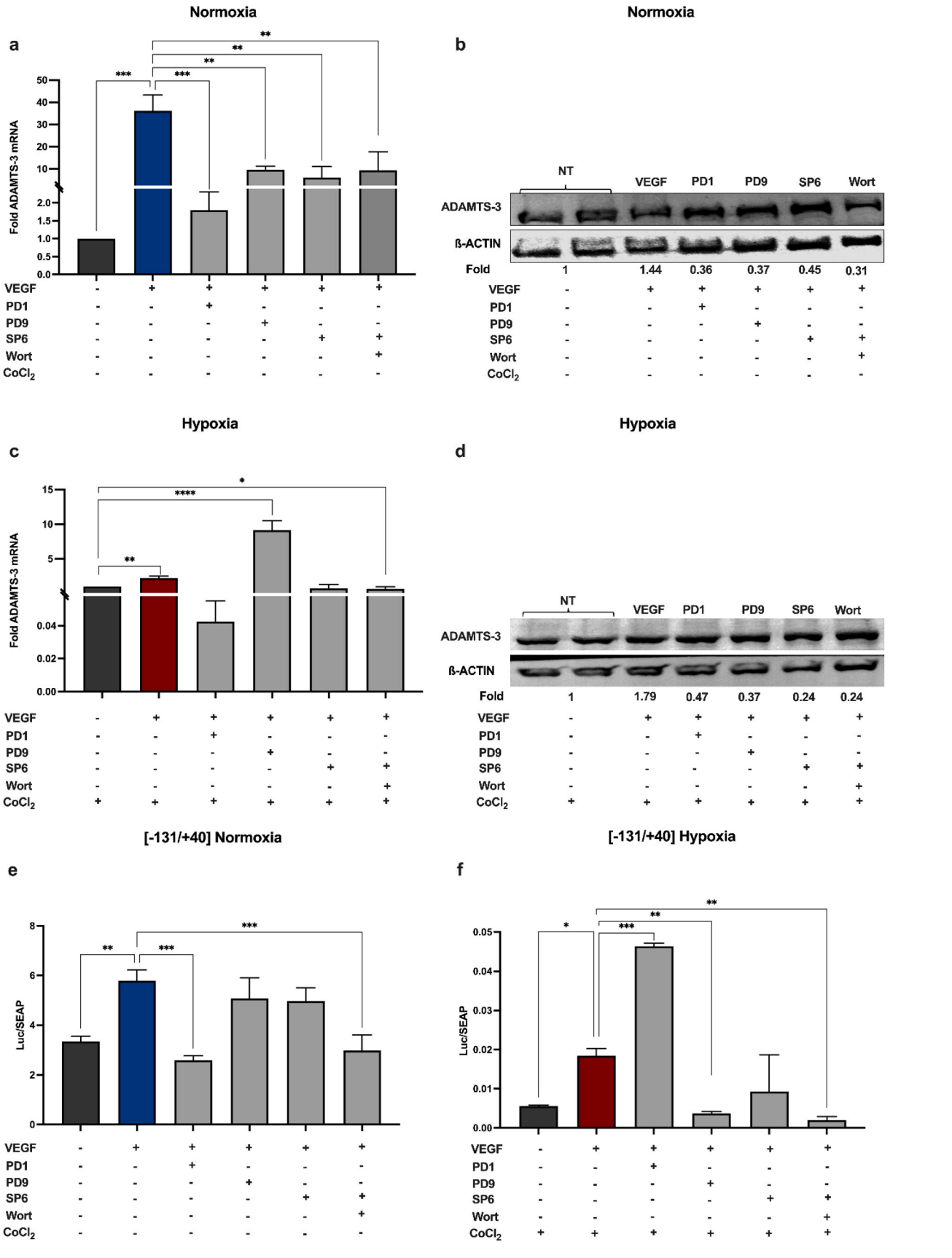
To define the oxygen-dependent regulation of ADAMTS3 transcription, HUVECs were transfected with the minimal −131/ + 40 ADAMTS3 promoter–luciferase construct, selected from four promoter fragments as the smallest region retaining VEGF responsiveness, and analyzed under normoxic and hypoxic conditions (Figs. 5e-5f). Under normoxia, VEGF stimulation significantly increased ADAMTS3 promoter activity, demonstrating that this proximal promoter fragment is sufficient to mediate VEGF-dependent transcriptional activation. This VEGF-induced promoter activation was markedly attenuated by pharmacological inhibition of p38 MAPK and PI3K signaling, indicating that VEGF-driven promoter activity under normoxia is mediated through multiple convergent signaling pathways (Fig. 5e).

Under hypoxic conditions, basal promoter activity remained low; however, VEGF-induced activation of the −131/ + 40 promoter was significantly enhanced compared with normoxia, revealing a strong potentiating effect of hypoxia on VEGF-dependent transcription. Importantly, hypoxia also reshaped the signaling dependency of promoter activation. Inhibition of MEK/ERK signaling almost completely abolished VEGF-induced promoter activity under hypoxia, whereas inhibition of PI3K resulted in partial but significant suppression (Fig. 5f). Together, these results demonstrate that hypoxia amplifies VEGF-induced ADAMTS3 transcription at the promoter level while imposing a stricter dependency on PI3K and MEK/ERK signaling, providing a mechanistic basis for the oxygen-sensitive regulation of ADAMTS3 expression in endothelial cells.

3.6. Bioinformatic analysis of the transcription effects of hypoxia on pathway activation and the VEGF signaling network in endothelial cells

To define hypoxia- and VEGF-responsive transcriptional programs in endothelial cells, three independent RNA-seq datasets (GSE186474, GSE190240, and GSE71967) from HUVECs were integratively analyzed. KEGG-based GSEA demonstrated that hypoxia induces a broad and coordinated reprogramming of endothelial signaling networks compared with normoxia (Fig. 6a). Significantly enriched pathways included HIF−1, PI3K–Akt, MAPK, and Rap1 signaling, alongside pathways governing cytoskeletal organization and cell–matrix interactions, such as focal adhesion, actin cytoskeleton regulation, and ECM–receptor interaction, indicating hypoxia-driven structural and migratory remodeling.

Beyond canonical angiogenic pathways, hypoxia also enriched gene sets related to vesicular trafficking, cellular clearance, and metabolic stress responses, including endocytosis, phagosome formation, oxidative phosphorylation, proteasome activity, and ferroptosis, reflecting adaptive endothelial stress responses. Notably, many of the enriched pathways overlapped with canonical VEGF downstream signaling, supporting a functional convergence between hypoxia-driven



(caption on next page)

Fig. 5. Changes in ADAMTS3 mRNA expression and promoter activity were analysed in HUVEC cells exposed to normoxic and hypoxic conditions. JNK, MAP kinase, PI3K, and p38 pathways are involved in VEGF induced VEGF-induced ADAMTS3 normoxic conditions (a) and hypoxic conditions (c) mRNA expression. Protein expression showed normoxic condition (b) and hypoxic condition (d). HUVECs were incubated overnight. Prior to VEGF stimulation, cells were incubated with specific inhibitors to block the relevant signalling pathways. Following 6 h of VEGF stimulation, mRNA expression was analysed by qRT-PCR; data were normalised using the hβ−2 gene as a reference. No cytokine or inhibitors were administered for the control group. Under normoxic conditions (−131/+40) (e) and under hypoxic conditions (−1340/+40) (f), promoter-truncated ADAMTS3 promoter constructs were transiently transfected into HUVECs. The cells were incubated overnight, then treated with 20 ng/mL VEGF in FCS-free medium. Following transient transfection, supernatant samples were collected at 48 h, and luciferase and SEAP activities were measured quantitatively. Experiments were repeated three times. All statistical analyses were performed using the one-way ANOVA test; $p \leq 0.05$ values were considered significant. The stars in the figures indicate the following levels of significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

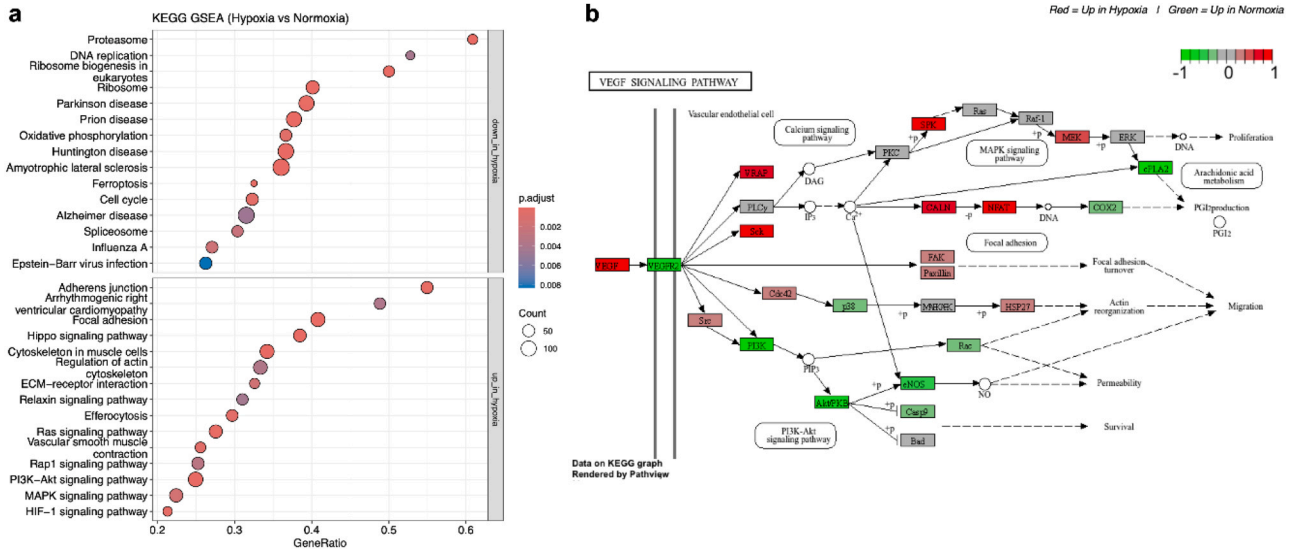


Fig. 6. KEGG-based bioinformatics analysis of the effects of hypoxia on VEGF-related molecular networks and gene expression dynamics. KEGG GSEA analysis shows pathways differentially enriched between hypoxic and normoxic conditions. A) Up in Hypoxia: Pathways increased under hypoxic conditions include HIF−1, MAPK, PI3K-Akt, Rap1, ECM–receptor interaction, regulation of actin cytoskeleton, focal adhesion, and Hippo signalling, which are associated with vascular permeability, cytoskeletal organisation, and matrix remodelling. Down in Hypoxia: Pathways suppressed by hypoxia represent processes related to translational activity, energy metabolism, and cell proliferation, such as Ribosome, Oxidative phosphorylation, DNA replication, Proteasome, Cell cycle, and Spliceosome. The analysis was performed using normalised expression values from the GSE186474 dataset. The horizontal axis represents Gene Ratio, the dot colour represents the adjusted p-value, and the dot size represents the number of genes. b) KEGG mapping showing changes in gene expression in the VEGF signalling pathway under hypoxic conditions. Genes upregulated under hypoxic conditions are shown in red, while those downregulated are shown in green. The analysis was generated using the PathView tool (hsa04370) based on log2FC values obtained from differential gene expression. The visualisation demonstrates that hypoxia induces significant transcriptional changes in key molecular components mediating VEGFR2 activation. The stars in the figures indicate the following levels of significance: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

transcriptional programs and VEGF-mediated endothelial regulation.

Pathway-level mapping onto the KEGG VEGF signaling pathway further revealed that hypoxia selectively reprograms VEGF signal flow (Fig. 6b). Hypoxia preferentially enhanced the PLCγ–PKC–MAPK axis and focal adhesion–associated modules, consistent with a pro-migratory and transcriptionally active endothelial phenotype, while components of the PI3K–Akt–eNOS pathway displayed relatively stronger activity under normoxic conditions. Together, these data indicate that hypoxia does not uniformly amplify VEGF signaling but instead redirects it toward distinct downstream branches, providing a systems-level framework for the context-dependent VEGF responses observed experimentally.

4. Discussion

VEGF-mediated signalling increases vascular permeability by coordinating endothelial proliferation, migration, and survival, thereby directing angiogenesis during vascular/lymphatic development, tissue repair, and homeostasis (Lee et al., 2025b; Melincovici et al., 2018). The current literature indicates that VEGF exhibits gene-specific and binding-dependent regulatory effects on different members of the ADAMTS family: ADAMTS1 functions as a feedback mechanism in retinal neovascularization (Xu et al., 2006), while ADAMTS4 has been defined as an anti-angiogenic regulator that inhibits VEGFR2 phosphorylation (Hsu et al., 2012). ADAMTS13 has been reported to exhibit a bidirectional effect, capable of both increasing VEGF levels and limiting VEGF signalling, depending on its binding state (Tang et al., 2017). These heterogeneous responses reveal that the ADAMTS gene family does not exhibit a uniform VEGF response; rather, each gene possesses distinct regulatory networks shaped by its unique promoter architecture and microenvironmental context.

Members of the pro-collagen N-peptidase subfamily, ADAMTS2/−3, regulate collagen fibrillogenesis by removing the N-propeptides of type I–III fibrillar pro-collagens, thereby shaping the structural organisation of vascular wall integrity and stromal architecture (Cal and López-Otín, 2015; Li et al., 2022). ADAMTS2 has been shown to exhibit anti-proliferative and anti-angiogenic properties, while ADAMTS3 plays a critical role in lymphangiogenesis and lymphatic vessel biogenesis (Dubail et al., 2010; Jeltsch et al., 2014; Ogino et al., 2017). However, data on how ADAMTS2/−3 expression is regulated in endothelial cells under angiogenic stimuli such as VEGF and hypoxia remain limited. Our group's previous studies have shown that hypoxia increases the mRNA and protein levels of these genes via functional HRE regions located in the ADAMTS2/−3 promoters, dependent on HIF−1α (Altuntaş et al.,

2023); however, the transcriptional and translational effects of VEGF on ADAMTS2/−3, the promoter-level counterparts of this effect, and its oxygen dependence have not yet been fully characterised.

In this study, the response of the ADAMTS gene family to hypoxia and VEGF was evaluated using a multidimensional approach. RNA-seq-based analyses revealed that ADAMTS2/−3 exhibited only a low-intensity and statistically insignificant increase in response to hypoxia. Analysis of the GSE71967 time series data revealed that the transcriptional response to VEGF varied depending on oxygen levels. Under normoxic conditions, the response to VEGF showed that ADAMTS2/−3 were significantly induced at early and mid-time points. Under hypoxic conditions, the early increase in VEGFA and HIF1A is consistent with the rapid adaptation of cells to low oxygen; the delayed increase in ADAMTS2/−3 indicates the initiation of secondary signaling. These results are consistent with previous findings indicating that VEGF can modulate ADAMTS gene expression in an oxygen-level-dependent manner (Jiang et al., 2023; Tayman et al., 2019). These findings are consistent with those from our experimental validation studies in HUVEC cells and support how VEGF's oxygen-sensitive regulatory effect on ADAMTS genes manifests at the cellular level. Our results show that VEGF is a potent inducer of ADAMTS2 and ADAMTS3 expression under both normoxic and hypoxic conditions, but that the magnitude, kinetics, and signaling dependency of this induction are profoundly shaped by oxygen availability. Under normoxia, VEGF-induced upregulation of both genes was accompanied by concordant increases in mRNA, protein, and promoter activity, indicating tightly coupled transcriptional and translational regulation. In contrast, under hypoxia, VEGF-driven transcriptional responses were markedly amplified—particularly for ADAMTS2-while protein accumulation showed more restrained increases, suggesting the involvement of post-transcriptional or translational control mechanisms that limit protein output under low-oxygen stress. The induction of ADAMTS3 under hypoxic conditions exhibited a more limited profile in terms of response intensity and duration compared to ADAMTS2. These distinct response profiles suggest that the two genes possess separate regulatory mechanisms within the VEGF–hypoxia axis and that ADAMTS2 may be a more sensitive target gene to environmental changes encompassing both normoxia and hypoxia.

Our study demonstrates for the first time that VEGF regulates ADAMTS2/−3 promoter activity in HUVEC cells in an oxygen-dependent manner, and that this regulation exhibits promoter fragment-specific differences. For ADAMTS2, VEGF responsiveness under normoxia was primarily mediated by the −658/ + 112 promoter fragment, indicating that distal regulatory elements are essential for angiogenic signaling in the absence of hypoxia. In contrast, hypoxia has shifted VEGF responsiveness to more proximal promoter regions, indicating the presence of cooperative or competitive interactions between HIF−1α-dependent HREs and VEGF-responsive transcription factors such as SP1, AP−1, and NF-κB (Pagès and Pouysségur, 2005). Similarly, ADAMTS3 promoter analysis revealed that minimal proximal elements (−131/+40) are sufficient for VEGF responsiveness under normoxia, whereas hypoxia broadens the spectrum of VEGF-responsive regions, enabling distal promoter fragments to contribute to transcriptional activation. This observation supports a model in which hypoxia increases chromatin accessibility or transcription factor cooperativity, allowing VEGF signaling to engage additional regulatory modules. Such hypoxia-induced promoter plasticity has been reported for multiple angiogenesis-associated genes and is considered a hallmark of endothelial adaptation to ischemic environments (Semenza, 2012). The literature reports that promoter responses to VEGF can exhibit region-specific differences depending on transcription factors such as Sp1, AP−1, and NF-κB, and that oxygen levels can modulate promoter activity through both HIF−1-mediated and HIF−1-independent pathways (Lee et al., 2025b).

A key finding of this study is the oxygen-dependent rewiring of VEGF downstream signaling pathways controlling ADAMTS transcription. The literature reports that VEGFR−2 activation simultaneously activates multiple downstream pathways, such as PLCγ–PKC–MAPK, PI3K–AKT, and p38-MAPKAPK2/3 (Yao et al., 2025). This study demonstrates that ADAMTS2 and ADAMTS3 are both VEGF-responsive, oxygen-sensitive genes whose expression is regulated through closely overlapping but non-identical signaling pathways. Under normoxic conditions, VEGF induces moderate increases in ADAMTS2 and ADAMTS3 mRNA, protein abundance, and promoter activity, with these effects being primarily mediated by PI3K, p38 MAPK, and JNK signaling, while MEK/ERK contributes more weakly and in a gene-dependent manner. Hypoxic exposure markedly elevates basal expression of both ADAMTS members and strongly potentiates VEGF-induced transcriptional activation. Notably, under hypoxia, pharmacological inhibition of PI3K, p38 MAPK, or JNK nearly abolishes VEGF-driven ADAMTS2 and ADAMTS3 expression, indicating a heightened and convergent pathway dependence in low-oxygen conditions. The strong concordance among mRNA, protein, and promoter-reporter data for both genes supports a predominantly transcriptional mode of regulation, mediated by VEGF-responsive cis-elements within their proximal promoter regions. Collectively, these findings identify ADAMTS2 and ADAMTS3 as hypoxia-sensitized downstream effectors of VEGF signaling, likely contributing to extracellular matrix remodeling and angiogenic adaptation in hypoxic microenvironments such as tumors and ischemic tissues.

The bioinformatic analyses conducted in this study provide independent, system-level validation of our experimental findings. In the ‘Hypoxia Exposure’ group, the activation of HIF−1, PI3K–Akt, MAPK, Rap1, ECM–receptor interaction, and focal adhesion pathways indicates that hypoxia triggered a coordinated transcriptomic response involving pro-angiogenic, ECM remodelling, and cytoskeletal reorganisation. Integrated RNA-seq and KEGG–GSEA analyses revealed that hypoxia induces a global reprogramming of VEGF signalling, enhancing the PLCγ–PKC–MAPK axis and focal adhesion-related pathways while relatively attenuating PI3K–Akt–eNOS signalling. This redistribution is consistent with our experimental observations that VEGF signalling under hypoxia prioritises transcriptional and migration programmes over survival, supporting the view that hypoxia directs the angiogenic response towards tissue remodelling and invasion rather than proliferation (Carmeliet and Jain, 2011).

These multifaceted pathway alterations suggest that ECM-regulating proteases such as ADAMTS2/−3 may play indirect yet significant roles in hypoxic adaptation. VEGF pathway mapping has demonstrated that hypoxia modulates VEGFR2-mediated signalling in a multi-layered manner: The marked activation of the VEGF/VEGFR2–PI3K–Akt–eNOS axis supports survival and angiogenic responses, while the relative suppression of the MAPK and Ca^{2+}/NFAT pathways may be interpreted as an adaptive regulation towards energy conservation under oxygen restriction. Collectively, these findings reveal that endothelial cells under hypoxia finely regulate proliferation, migration, and permeability processes, with the VEGF signalling pathway at the core of this adaptation.

Validation experiments conducted in osteosarcoma cell lines (MG−63, SAOS−2) demonstrated that VEGF can increase ADAMTS2 expression and ADAMTS3 independently of cell type, confirming that this protease's VEGF-responsive transcriptional response is not specific to endothelial cells. This finding is consistent with studies reporting that ADAMTS2 can be induced by pro-angiogenic and pro-fibrotic signals in various mesenchymal and stromal cells (Li et al., 2022; Wang et al., 2003). These results are consistent with our team's previous studies conducted on MG−63 and SAOS−2 cells using IL−1α and IL−6; both cytokines produced time-dependent increases in ADAMTS2/−3 expression, but the intensity and duration of the response varied depending on the cell type and the nature of the stimulus (Alper and Kockar, 2014; Alper et al., 2015). Monitoring HIF1A levels is important for the correct assessment of the response to hypoxia. Previously, our team validated the model by demonstrating an increase in HIF1A mRNA

in HUVEC cells under chemically mimicked hypoxic conditions (using $CoCl_2$) (Altuntaş et al., 2023). In the present study, the effect of hypoxia was directly examined in SAOS−2 cells, and an increase in HIF1A mRNA levels was observed under VEGF-treated hypoxic conditions. Thus, it was demonstrated that each hypoxic stimulation was successfully achieved and that SAOS−2 cells were able to respond to the VEGF–HIF axis. This finding suggests that the regulation exhibited by ADAMTS genes under hypoxia is not limited to endothelial cells alone, and that HIF1A activity should be evaluated in conjunction with VEGF signaling in different cell types.

In conclusion, this study identifies ADAMTS2/−3 as integrative molecular hubs at the intersection of hypoxia and VEGF signalling in endothelial cells. By integrating mRNA, protein, promoter-reporter, and bioinformatic analyses, we demonstrate that oxygen availability reshapes the VEGF signalling architecture and how angiogenic signals are translated into ECM remodelling programmes. These findings provide a mechanistic framework for how endothelial cells finely tune matrix remodelling in ischaemic and tumour-associated microenvironments and suggest that the VEGF–ADAMTS axis could be a potential target for modulating pathological angiogenesis. Overall, our data demonstrate that ADAMTS2/−3 is regulated in an oxygen-sensitive and pathway-specific manner within the endothelial response to VEGF and hypoxia; however, they emphasise the need for further mechanistic studies to fully characterise the functional contributions of these proteases. In addition relatively low signal intensity represent a technical limitation of the ADAMTS2 blot analysis.

CRediT authorship contribution statement

Candan Altuntaş: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Meltem Alper:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yasemin Kalfa:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Feyza Nur SAV:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Köçkar Feray:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Compliance with ethical standards

The article contains no research in which animals were used.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tice.2026.103636.

Data availability

Data will be made available on request.

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