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ORIGINAL PAPER

TNF-α-Induced Upregulation of ADAMTS-8 Expression in SW480 Cells: Implications for Intracellular Signaling Pathways and Transcription Factor Activity

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Abstract

Tumor necrosis factor-alpha (TNF-α) is associated with the metastatic phenotype and regulates cellular communication in Colorectal Cancer (CRC). This study investigates the effects of TNF-α on the expression of the ADAMTS-8 gene in SW480 colorectal cancer cells, focusing on both mRNA and protein levels, as well as the underlying intracellular signaling pathways activated in response to TNF-α. SW480 cells were treated with TNF-α at varying concentrations (10, 20, and 40 ng/mL) for 24, 48, and 72 h. Utilizing the MTT assay, a significant reduction in cell viability was observed at 48 and 72 h across all doses, indicating a cytostatic effect of TNF-α. The transcriptional activity of ADAMTS-8 was assessed through transient transfection of full-length and truncated promoter constructs, revealing that TNF-α significantly upregulated ADAMTS-8 expression through specific promoter regions. Quantitative RT-PCR and Western blot analyses demonstrated a time- and dose-dependent increase in both mRNA and protein levels of ADAMTS-8 in cells treated with 10 and 20 ng/mL of TNF-α. TNF-α strongly increased the transcriptional activity of several truncated ADAMTS-8 promoter constructs. Further exploration of intracellular signaling pathways revealed the activation of NF-κB, PI3K, and SAPK/JNK pathways, with limited activation of p38/MAPK. Inhibition studies employing specific pathway inhibitors indicated that blockade of NF-κB, PI3K, p38/MAPK, and JNK signaling pathways significantly suppressed TNF-α-induced ADAMTS-8 expression at both mRNA and protein levels. Additionally, Chromatin Immunoprecipitation (ChIP) assays confirmed the binding of NF-κB, STAT-3, ELK-1, c-Jun, and ATF-1 to the ADAMTS-8 promoter in response to TNF-α treatment. These findings suggest a multifaceted regulatory mechanism wherein TNF-α promotes ADAMTS-8 expression by activating key transcription factors and associated signaling cascades—in silico analysis revealed that ADAMTS-8 is upregulated in colorectal adenocarcinoma and positively correlates with TNF expression, suggesting a potential link between inflammatory signaling and favorable prognostic outcomes. Knockdown of the ADAMTS-8 markedly promoted cell growth. When combined with ADAMTS-8 silencing, TNF-α attenuated the proliferation effect, indicating a potential interaction between TNF-α signaling and ADAMTS-8 pathways and supporting tumor suppressor function of ADAMTS-8.

Keywords ADAMTS-8 · TNF · Colorectal cancer · NF-κB · PI3K · And SAPK/JNK

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Abbreviations

ADAMTS 8	ADAM metallopeptidase with thrombospondin type 1 motif 8
TNF-α	Tumour Necrosis Factor alpha
NF-κB	Nuclear Factor kappa B
ATF	Activating transcription factor
CREB	cAMP Response Element-Binding

Introduction

Colorectal cancer (CRC) is one of the most common cancers in the world [1]. Although the exact causes of colorectal tumor formation are unclear, factors such as inflammatory bowel disease (IBD), intestinal injury, and oxidative stress are recognized as significant risk factors for colonic inflammation and consequently colon cancer occurrence [2]. This persistent inflammatory state can disrupt the intestinal balance, and chronic inflammation leads to damage of genetic material in colon cells, leading to the development of colorectal cancer. For example, individuals with inflammatory bowel disease (IBD) have been found to have a 60% higher risk of colorectal cancer compared to other people [3]. Moreover, the imbalance between different types of cytokines disrupts the natural signaling mechanisms of normal cells, contributing to the disease's complex nature [4]. Proinflammatory cytokines are synthesized in the colon because of local inflammation. These proinflammatory cytokines lead to complex interactions between mucosal cells, macrophages, and intestinal cells in the intestinal microenvironment. These interactions cause persistent inflammation through activating STAT3, one of the major oncogenic transcription factors, and NF-κB [5, 6]. Inflammation is clearly a natural component of colorectal cancer progression. Tumor necrosis factor-alpha (TNF-α) promotes inflammation and contributes to cancer progression [7]. Elevated TNF-α levels have been associated with colorectal cancer (CRC) metastasis [8–10].

The ADAMTS family has been defined as a 19-member family of secretory, multi-structural domain and matrix-associated zinc ion-dependent proteases [11]. ADAMTS family members are involved in several important functions, such as collagen maturation, mucin degradation, regeneration and inflammation, inhibition of angiogenesis, which are among the basic physiological processes of the body [12–16]. ADAMTS has been found to regulate cancer-related biological events by affecting important steps such as cell proliferation, apoptosis, migration, and invasion in cancer processes (tumor formation, development, and invasion) [17–19]. ADAMTS-8 synthesizes an 889 amino acid protein with antiangiogenic function [20]. ADAMTS-8 expression has been reported to be downregulated in various tumors, including esophageal squamous cell carcinoma, colorectal, lung, and breast cancers, primarily due to epigenetic silencing. Other studies have indicated that ADAMTS-8 upregulation can inhibit the progression of lung, breast, and colorectal cancers both in vitro and in vivo [19, 21–23]. There is insufficient information about the molecular mechanism of ADAMTS-8 up-regulation in colorectal cancer.

The purpose of this investigation was to clarify TNF-α-mediated regulation of ADAMTS-8 expression. For this purpose, we first determined the proliferative effect of TNF-α in SW480 cells. It was found that TNF-α cytokine up-regulated ADAMTS-8 expression at mRNA and protein levels. For transcriptional analyses, luciferase reporter assays were performed using five different-length 5' truncations of the ADAMTS-8 promoter. TNF-α-mediated regulation of ADAMTS-8 was found to be mediated by activation of MAPK, NFκB, PI3K, and SAPK/JNK pathways. In addition, bioinformatic analyses identified transcription factors that likely bind to the ADAMTS-8 promoter. Then, Chromatin Immuno-Precipitation showed that inflammation-related transcription factors, NFκB, STAT-3, ELK-1, c-Jun, and ATF-1 functionally bind to the ADAMTS-8 promoter.

Additionally, we explored the clinical relevance of ADAMTS-8 expression in CRC, focusing on its potential association with inflammation and patient prognosis. Further, knockdown of the ADAMTS-8 markedly promoted cell growth. When combined with ADAMTS-8 silencing, TNF-α attenuated the proliferation effect, indicating a potential interaction between TNF-α signaling and ADAMTS-8. This study offers a new perspective for understanding the molecular mechanisms of colorectal cancer and developing potential treatment strategies.

Materials and Methods

Cell Culture

SW480 (Human Colorectal Cancer Cell Line) cells were maintained in high-glucose DMEM medium supplemented with 10% FBS at 37 °C in 5% CO_2. Cells between passages 3 and 10 were employed for experimental procedures. TNF-α was purchased from PeproTech, Inc. (USA).

MTT Assay

The effect of TNF-α on cell proliferation in the SW480 cell line was determined by MTT analysis. First, SW480 cells were distributed at 3 × 10^4 per well in a 96-well plate. Cells were serum-starved for one hour with 0.1% BSA. Three distinct TNF-α concentrations (10, 20, and 40 ng/mL) were then administered. Untreated cells were designated as

the control group. After incubation with TNF-α for 24, 48, and 72 h, the amount of formazan crystals formed within the cells was measured. A spectrophotometer was used to test their absorbance at a wavelength of 550 nanometers [24, 25].

Transcriptional Activity Assays

SW480 cells were cultured by seeding them in 25 cm^2 culture dishes at 2 × 10^6 cells per dish. The cells were incubated with 5% CO_2 at 37 °C overnight for surface attachment. Transient transfection experiments were performed using ADAMTS-8 promoter reporter constructs, including pMET_TS-8[− 220/+9], pMET_TS-8[− 223/+323], pMET_TS-8[− 410/+323], pMET_TS-8[− 662/+323], and pMET_TS-8[− 851/+323], which were generated through a PCR-based approach (Supp. Figure 1). Calcium-phosphate precipitation was used for transient transfection with 10 µg promoter-luciferase DNA constructs and five µg secreted human alkaline phosphatase (SEAP) internal control into the SW480 cells [26]. In addition, 0.5 µg of pMetLuc control as a positive control and pMetLuc vectors as a negative control were also transfected into a 12-well plate. After overnight, the transfected cells were then removed with Trypsin-EDTA and counted. They were divided into 96-well plates as 3 × 10^4 cells. For repeated incubation, cells were placed in an incubator with 5% CO2 and a temperature of 37 °C. The cells were then transferred to medium containing 0.1% BSA and serum-starved. Serum-starved cells were treated with 20 ng/mL of TNF-α. Then, at the end of 72 h incubation periods of transfection, the medium was taken to determine the released luciferase and SEAP activities. The medium samples were quantified using a luminometer with the Ready-to-Glow Secreted Reporter System Kit (Takara). Transfection experiments were performed three times at different times for each promoter fragment. In each experimental repetition, promoter fragments were measured in at least three replicates [24].

qRT-PCR

Total RNA was isolated from SW480 cells using GeneJET™ RNA Purification Kit. Reverse transcription reaction was carried out using one microgram of total RNA, oligo(dT), and Reverse Transcriptase (200 U) (Thermo). To carry out the real-time PCR reaction, 6.25 µL of Light Cycler- RealQ Plus 2x Master Mix Green (Ampliqon), 1 µl of ADAMTS-8 expression primers, and 1 µL of cDNA were added to the reaction tube, and the reaction volume was completed to 12.5 µL. Human β-2 microglobulin was used for normalization. The primer sequence used for the expression analysis is shown in Table 1. Ct values obtained in triplicate studies were analyzed by the Livak (2-ΔΔCT) method, and gene expression levels were determined. Data were analyzed using the Graphpad Prism 9 program., values of $p \leq 0.05$ were statistically considered significant [26].

Table 1 Primers used to determine ADAMTS-8 mRNA expression

Primer name	Primer sequence
ADAMTS-8 F	5'-GGAGCGAGTTCAAAAGTGTTC-3'
ADAMTS-8 R	5'-AGACCTTCCTGCAGGAGTTG − 3'
Hβ-2 F	5'-TTTCTGGCCTGGAGGCTATC-3'
Hβ-2 R	5'-CATGTCTCGATCCCACTTAACT-3'

Western Blot Analysis

Protein extracts were obtained from 5 × 10^5 SW480 cells with RIPA buffer. Protein extracts were briefly obtained using the following steps: cells were scraped with RIPA buffer and sonicated for 2 min in an ice-cooled sonicator. For protein extraction, samples were centrifuged at 13,500 rpm for 15 min at + 4 °C. Proteins (10–40 µg) were loaded onto a 10% SDS-polyacrylamide gel and run at 80 V. Proteins were transferred to a PVDF membrane (Millipore) at + 4 °C using the Bio-Rad Trans-Blot system (Bio-Rad) at 80 mA overnight. After blocking with 5% (w/v) skimmed milk powder, the membranes were labeled with ADAMTS-8 (Invitrogen/OTI2H5 1:500) and β-Actin antibodies for normalization (1:5,000) overnight at + 4 °C. Membranes were incubated with Anti-mouse IgG antibody (1:10,000; Sigma) and treated with ECL (Thermo) to visualize protein bands. Densitometric analysis was performed using ImageJ software [27], and band intensities were normalized to β-actin to provide a consistent internal reference.

Pathway Studies

Pathway analysis was performed to demonstrate which pathway is activated in SW480 cells upon TNF-α treatment. Cells were incubated with TNF-α for short periods (5 min, 15 min, and 30 min) after serum-starvation with 0.1% BSA for one hour. Western blot analysis was used to evaluate PI3K, p38 MAPK, NF-κB, and SAPK/JNK pathways. The phosphorylation pattern was analyzed using phosphorylated antibodies and compared to total antibodies in western blotting. The antibodies used for this purpose are indicated in Supplementary Table 1. Pathway inhibition studies were performed to elucidate which intracellular pathway plays a role in the TNF-mediated upregulation of ADAMTS-8 gene expression. After serum starvation with 0.1% BSA, cells were incubated with specific pathway inhibitors for 45 min. These are; 1µM of Wortmannin (Cell signaling 99515) for Phosphoinositide 3-kinase (PI3K), 10 µM of PD98059 (Cell Signaling, 9900 S) for MEK, 12.5µM of PD169316 (Biovision,1666-5) for p38 MAPK, 20µM of SP600125

(Santa Cruz, sc200635) for JNK, 5μM of BAY 11-7082 (Santa Cruz, sc202490) for NF-κB. 20 ng/mL of TNF-α was applied to the cells after 45 min of specific pathway inhibitor incubation and incubated for 6 h. Then, the pellets and extracts were used to investigate the expression analysis of the ADAMTS-8 gene at the mRNA and protein levels [28].

Chromatin Immunoprecipitation (ChIP) Assay

EZ-ChIP Kit (17–371, Upstate, Millipore, MA, USA) was used. TNF-α treatment was followed by a 10-minute room temperature fixation with 1% formaldehyde. For protein analysis, cells were lysed and sonicated. Lysates were precipitated with protein A/G agarose at 4 °C for 1 h. 1 μg of anti-c-Jun, anti-STAT-3, anti-ELK-1 antibody, or normal mouse/rabbit immunoglobulin G was added, and protein complexes were gradually washed with buffers with different salt concentrations. The antibody suppliers and catalog numbers are stated in the supplementary Table 1. After the washing step, the protein/DNA complexes' crosslinking was undone. Subsequently, DNA purification was performed utilizing a spin column, followed by qPCR analysis [29]. Immunoprecipitated DNA (IP) and input DNA were subjected to 35 cycles of PCR amplification with the specific primers indicated in Supp Table 2. A corrected input value was obtained by reducing the input Ct value by 1% on a logarithmic scale. The ΔCt value was calculated by subtracting the IP Ct value from the corrected input value. The percentage of the input value was calculated using the formula 100*2^(ΔCt). The fold change was computed as the percentage of the input value divided by the percentage of the input value determined as a negative control (IgG). The negative control is considered a fold and assigned a value of 1 [30].

The mRNA Expression of ADAMTS-8 in Colorectal Adenocarcinoma (COAD) Versus Normal Tissues

Colon Adenocarcinoma (TCGA-COAD) tumor data and Genotype-Tissue Expression (GTEx) project normal colon tissue data were used to compare ADAMTS8 gene expression levels in normal and tumor colon tissues. Data analysis was performed using the web-based platform Gene Expression Profiling Interactive Analysis 2 (GEPIA2) tool (http://gepia2.cancer-pku.cn/#analysis, accessed on 24 July 2025) [31].

The expression of the ADAMTS8 gene was analyzed using the "Box Plot" module in the GEPIA2 interface. The default parameters ($|Log_2FC|$ Cutoff = 1, p-value Cutoff = 0.01) were retained for statistical analysis. Expression data were presented in log2(TPM + 1) format. The "Match TCGA normal and GTEx data" option was selected in the study to enable direct comparison between TCGA tumor samples and GTEx normal tissue samples. GEPIA2 automatically generated graphical outputs. A t-test-based statistical evaluation of the ADAMTS8 expression difference between tumor and normal tissue groups was performed by the GEPIA2 algorithm, yielding a significance level of $p < 0.001$. The data points in the figure represent individual samples, while the boxes indicate the median and interquartile range. Significant differences are marked with a red asterisk (*).

Survival Analysis Related To ADAMTS-8 Expression in COAD

To evaluate the prognostic effect of ADAMTS8 gene expression on overall survival in patients with colorectal adenocarcinoma (COAD), analyses were performed using the GEPIA2 (Gene Expression Profiling Interactive Analysis 2) platform [31].

Based on RNA sequencing data from the TCGA (Cancer Genome Atlas) project, patients were divided into high and low expression groups using a median value-based threshold based on ADAMTS8 expression levels in tumor tissues. Survival curves were constructed using the Kaplan–Meier method, differences between groups were evaluated using the log-rank test, and hazard ratios (HR) were calculated using the Cox regression model with 95% confidence intervals. Analyses were performed using overall survival data with a follow-up period of 150 months (approximately 12.5 years), and confidence intervals were shown as dashed lines in the graphs. All analyses were conducted using the GEPIA2 web interface (http://gepia2.cancer-pku.cn/#survival, accessed on July 24, 2025) [31].

Interaction Analysis of ADAMTS-8 with Inflammation-Related Genetic Mechanisms

Correlation analysis was performed to evaluate the relationship between ADAMTS8 gene expression and TNF gene expression using the "Gene_Corr" module of the TIMER 2.0 (Tumor Immune Estimation Resource) platform. This analysis calculated the Spearman correlation coefficient for numerous cancer types in the TCGA dataset, and statistical significance ($p < 0.05$) was considered. The correlation between the expression levels of TNF and ADAMTS8 genes for each tumor type was evaluated using Spearman's ρ (rho) coefficient; the results were classified as positive (blue), negative (red), or insignificant (gray) according to the direction of the correlation. The analyses were performed using the TIMER 2.0 web interface (http://timer.cistrome.org/, accessed on August 1, 2025) [32].

Construction of shADAMTS-8 Expression Plasmid and Transfection

shRNA sequences targeting human ADAMTS-8 were designed using an online RNAi design tool. Oligonucleotides containing the shRNA hairpin sequence and restriction enzyme overhangs were synthesized (Macrogen, Korea). The annealed oligonucleotides were ligated into the pLKO.1-puro vector (Addgene) digested with AgeI and EcoRI, following the manufacturer's protocol. The ligation products were transformed into *E. coli* DH5α competent cells, and positive clones were screened by restriction digestion. pLKO.1-ADAMTS-8 plasmid was transfected using Lipofectamine 2000 (Thermo Fisher Sci.) following the manufacturer's protocol. QRT-PCR confirmed knockdown efficiency.

Results

TNF-α Upregulates ADAMTS-8 Gene Expression

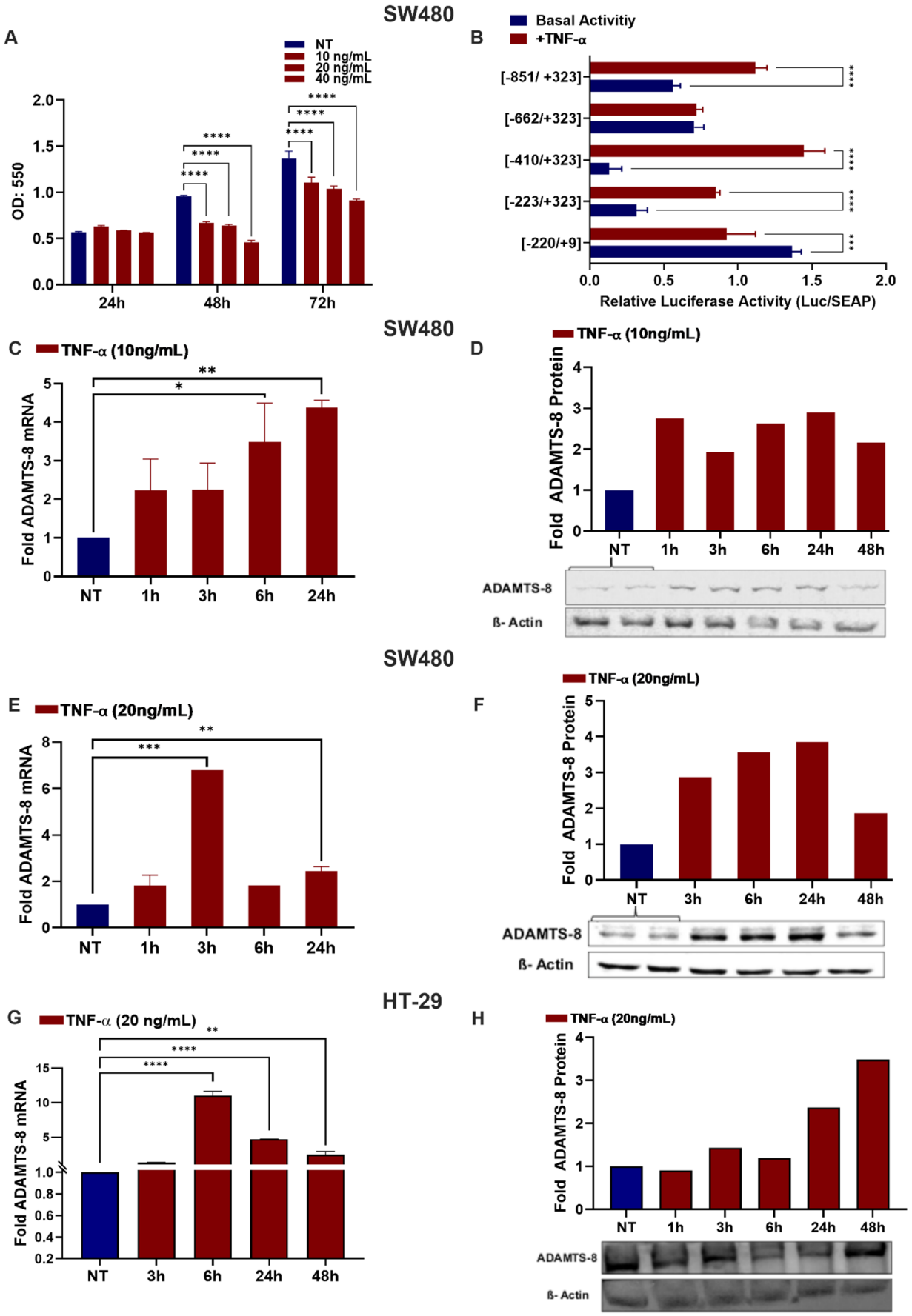
The cell proliferative effect of the TNF-α cytokine in SW480 was evaluated by MTT assay. At 24, 48, and 72 h, three TNF-α concentrations—10, 20, and 40 ng/mL—were administered. Untreated SW480 cells were used as controls. There is no statistically significant difference in SW480 cells for 24 h incubation. Whereas, for 48 and 72 h, TNF-α reduced SW480 cell proliferation in a statistically significant manner at all doses (Fig. 1A).

To investigate whether TNF-α affects ADAMTS-8 transcriptional activity, the full-length and four truncated promoter constructs of ADAMTS-8 were transiently transfected into SW480 cells. After 48 h of transfection, 20 ng/mL of TNF-α was treated for a further 24 h, and reporter activities were determined. The basal activities of ADAMTS-8 promoter fragments were compared with the TNF-α-treated promoter activity of all constructs. We determined TNF-α-mediated up-regulation of ADAMTS-8 for pMET_TS-8[-223/+323], pMET_TS-8 [-410/+323], and pMET_TS-8 [-851/+323] promoter fragments (Fig. 1B, Supp. Figure 1). These results suggest that TNF-α-activated transcription factors could bind to these ADAMTS-8 promoter regions. To investigate the effect of TNF-α on ADAMTS-8 expression at the mRNA level, total RNA was isolated from SW480 cells treated with 10 ng/mL and 20 ng/mL of TNF-α cytokine for 1, 3, 6, 24, and 48 h. The results revealed a significant increase in ADAMTS-8 expression at 10 ng/mL of TNF-α, particularly at 6 and 24 h. Likewise, an increase in the expression of ADAMTS-8 was observed in the mRNA expression of SW480 cells treated with 20 ng/mL TNF-α in 1, 3, 6, and up to 24 h compared to the control group (Fig. 1C). Also, TNF-α-mediated ADAMTS-8 expression at both 10 ng/mL and 20 ng/mL was induced at the protein level using Western blot. Induction at the ADAMTS-8 protein level was determined at both doses at all time points (Fig. 1D and F). The regulatory effect of TNF-α on ADAMTS-8 mRNA and protein expression was also investigated in HT-29 cells. Treatment with TNF-α (20 ng/mL) led to an upregulation of ADAMTS-8 mRNA expression beginning at 3 h post-incubation, with the highest levels observed at 6 h and sustained up to 48 h. The induction of ADAMTS-8 protein expression by TNF-α was predominantly evident at 24 and 48 h of incubation (Fig. 1G and H).

Intracellular Signaling Pathways Regulating TNF-α-mediated ADAMTS-8 Expression

Intracellular pathway analyses were performed to determine which intracellular pathway is activated by TNF-α administration. TNF-α is widely known to activate gene expression via the NF-κB signaling pathway. However, it has been suggested that not only NF-κB but also other intracellular pathways contribute to TNF-α signaling in different cancers. In SW480 cells, activation of NF-κB, PI3K, p38/MAPK, and SAPK/JNK cell pathways was investigated at 5, 15, and 30 min after 20 ng/mL TNF-α treatment. It was determined that NF-κB, PI3K, and SAPK/JNK pathways were phosphorylated and activated after TNF-α treatment in SW480 cells (Fig. 2A). The p38/MAPK pathway was limitedly phosphorylated upon TNF-α stimulation. In SW480 cells, the p65 subunit of NF-κB, the p55 subunit of PI3K, and the p54 subunit of the SAPK/JNK pathway were phosphorylated at the end of 15 min, 5 min, and 15 min of stimulation with TNF-α cytokine, respectively (Fig. 2A). These results suggest that TNF-α treatment activates all tested intracellular pathways, mainly NF-κB, PI3K, and SAPK/JNK pathways.

We evaluated the impact of several signaling pathway inhibitors on ADAMTS-8 mRNA and protein expression to investigate the signaling pathways via which TNF-α triggers ADAMTS-8 expression. SW480 cells were pre-treated with PD1 (PD169316), PD9 (PD98059), SP6 (SP600125), WORT (Wortmannin), and BAY (BAY11-7082) chemical inhibitors for p38/MAPK, MEK, JNK, PI3K, and NF-κB, respectively. Subsequently, cells were stimulated with TNF-α for 6 h, after which protein levels were measured using Western blot analysis. Specific inhibitors of p38/MAPK, MEK, JNK, PI3K, or NF-κB pathways significantly reduced TNF-induced ADAMTS-8 protein levels, indicating the role of these pathways upon the TNF stimulation (Fig. 2B). The expression of ADAMTS-8 was increased by the addition of TNF-α, and its mRNA expression was significantly suppressed by inhibitors of p38/MAPK, MEK, JNK,


SW480
A
NT
10 ng/mL
20 ng/mL
40 ng/mL
OD: 550
24h
48h
72h
B
Basal Activitiy
+TNF-α
[-851/ +323]
[-662/+323]
[-410/+323]
[-223/+323]
[-220/+9]
Relative Luciferase Activity (Luc/SEAP)
SW480
C
TNF-α (10ng/mL)
Fold ADAMTS-8 mRNA
NT
1h
3h
6h
24h
D
TNF-α (10ng/mL)
Fold ADAMTS-8 Protein
ADAMTS-8
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SW480
E
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Fold ADAMTS-8 Protein
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HT-29
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TNF-α (20 ng/mL)
Fold ADAMTS-8 mRNA
H
TNF-α (20ng/mL)
Fold ADAMTS-8 Protein
ADAMTS-8
ß- Actin

Fig. 1 TNF-α upregulates ADAMTS-8 expression in a dose-dependent manner. (**A**) The MTT assay was used to evaluate cell viability. After an hour of serum starvation in 0.1% BSA, SW480 cells were treated for 24, 48, and 72 h with different TNF-α concentrations (10, 20, and 40 ng/mL). Untreated cells were used as a control. Statistical analysis was performed with ANOVA (one-way). **** represents $p > 0.001$. (**B**) Transient-transfection experiments were carried out with ADAMTS-8 promoter reporter constructs, pMET_TS-8[−220/+9], pMET_TS-8[−223/+323], pMET_TS-8[−410/+323], and pMET_TS-8[−662/+323] and pMET_TS-8[−851/+323]. After one hour of serum starvation in 0.1% BSA, the transfected cells were treated with 20 ng/mL TNF-α for 24 h. Untreated cells served as a control to indicate basal activity. The data represent the mean of three independent values ± SD. **(C) and (E)** Time- and dose-dependent mRNA expression change of TNF-α-mediated ADAMTS-8. After the cells were serum-starved with 0.1% BSA for one hour, they were subjected to TNF-α stimulation at 10 ng/mL (Fig. 1C) and 20 ng/mL for 1, 3, 6, and 24 h (Fig. 1E). The non-stimulated group (non-treated) was considered the control. qRT-PCR was performed with ADAMTS-8 primers and β-2 M primers. Data are expressed as the mean of three independent replicates ± SD. **(D) and (F)** The protein level of ADAMTS-8 was measured by Western blotting at various time intervals: 1 h, 3 h, 6 h, 24 h, and 48 h. with 10 ng/mL (Fig. 1D) and 20 ng/mL TNF-α (Fig. 1F). The resulting bands was analyzed by Image J (NIH) and normalized with β-actin values

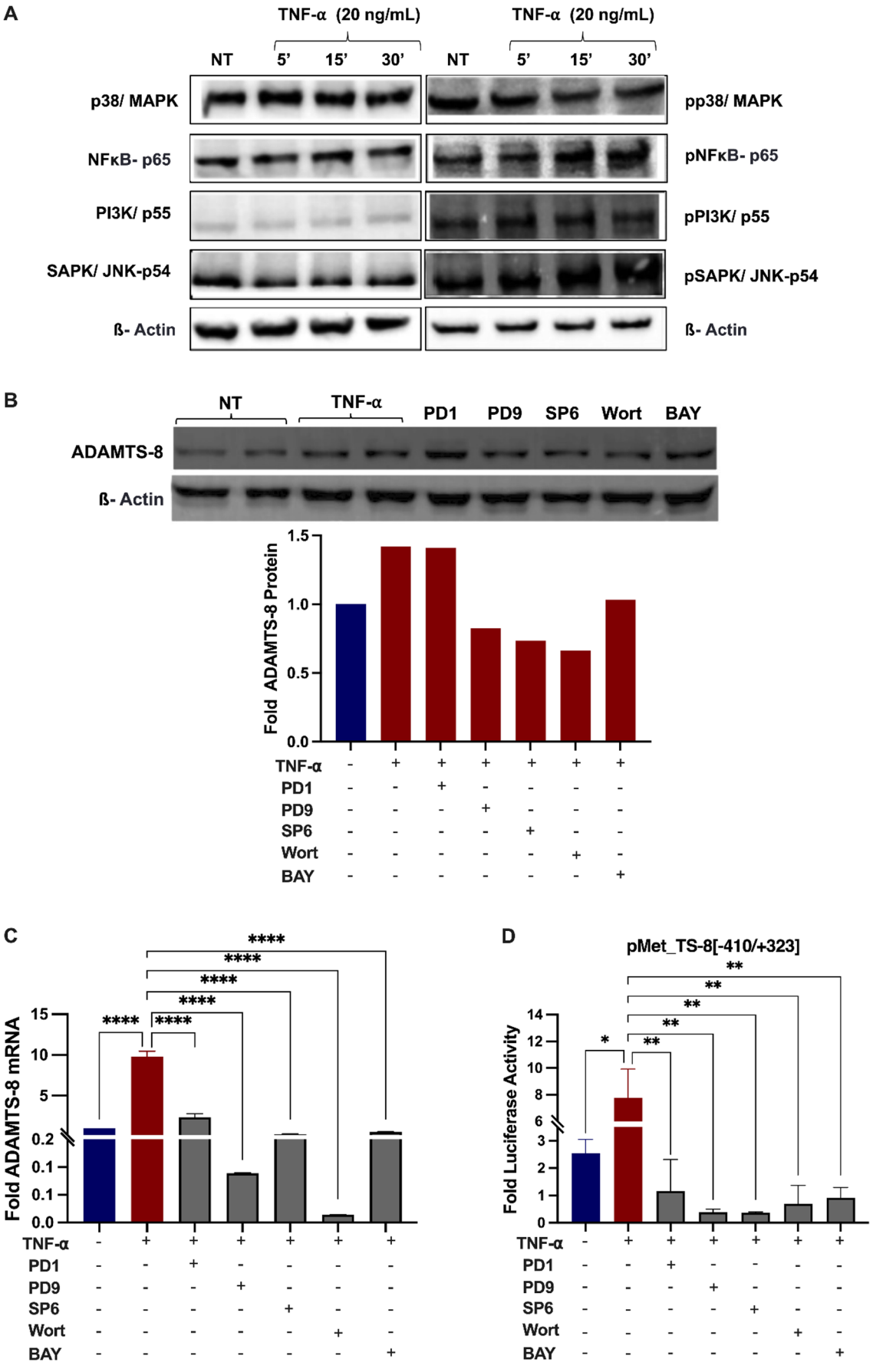
PI3K or NF-κB activity. (Fig. 2C). When TNF-α activates intracellular pathways, the last target protein is a transcription factor that controls gene expression. TNF-α treatment induced ADAMTS-8 transcriptional activity through different truncated promoter fragments. Accordingly, the promoter fragments affected by TNF-α stimulation are pMET_TS-8 [-223/+323], pMET_TS-8 [-410/+323], and pMET_TS-8 [-851/+323] (Fig. 1B). Therefore, pMET_TS-8 [-410/+323], the promoter fragment of ADAMTS-8 that is most affected by TNF-α, was used in pathway inhibition studies. The promoter construct pMet_TS-8_-410/+323 was transiently transfected into SW480. After serum starvation with 0.1% BSA, specific pathway inhibitors were used to elucidate the target TF binding sites activated by TNF-α mediated pathway. Similarly, p38/MAPK, MEK, JNK, PI3K, or NF-κB signaling pathways abolished transcriptional activation. This suggests that a signaling pathway stimulated by TNF-α in this region is the binding site of the target transcription factor or factors.

NFκB, STAT-3, ELK-1, c-Jun and ATF-1 Binds and Regulates TNF-α -mediated ADAMTS-8 Transcriptional Activity

We predicted the possible TNF-responsive transcription factors in the ADAMTS-8 promoter region using the allgen database to confirm further whether these TFs might control ADAMTS-8 transcriptional expression by directly binding to its promoter region [33]. The ADAMTS-8 promoter [-851/+323] region was analyzed in silico for the binding sites of transcription factors NFκB, STAT-3, ELK-1, c-Jun, and ATF-1 (Supp Fig. 2). The actual binding was confirmed by ChIP assay.

ChIP assay was performed in TNF-α-treated SW480 cells by immunoprecipitation with antibodies against NFκB, STAT-3, ELK-1, c-Jun, and ATF-1 and compared with IgG-precipitated samples as a control. Figure 2A illustrates that the phosphorylation levels of p-JNK1/2, p-ERK1/2, and p-p38 were markedly elevated in TNF-α-treated cells in a time-dependent manner. One of the main transcription factors activated by TNF-α is the NFκB transcription factor. NFκB transcription factors have three potential consensus sites in the promoter regions located in the ChIP1, ChIP4, and ChIP6 regions. However, the ChIP assay showed that the NFκB transcription factor could only bind to the site in the ChIP1 region as a result of TNF-α treatment (Fig. 3A). STAT3, another transcription factor activated in inflammation processes, was also examined by ChIP assays for its contribution to TNF-α activated ADAMTS-8 transcriptional regulation. Numerous STAT binding sites belonging to different STAT members (STAT1, STAT3, STAT4, STAT5, and STAT6) were found in all ChIP sites examined along the ADAMTS-8 promoter. All potential binding sites of all STAT members were analyzed for STAT binding. This is because STAT family members can bind to consensus regions as homodimers with themselves and as heterodimers between members of the same family. However, despite many of the STAT sites, only ChIP2, ChIP3, and ChIP4 sites are bound by STAT transcription factors (Fig. 3B). There are three types of serine-threonine kinases known as mitogen-activated protein kinases (MAPKs) in mammals: p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinases 1 and 2 (ERK1/2). It has been shown that p38 activation influences the regulation of macrophage lipid production and plaque development [34]. MAPKs can phosphorylate and activate ETS-like transcription factor 1 (Elk-1), which belongs to the ETS domain family [35]. Multiple putative ELK-1 binding sites in the ADAMTS-8 promoter suggest that the ADAMTS-8 promoter may be a gene regulated by p38, one of the mitogen-activated protein kinases. After TNF-α treatment, ELK-1 was found to bind to consensus binding elements in ChIP1, ChIP2, ChIP3, ChIP4, and ChIP6 regions of the ADAMTS-8 promoter (Fig. 3C).,

Examples of classical MAPKs that have a range of effects on the control of the cancer cell cycle and the induction of apoptosis include ERK1/2, JNK-1/2/3, and p38-α/β/γ/δ. According to some research, JNKs have pro-oncogenic qualities, but other studies show that they have tumor-suppressive effects. It has been demonstrated that JNK controls apoptosis by encouraging c-Jun phosphorylation [36]. C-jun transcription factor can bind to ChIP2, ChIP3, and ChIP4 sites upon TNF-α stimulation (Fig. 3D).


A
TNF-α (20 ng/mL)
TNF-α (20 ng/mL)
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NFκB- p65
pNFκB- p65
PI3K/ p55
pPI3K/ p55
SAPK/ JNK-p54
pSAPK/ JNK-p54
ß- Actin
ß- Actin
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NT
TNF-α
PD1
PD9
SP6
Wort
BAY
ADAMTS-8
ß- Actin
Fold ADAMTS-8 Protein
1.5
1.0
0.5
0.0
TNF-α
PD1
PD9
SP6
Wort
BAY
C
Fold ADAMTS-8 mRNA
15
10
5
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TNF-α
PD1
PD9
SP6
Wort
BAY
D
pMet_TS-8[-410/+323]
Fold Luciferase Activity
14
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1
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TNF-α
PD1
PD9
SP6
Wort
BAY

Fig. 2 Intracellular signaling pathways regulating TNF-α-mediated ADAMTS-8 expression (**A**) TNF-α was applied to SW480 cells for 5, 15, and 30 min after serum-starvation for 1 h. The impact of TNF-α therapy on prospective pathway inhibitors implicated in intracellular signal transduction was assessed using Western blot analysis. NT represents the non-treated control group. (**B**) SW480 cells were serum-starved for 1 h before TNF-α stimulation and treated with specific inhibitors. TNF-α upregulates ADAMTS-8 expression in a dose-dependent manner. (A) Cell viability was assessed using the MTT assay. Western blotting was used to quantify the bands of ADAMTS-8 proteins, which were then adjusted to levels of β-actin. Image J (NIH) was used to evaluate each band. (**C**) SW480 cells were treated for 6 h with specific inhibitors, namely PD1, PD9, SP6, WORT, and BAY, before stimulation with TNF-α. Then, after RNA extraction, ADAMTS-8 mRNA expression was determined by qRT-PCR. Results are the mean ± SE for the triplicate experiment (Fig. 2C). (**D**) For pathway analysis on TNF-α-mediated ADAMTS-8 gene transcriptional activity, pMet_TS-8_-410/+323 was transiently transfected into SW480. After serum starvation with 0.1% BSA, specific PD1, PD9, SP6, WORT, and BAY pathway inhibitors were applied to cells. After 6 h of TNF-α treatment, ADAMTS-8 promoter activities were determined by Luciferase and SEAP activities (Fig. 2D). For calculation, fold changes were determined by comparing the groups indicated in the graph. These experiments were carried out in 3 repetitions. Statistical analyses were performed with one-way ANOVA in the Graphpad program. (p values: < 0.05 (*), < 0.01 (**)

The ATF/CREB family of bZIP transcription factors includes activating transcription factor 1 (ATF1). It mediates transcriptional responses to various extracellular signals, maintains cell viability, and regulates cell transformation. Moreover, ATF1 contributes significantly to the development and spread of tumors in colorectal cancer (CRC), with high expression levels serving as an indicator of poor prognosis for patients [37]. ATF1 functions as a key driver by activating some target genes related to apoptosis and the Wnt, MAPK, and TGF-β pathways, which enhance the risk of developing colorectal cancer. Although the ATF-1 transcription factor has binding motifs in ChIP2, ChIP3, and ChIP4 regions, it was shown by ChIP assay that it binds only to potential binding sites in ChIP3 and ChIP4 regions (Fig. 3E).

ADAMTS-8 Expression Correlates with TNF-Mediated Inflammatory Signaling and Prognostic Outcomes in Colorectal Adenocarcinoma (COAD)

We analyzed ADAMTS-8 expression in Colorectal Adenocarcinoma (COAD) from RNA-sequencing data from the TCGA dataset accessed via the GEPIA2 platform (http://gepia2.cancer-pku.cn/, accessed on 24 July 2025) [31]. In patients with colorectal adenocarcinoma (COAD), the expression level of the *ADAMTS-8* gene in tumor tissues was observed to be significantly increased compared to normal colon tissues (Fig. 4A). According to the Kaplan-Meier survival analysis (http://gepia2.cancer-pku.cn/#survival, accessed on July 24, 2025) [31]. ADAMTS was conducted to evaluate the relationship between overall survival and ADAMTS-8 expression level. The survival time was longer in COAD patients with high ADAMTS-8 expression compared to the low expression group (Fig. 4B). This difference was statistically borderline significant (HR = 0.62; $p = 0.057$; log-rank test), suggesting that ADAMTS-8 may be a gene with prognostic potential. In addition, Spearman correlation analysis (https://timer.cistrome.org/, accessed on July 24, 2025) [32], conducted to evaluate the interaction of ADAMTS-8 with inflammation-related genetic mechanisms, revealed a positive and significant correlation between ADAMTS-8 and TNF genes in COAD patients ($\rho = 0.165$, $p < 0.05$; Fig. 4C). TNF is an important cytokine that regulates pro-inflammatory signaling in the tumor microenvironment and is thought to play a role in inflammation-related pathological processes, along with ADAMTS-8 in CRC. Within the scope of this study, in silico analyses based on patient tissues were performed to evaluate the relationship between ADAMTS-8 and TNF and its prognostic potential in a clinical context. These analyses provide preliminary data for identifying biomarker candidates that could guide future clinical studies.

ADAMTS8 Depletion Modulates TNF-α–Mediated Proliferation in Colorectal Cells

We investigated the effect of the ADAMTS-8 silencing on SW480 cell proliferation with/without TNF-α (20 ng/mL) stimulation. Knockdown of the ADAMTS-8 was confirmed by qRT-PCR (Fig. 5A). TNF-α treatment alone did not induce a statistically significant change compared with the non-treated group (NT) ($p > 0.05$). Knockdown of the ADAMTS-8 led to a substantial increase in the SW480 cell proliferation ($p < 0.001$). Cell proliferation reduced with TNF-α treatment in the ADAMTS-8 knockdown group, yet remained significantly higher than NT (non-treated group) ($p < 0.001$). These findings indicated that silencing ADAMTS-8 enhances cell proliferation, while TNF-α partially suppresses this effect (Fig. 5B).

Discussion

ADAMTS proteases are involved in various pathological conditions, such as cancer metastasis, inflammatory disorders, respiratory diseases, and cardiovascular diseases. They exert their effects by regulating critical processes, including cell proliferation, apoptosis, migration, and the remodeling of the extracellular matrix. It has been observed that ADAMTS-8, known for its anti-angiogenic properties, particularly exhibits decreased expression through promoter methylation in specific cancer types. Reduced expression of

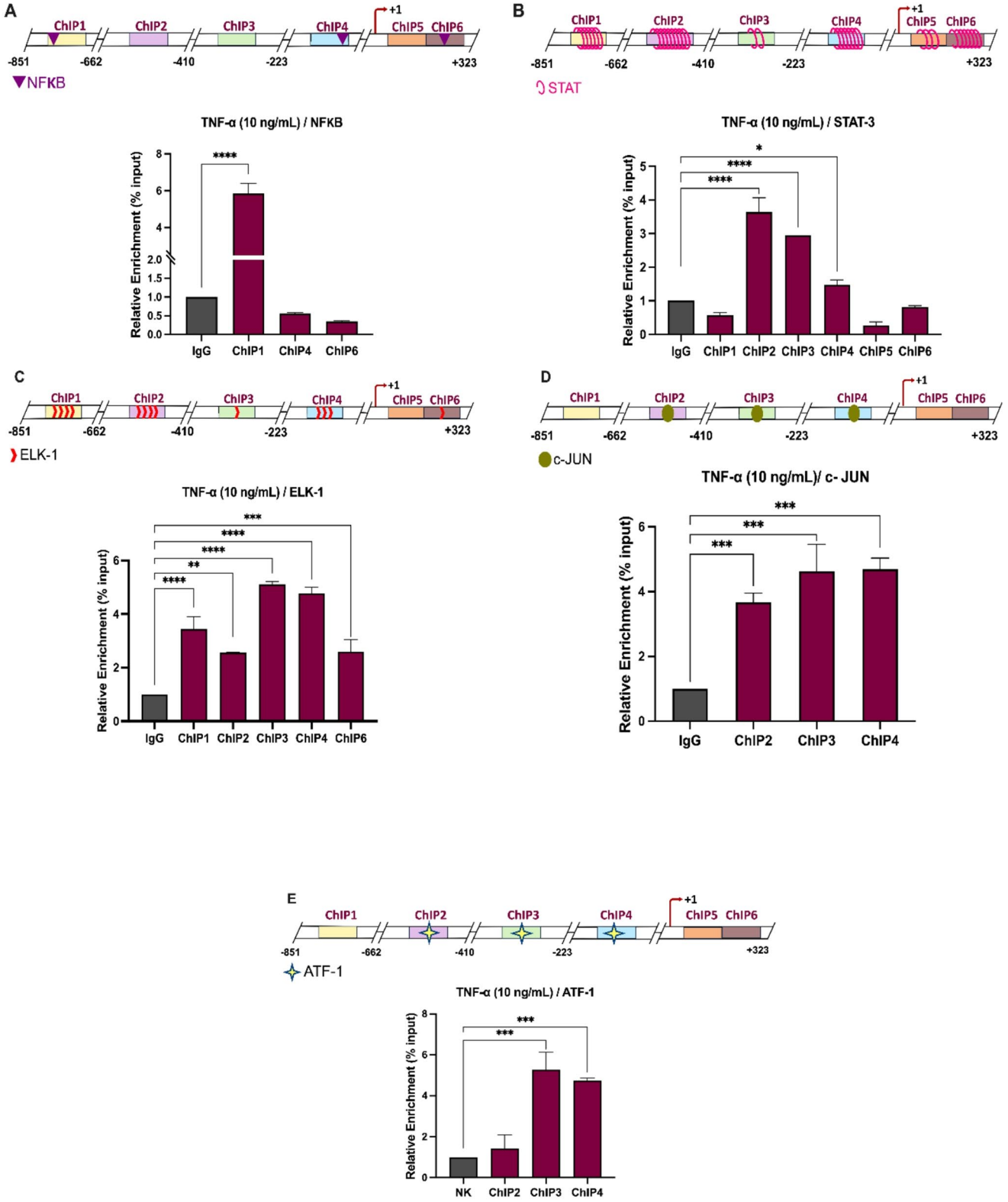

Fig. 3 Diagrammatic illustration of the interaction of NF-κB, STAT-3, ELK-1, c-Jun, and ATF-1 with the ADAMTS-8 promoter and qRT-PCR analysis. SW480 cells were left in medium with 0.1% BSA for 1 h for serum starvation. They were subjected to 10 ng/mL TNF-α stimulation for 24 h. SW480 cells are sonicated, chromatin fixed with formaldehyde, and precipitated with antibodies specific for NFKB (**A**), STAT-3 (**B**), ELK-1 (**C**), c-Jun (**D**), and ATF-1 (**E**). Protein-DNA complexes were precipitated with an IgG-specific antibody as a control. Data are normalized to levels of precipitated chromatin with % input, and error bars represent (±SEM)

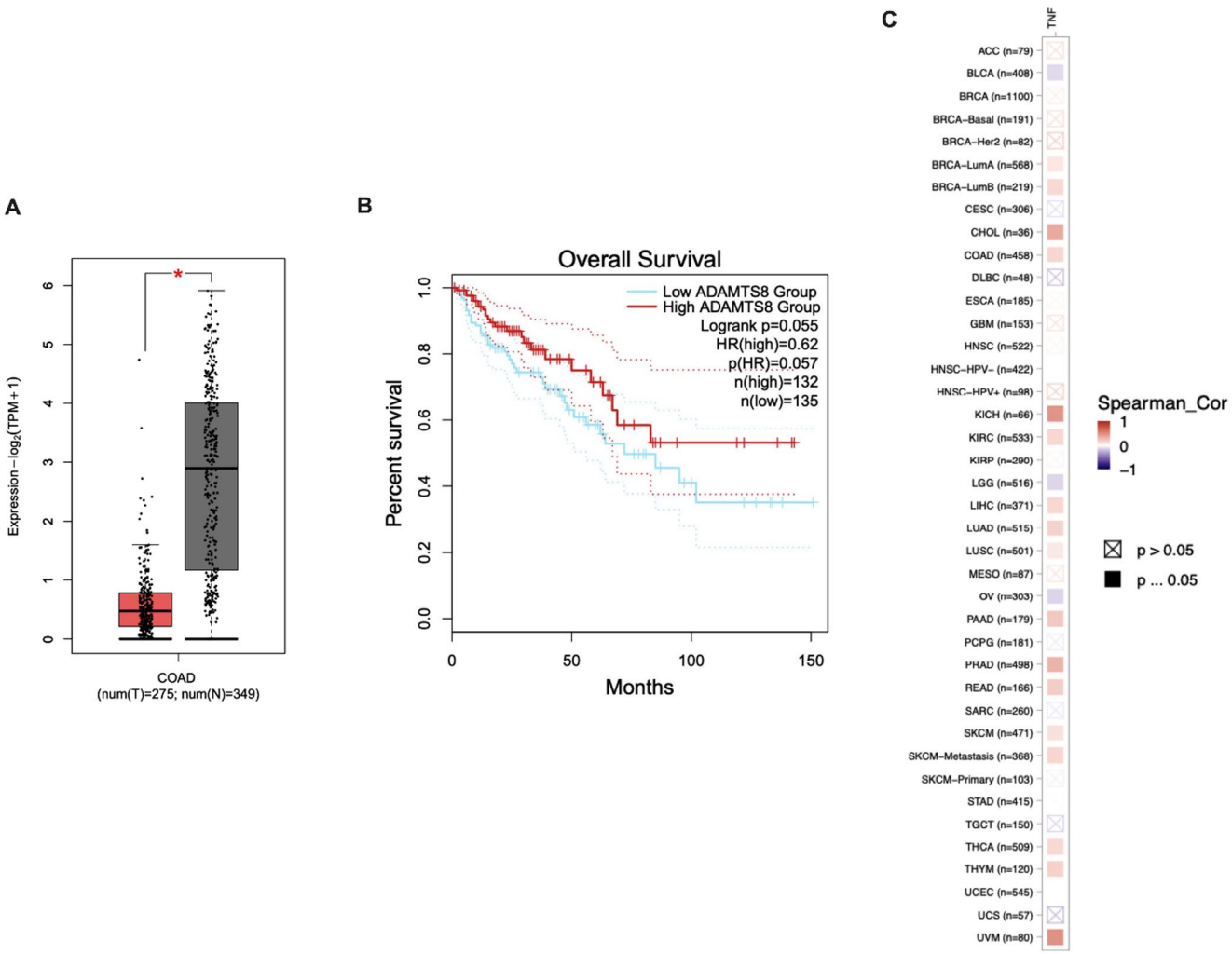

Fig. 4 (**A**) The mRNA expression of ADAMTS-8 Colorectal Adenocarcinoma (COAD) via the Gene Expression Profiling Interactive Analysis2 (GEPIA2) platform (http://gepia2.cancer-pku.cn/, accessed on 24 July 2025) [31]. Data presented as red (tumor) and gray (normal tissue) box-plots for ADAMTS-8.The black dots represent the raw data. Significant differences are shown with asterisks: * $p < 0.05$; T, tumor; N, normal. (**B**) The correlation of *ADAMTS-8* gene expression (low vs. high expression level) with patient overall survival (OS) in COAD. The survival curves were retrieved from the Kaplan–Meier plotter (http://gepia2.cancer-pku.cn/#survival, accessed on July 24, 2025) [31]. (**C**) Interaction of ADAMTS-8 with inflammation-related genetic mechanisms. Spearman correlation analysis was conducted to evaluate the relationship between ADAMTS-8 and TNF genes in COAD patients (https://timer.cistrome.org/, accessed on July 24, 2025) [32]

ADAMTS-8 is frequently linked to the metastasis and invasion of cancer cells [38]. Li and colleagues have also demonstrated that ADAMTS-8 functions as a tumor suppressor, particularly in colorectal cancer.

Inflammation is crucial in tumor formation, progression, and metastasis [39]. The connection between proinflammatory cytokines and colorectal cancer has been demonstrated in numerous studies [40, 41]. Tumor necrosis factor-alpha (TNF-α) contributes to several physiological and pathological processes, including cell proliferation, inflammation, immunological responses, autoimmune reactions, and cell death [42]. Studies have indicated that increased concentrations of TNF-α in the tumor microenvironment promote cancer metastasis and that its expression is elevated in colorectal, skin, breast, ovarian, and prostate cancers [43, 44].

In this study, we found that TNF-α, which is highly significant in colon cancer, also exerts significant inducing effects on the transcription of the ADAMTS-8 gene. Initially, the impact of TNF-α on cell viability in colorectal cancer was assessed using the MTT assay. The TNF-α therapy did not significantly impact the colorectal cancer cell line SW480 cells' ability to proliferate. However, Alotaibi and colleagues showed that TNF-α enhanced cell proliferation in SW480 cells at physiologically relevant doses (1, 10, and 100 pg/mL), but not in HCT116 cells. They attributed

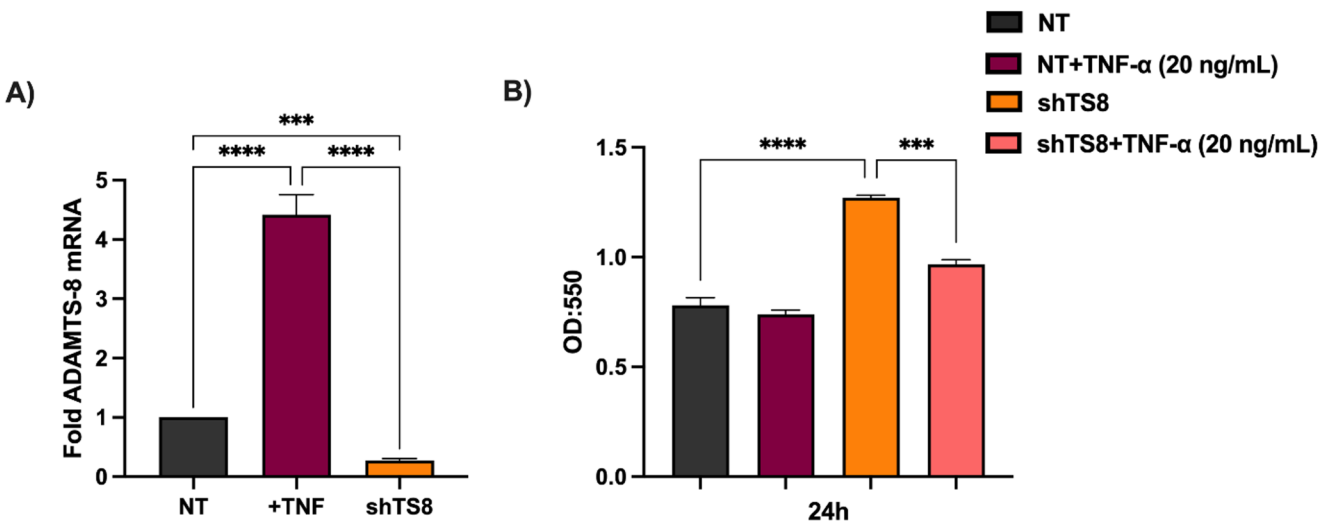

Fig. 5 (**A**) Confirmation of the ADAMTS-8 mRNA expression level after silencing. (**B**) Effect of ADAMTS-8 silencing and TNF-α treatment on SW480 cell proliferation. As indicated, cells were transfected with shADAMTS-8 plasmids and treated with TNF-α (20 ng/mL). Cell proliferation was assessed by MTT. Assays performed at least in triplicate. Statistical analysis was performed using one-way ANOVA (*$p \leq 0.05$, ***$p \leq 0.001$, ****$p \leq 0.0001$)

this differential cellular response to differing metastatic phenotype origins of the two cell types [45].

Gene expression of ADAMTS family members in cancer processes is dysregulated [46]. The expression of ADAMTS-8 in colorectal cancer was found to be silenced through promoter methylation [19]. According to survival analyses, patients with higher levels of ADAMTS-8 expression have a greater chance of survival than those with lower levels [47]. Here, treatment of TNF-α on SW480 and HT-29 cells increased ADAMTS-8 expression at the mRNA and protein levels. Phenotypically, HT-29 cells are derived from a Dukes' B (stage II) primary tumor, show moderate proliferation, whereas SW480 originates from a Dukes' C (stage III) primary tumor with lymph node involvement, exhibits higher proliferation rates, and demonstrates a more mesenchymal morphology and increased metastatic potential in vivo. These molecular and behavioral differences make HT-29 a model for BRAF-driven, differentiated CRC, while SW480 is representative of KRAS-driven, aggressive subtypes, making both valuable in preclinical studies targeting CRC heterogeneity [48–50].

Our in silico analysis of TCGA-COAD data reveals that ADAMTS-8 expression is significantly elevated in colorectal adenocarcinoma tumor tissues compared to normal colon mucosa (Fig. 4A), and that higher expression is associated with a trend toward improved overall survival (HR = 0.62, $p = 0.057$; Fig. 4B). Moreover, we identified a modest but statistically significant positive correlation between ADAMTS-8 and TNF expression ($\rho = 0.165$, $p < 0.05$; Fig. 4C). These observations suggest a complex role for ADAMTS-8 in COAD, potentially integrating inflammatory signaling with patient prognosis.

In vitro silencing of ADAMTS-8 markedly promoted cell growth within 24 h, suggesting that ADAMTS-8 may normally act as a negative regulator of proliferation. TNF-α treatment alone had no significant impact on cell viability at this time point. However, when combined with ADAMTS-8 silencing, TNF-α attenuated the proliferation-enhancing effect, indicating a potential interaction between TNF-α signaling and TS8 pathways.

These results support the hypothesis that TS8 may function as a tumor suppressor-like gene by restraining cell growth. The partial reversal of the proliferative phenotype by TNF-α suggests that inflammatory signaling could influence TS8-dependent mechanisms.

It was reported for the first time that TNF-α induced ADAMTS-8 promoter activity in SW480 cells (Fig. 1D). The activation of NF-κB, which is essential for inflammation and apoptosis, is one of the several ways that TNF-α works [51]. It initiates the promotion of intracellular pathways such as p38/MAPK, JNK, and transcription factors active in these pathways [52]. These factors' pro-inflammatory signaling is a defense mechanism against adverse events like infection [53]. In addition, it activates pro-survival signaling through PI3K and protein kinase B Akt [54]. In a study in SW620 cells, TNF-α cytokine was found to activate p38 MAPK, PI3K/Akt, and NF-κB pathways [55]. In parallel with these findings, we found that p38/MAPK, NF-κB, PI3K, and SAPK/JNK signaling pathways were phosphorylated in SW480 cells stimulated with TNF-α cytokine (Fig. 2a). In addition to these findings, we analyzed the pathways responsible for the increased ADAMTS-8 expression in TNF-α-induced SW480 cells. For this purpose, we determined the altered ADAMTS-8 expression by

applying specific pathway inhibitors for p38/MAPK, p38/MAPK, MEK, NF-κB, PI3K, and JNK. Specific pathway inhibitors significantly decreased ADAMTS-8 mRNA and protein expression. This confirms the activated signaling pathways. In addition, we have investigated the signaling pathways regulating TNF-induced ADAMTS-8 promoter activity. Our results were in line with the mRNA and protein data that TNF-mediated ADAMTS-8 promoter activity reversed the increased activity with specific pathway inhibitors compared to the corresponding controls. When we analyzed the ADAMTS-8 promoter in silico, we observed that it is rich in binding sites for NF-κB, STAT-3, ELK-1, c-Jun, and ATF-1 transcription factors. To confirm the functional binding of these transcription factors, we conducted ChIP analyses by preparing specific primers for the ADAMTS-8 promoter. As shown in Fig. 1B, the TNF-α treatment activates differentially on the transfected different lengths of ADAMTS-8 promoter fragments. pMET_TS-8[-223/+323], pMET_TS-8[-410/+323] and pMET_TS-8[-851/+323] promoter fragments are activated by TNF-α treatment, while pMET_TS-8[-220/+9] and pMET_TS-8[-663/+323] promoter fragments are not responsive to TNF-α. In this context, ChIP and transfection assays were evaluated together, and potential transcription factors were interpreted regarding TNF-α activation.

While pMET_TS-8[-223/+323] is activated by TNF-α, pMET_TS-8[-220/+9] is not activated by TNF-α. While all promoter fragments contain common sequences, the +9 and +323 regions are found in the pMET_TS-8[-223/+323] promoter fragment. The +9 and +323 regions also contain the ChIP5 and ChIP6 regions. Therefore, it is likely that the regions responsible for TNF-activation are also located within the ChIP5 and ChIP6 regions. Among the transcription factors analyzed by ChIP analysis, the ELK-1 binding site is located between +284/+292. It was determined that ELK-1 binds to its consensus sites in the CHIP6 region. This binding contributes to the transcriptional activity mediated by TNF-α. However, there are NFκB and STAT binding sites in the ChIP6 region and STAT binding sites in the ChIP5 region (Supp. Table 3). However, these sites are not functional in TNF-α-mediated transcriptional activation (Fig. 3A and B). ChIP analysis showed that the ChIP4 site in the pMET_TS-8[-223/+323] region binds to the respective consensus regions of NFκB, STAT, ELK-1, c-jun, and ATF-1 and transcription factors (Fig. 3A, B, C and D, and 3E). NFκB and STAT sites in the ChIP4 region were shown to be non-functional, but ELK-1, c-jun and ATF-1 transcription factors were shown to bind to these sites upon TNF-α stimulation.

The promoter fragment with the highest increase in TNF-α is pMET_TS-8 [-410/+323]. This promoter region contains ChIP3, ChIP4, ChIP5 and ChIP6 regions. In the ChIP3 region, STAT, ELK-1, c-jun and ATF-1 binding sites appear to be functionally bound (Supp Table 3) (Fig. 3B, C, D and E). Transfection and reporter assays have shown that TNF-α-mediated transcriptional activity is greatly enhanced by the inclusion of this region. Therefore, the interactions of these transcription factors in the ChIP region are important for TNF-α and ADAMTS-8 transcriptional activation.

pMET_TS-8[-663/+323] promoter region is not activated by TNF-α. However, it was determined by ChIP analysis that STAT, c-Jun, and ELK-1 transcription factors bind to the ChIP2 site in this region. The reason why these transcription factors do not contribute to TNF-α-mediated transcriptional activity despite binding is the presence of repressor transcription factors that may be present in the region.

The largest ADAMTS-8 promoter fragment is pMET_TS-8 [-851/+323]. This region, as well as all ChIP regions, contains the ChIP1 region. NFκB and ELK-1 transcription factors functionally bind to this region (Supp Table 3) (Fig. 3A and C). STAT transcription factors do not bind to this region.

NF-κB activation is typically triggered by cellular stress, infection, or inflammation. Activation of this signaling pathway can occur through the influence of external stimuli like TNF binding [56]. The ADAMTS-8 promoter contains an NF-κB binding site. Pathway inhibition and phosphorylation studies have demonstrated TNF-α mediated activation of the NF-κB pathway. ChIP analysis revealed that NF-κB functionally binds to the [-851/-662] region of the ADAMTS-8 promoter, correlating with the in silico analysis. This suggests that NF-κB has the potential to regulate ADAMTS-8 transcription during inflammatory processes in colorectal cancer. A number of growth factors and inflammatory cytokines activate the crucial JAK-STAT signaling pathway. JAK and its downstream transcription factor, STAT, are phosphorylated when cytokines attach to their receptor at the cell surface and cause the receptor to dimerize. After dimerizing and moving to the nucleus, phosphorylated STAT attaches itself to the promoters of its target genes to create enhanceosomes. With the aid of other transcriptional coactivators, these enhanceosomes initiate transcription [57]. Numerous physiological functions, including inflammation, cell survival, and proliferation, are mediated by STAT3 [58]. Under TNF-α treatment, we substantiated through ChIP analysis the binding of the STAT-3 transcription factor to the [-662/-410] and [-223/+1] regions of the ADAMTS-8 promoter, which boasts numerous potential binding sites.

ELK-1 is a significant transcription factor functioning at the terminal stage of the MAPK pathway and is a member of the ETS family [59]. The ADAMTS-8 promoter contains numerous potential ELK-1 binding sites. Following TNF-α

treatment, pathway inhibition studies have demonstrated the activation of the P38/MAPK pathway. Through ChIP analysis, we found that the ADAMTS-8 promoter is notably enriched with the ELK-1 transcription factor.

Jun proteins can form heterodimers with Fos proteins and ATF members, while also capable of forming homodimers. These transcription factors facilitate each other's binding and indirectly regulate transcription, aside from binding to similar regions [60, 61]. Our bioinformatics analyses revealed that the [-637/-503], [-353/-234], and [-87/+38] regions of the ADAMTS-8 promoter contain c-Jun binding sites. However, ChIP analysis demonstrated functional binding of c-Jun to all identified regions of the ADAMTS-8 promoter under TNF-α treatment. This suggests that binding at the [-687/-792], [+ 91/+232], and [+ 188/+312] regions may occur indirectly.

ATF-1 is a response element present in the PI3K and MAPK signaling pathways [62]. Through bioinformatics analysis, we identified ATF's binding to the [-662/-410] and [-223/+1] regions of the ADAMTS-8 promoter. However, in the ChIP analysis conducted to determine functional bindings, we observed that ATF-1 was intensively bound to other regions of the ADAMTS-8 promoter [687/792], [353/234], [+ 91/+232], [+ 188/+312].

In summary, TNF-α upregulates ADAMTS-8 mRNA and protein expressions as well as the transcriptional activity of its promoter. In addition, TNF-α activates p38/MAPK, NF-κB, PI3K, SAPK/JNK parallel signaling pathways in SW480 cells. With inhibitor studies, the transcriptional activity of the promoter was examined in addition to the mRNA, protein level, where p38/MAPK, MEK, JNK, PI3K, NF-κB are critical in the upregulation of ADAMTS-8. In particular, the use of p38/MAPK, MEK, JNK, PI3K and NF-κB inhibitors caused a significant reduction in ADAMTS-8 gene expression. Furthermore, we identified the ADAMTS-8 promoter binding sites of transcription factors such as NF-κB, STAT-3, ELK-1, c-JUN, ATF-1, which are associated with signaling pathways activated in TNF-α-mediated ADAMTS-8 upregulation. The interactions of these transcription factors with TNF-α at the ADAMTS-8 promoter were determined by ChIP assay. ChIP results revealed that NF-κB, STAT-3, ELK-1, c-JUN, ATF-1 were significantly enriched in the ADAMTS-8 promoter and these interactions increased under TNF-α stimulation. These findings suggest that TNF-α acts directly on the promoter region through signaling pathways that regulate ADAMTS-8 expression and thus plays a crucial role in gene regulation of ADAMTS-8. This study underscores the complex interplay between cytokine signaling and gene expression in cancer cells, highlighting the need for further investigation into the role of ADAMTS-8 in the tumor microenvironment and its potential implications in colorectal cancer progression. Clinical validation in independent cohorts should be performed to support the potential biomarker function of ADAMTS-8 in colorectal cancer. Further studies focusing on downstream signaling pathways and longer time points are required to fully elucidate the molecular interactions between ADAMTS-8 and TNF-α in regulating cell proliferation.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics Approval The article does not contain research in which animals or humans were used, so ethical approval is not required for this study.

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