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YY1 positively regulates human UBIAD1 expression

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ABSTRACT

Vitamin K is involved in bone formation and blood coagulation. Natural vitamin K compounds are composed of the plant form phylloquinone (vitamin K_1) and a series of bacterial menaquionones (MK-n; vitamin K_2). Menadione (vitamin K_3) is an artificial vitamin K compound. MK-4 contains 4-isoprenyl as a side group in the 2-methyl-1,4-naphthoquinone common structure and has various bioactivities. UbiA prenyltransferase domain containing 1 (UBIAD1 or TERE1) is the menaquinone-4 biosynthetic enzyme. UBIAD1 transcript expression significantly decreases in patients with prostate carcinoma and over-expressing UBIAD1 inhibits proliferation of a tumour cell line. UBIAD1 mRNA expression is ubiquitous in mouse tissues, and higher UBIAD1 mRNA expression levels are detected in the brain, heart, kidneys and pancreas. Several functions of UBIAD1 have been reported; however, regulation of the human UBIAD1 gene has not been elucidated. Here we report cloning and characterisation of the human UBIAD1 promoter. A 5′ rapid amplification of cDNA ends analysis revealed that the main transcriptional start site was 306 nucleotides upstream of the translation initiation codon. Deletion and mutation analyses revealed the functional importance of the YY1 consensus motif. Electrophoretic gel mobility shift and chromatin immunoprecipitation assays demonstrated that YY1 binds the UBIAD1 promoter *in vitro* and *in vivo*. In addition, YY1 small interfering RNA decreased endogenous UBIAD1 mRNA expression and UBIAD1 conversion activity. These results suggest that YY1 up-regulates UBIAD1 expression and UBIAD1 conversion activity through the UBIAD1 promoter.

1. Introduction

Vitamin K plays important roles in bone formation and blood coagulation. The two molecular forms of natural vitamin K differ in the alkyl side chain at the 3′ position of the 2-methyl-1,4-naphthoquinone common structure (menadione; vitamin K_3). The plant form, called phylloquinone (PK, vitamin K_1), contains a phytyl group as a side chain and the bacterial form menaquinone-n (MK-n) contains a polyisoprenyl group as the side chain [1]. MK-4, which contains 4-isoprenyl as a side group in the 2-methyl-1,4-naphthoquinone common structure, has various bioactivities [2].

UbiA prenyltransferase domain containing 1 (*UBIAD1*) encodes a 36.8 kDa membrane protein of 338 amino acids. UBIAD1 has been localised to chromosome 1p36. The protein is comprised two exons and one intron and is 57% homologous with the *Drosophila* protein *heix* [3]. UBIAD1 is ubiquitously expressed in mouse tissues. In particular, UBIAD1 mRNA expression levels are higher in brain, heart, kidneys and pancreas than in other organs [4]. UBIAD1 is localised to the mitochondria and/or endoplasmic reticulum in different cell types [4,5].

Recently, our group reported that UBIAD1 converts PK and vitamin K_3 to MK-4 in several cell lines. Furthermore, another group reported that UBIAD1 expression levels decrease in prostate carcinoma and that UBIAD1 overexpression suppresses proliferation in a tumour cell line [6]. Thus, several functions of UBIAD1 have been reported. However, regulation of the human *UBIAD1* gene is not understood.

Abbreviations: UBIAD1, UbiA prenyltransferase domain containing 1; YY1, Yin yang-1; EMSA, electrophoretic gel mobility shift; ChIP, chromatin immunoprecipitation; siRNA, small interfering RNA; PK, phylloquinone; MK-n, menaquinone-n; 5′-RACE, 5′-rapid amplification of cDNA ends; PCR, polymerase chain reaction.

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Transcription factor Yin yang-1 (YY1 or NF-E1) is ubiquitously expressed as a zinc finger protein in the GLO Kruppel-related family [7,8]. YY1 binds to the 5′-CAANATGGC-3′ consensus sequence and is involved in repression and activation of several genes that play roles in various biological processes [9]. Furthermore, YY1 positively regulates expression of several oncogenes, including *c-Myc*, *c-Fos* and *transforming growth factor-β* [10–12]. YY1 also positively regulates several tumour suppressor genes, including *death receptor 5*, *p21* and *p16* [13–16]. Moreover, YY1 functions as a negative cell growth regulator by inhibiting c-Myc function [17]. Thus, YY1 regulates both oncogene and tumour suppressor genes through different mechanisms depending on the tissue.

The aim of this study was to clarify regulation of the UBIAD1 gene. We used human osteosarcoma MG-63 and human embryonic kidney 293 (HEK293) cells because MG-63 is a vitamin K-targeted tissue-derived cell line, and HEK293 cells express high levels of UBIAD1. We isolated and characterised the human UBIAD1 promoter with respect to the YY1 transcription factor-binding site that we identified.

2. Materials and methods

2.1. Materials

Restriction enzymes were purchased from Takara Bio Inc. (Shiga, Japan). Foetal bovine serum (FBS) was purchased from Gibco (Grand Island, NY, USA). Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin, trypsin/EDTA and other chemicals were purchased from Nacalai Tesque, Inc. (Kyoto Japan).

2.2. Cell culture

MG-63 and HEK293 cells were maintained in DMEM supplemented with 10% FBS, 100 IU/ml penicillin and 100 μg/ml streptomycin.

2.3. 5′-Rapid amplification of cDNA ends (RACE)

Total RNA from HEK293 cells was prepared using ISOGEN II (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. 5′-RACE was performed using the RLM-GeneRacer kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The amplified fragments were cloned into the pCR4-TOPO vector. Twenty clones were randomly selected for purification and DNA sequencing.

2.4. Plasmid constructs

The UBIAD1 promoter sequences were amplified by polymerase chain reaction (PCR) in a reaction mixture containing human genomic DNA (Clontech, Palo Alto, CA, USA), UBIAD1 primers (Supplementary materials) and KOD FX (Toyobo, Osaka, Japan) to generate the UBIAD1 promoter constructs. The promoter was cloned into the pGL4.10 vector (Promega, Madison, WI, USA). The resulting construct was named pGL4.10 (−3073/+353). A series of UBIAD1 promoter deletion constructs were generated by PCR using oligonucleotides (Supplementary materials). pGL4.10 (−3073/+353) was digested with *Kpn*I and self-ligated to generate pGL4.10 (−1626/+353). The mutation construct for the YY1-binding site was generated by PCR using oligonucleotides (Supplementary materials). The YY1 gene was amplified by PCR in a reaction mixture containing cDNA derived MG-63 total RNA, primers and KOD FX to generate the human YY1 expression construct. All constructs were verified by DNA sequencing.

2.5. Transfection and dual luciferase assay

MG-63 cells were cultured for 24 h in 12-well plates (2×10^5 cells/well) and HEK293 cells were cultured for 24 h in 12-well plates (4×10^5 cells/well). The cells were then incubated with a reaction mixture containing 0.25 μg of the reporter gene construct, 0.1 μg pGL4.74 [hRluc/TK] (Promega) and 0.75 μl Lipofectamine 2000 (Invitrogen). The cells were harvested 24 h later and luciferase activity was determined with a luminometer.

2.6. Electrophoretic gel mobility shift assay (EMSA)

Nuclear extracts were prepared from MG-63 and HEK293 cells using a Nuclear Extract Kit (Active Motif, Carlsbad, CA, USA), according to the manufacturer's instructions. *In vitro* translated YY1 was prepared using the T_NT Quick Coupled Transcription/Translation System (Promega) and 1 μg of the YY1 expression vector. EMSA was performed by incubating 5 μg of nuclear extract in a binding buffer containing 12 mM Hepes-NaOH (pH 7.9), 12% glycerol, 60 mM KCl, 1 mM EDTA, 1 mM DTT, 4 mM Tris–HCl (pH 8.0), 2 μg poly dI-dC (Sigma–Aldrich, St. Louis, MO, USA), 1 μg bovine serum albumin and ^{32}P-labelled oligonucleotides for 30 min at 4 °C. The oligonucleotides were annealed, labelled with T4 polynucleotide kinase and [γ-^{32}P] ATP and purified with G-50 micro columns (GE-Healthcare, Piscataway, NJ, USA) to generate EMSA probes. Excess oligonucleotides (10- or 50-fold) or antibodies were pre-incubated with the nuclear extract for 10 min before adding the oligonucleotide probe for the competition and supershift assays. Antibodies to YY1 and control rabbit IgG used as the control antibody were purchased from Abcam (Cambridge, MA, USA) and Cell Signaling Technology (Danvers, MA, USA), respectively. Gel electrophoresis was conducted at 120 V using 4% native polyacrylamide gels and 0.5 × TBE buffer.

2.7. Chromatin immunoprecipitation (ChIP) assay

The cells were fixed in formalin (final concentration, 1%) at room temperature for 5 min. Fixation was completed after adding glycine (final concentration, 0.125 M) and the incubation was continued for an additional 5 min. The cells were washed twice using ice-cold phosphate-buffered saline and collected. The cell pellets were suspended in LB1 [50 mM Hepes-KOH (pH 7.5), 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100 and protease inhibitor cocktail (PIC; Roche, Manheim, Germany)] and remained on ice for 10 min. After discarding LB1, the pellets were suspended in LB2 [10 mM Tris–HCl (pH 8.0), 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA and PIC] and remained on ice for 5 min. After discarding LB2, the pellets were dissolved in LB3 [10 mM Tris–HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% Na-deoxycholate, 0.5% N-lauroylsarcosine and PIC]. The cell lysates were sheared by sonication and the sheared DNA was analysed on a 2% agarose gel. The DNA–protein complexes were immunoprecipitated with 3 μg anti-YY1 or control rabbit IgG. PCR was performed using the precipitated DNA fragments and primers for the UBIAD1 promoter, which was the YY1 site. PCR products were detected on a 2% agarose gel.

2.8. siRNA-mediated YY1 knockdown

YY1 was silenced by transfecting MG-63 and HEK293 cells with YY1 siRNA (Santa Cruz Biotechnology, Santa Cruz, CA, USA) using Lipofectamine RNAiMAX (Invitrogen) and analysing expression after 48 h. We used the Stealth RNAi negative control duplexes as the control (Invitrogen).

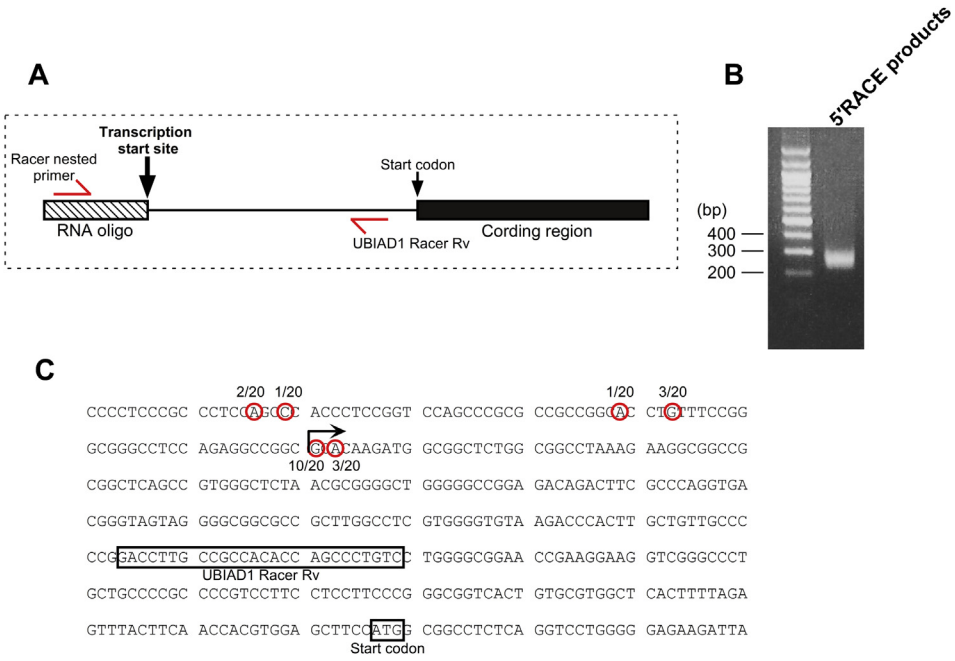

Fig. 1. 5′-Rapid amplification of cDNA ends (RACE) analysis of the human UbiA prenyltransferase domain containing 1 (UBIAD1) gene transcription start site. (A) Schematic representation of the primers used in the 5′-RACE analysis. (B) Ethidium bromide staining of the polymerase chain reaction products on an agarose gel. (C) The 5′-flanking region of the human UBIAD1 coding region and the start codon are shown. Putative transcription start sites are indicated by circled nucleotides. Ratios indicate the number of clones (n = 20). Arrow indicates the main human UBIAD1 gene transcription start site.

2.9. Quantitative real-time RT-PCR

Total RNA was prepared using ISOGEN II, according to the manufacturer's instructions. Total RNA was mixed and converted to cDNA using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo) to generate cDNA. Primers were designed using Primer3Plus and synthesised by Invitrogen. All real-time PCR primer sequences used are listed in Supplementary materials. Real-time PCR was performed on a CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA) using FastStart Universal SYBR Green Master (ROX) (Roche).

2.10. Western blot analysis

MG-63 or HEK293 cells were lysed in buffer containing 25 mM Tris–HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 1% NP-40, 1% Na-deoxycholate, 0.1% SDS and PIC and total protein concentrations were measured using a BCA protein assay reagent kit (Thermo Scientific, Rockford, IL, USA). Lysates were resolved on 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Anti-YY1 and anti-GAPDH HRP-DirecT antibodies were obtained from Medical and Biological Laboratories (Nagoya, Japan).

2.11. UBIAD1 conversion assay

The UBIAD1 conversion assay was performed as previously described (7).

2.12. Bioinformatics analysis

We predicted the UBIAD1 promoter transcription factor-binding sites using the Transcription Element Search System (TESS) website (http://www.cbil.upenn.edu/tess). The genome was aligned at the UBIAD1 locus using the University of California Santa Cruz (UCSC) genome browser (http://genome.ucsc.edu).

2.13. Statistical analysis

Data are expressed as means ± standard errors. Data were analysed using Student's *t*-test. In all cases, P-value <0.05 was considered as statistically significant.

3. Results

3.1. Identification of the human UBIAD1 gene transcription start sites

The human *UBIAD1* gene transcription start site was identified by 5′-RACE analysis using total RNA from HEK293 cells. As shown in Fig. 1A and B, the transcription start site was predicted to be approximately 250 bp from the UBIAD1 Racer primer. PCR products were cloned into the cloning vector. The transcription start site was identified by sequence analysis and mapped to 306 bp (10/20 randomly selected clones), 304 bp (3/20 clones), 334 bp (3/20 clones), 371 bp (2/20 clones), 368 bp (1/20 clones) and 338 bp (1/20 clone) upstream of the start codon (Fig. 1C). The most frequent transcription start site was designated as base pair number +1.

3.2. The YY1-binding sequence is required for UBIAD1 promoter activity

The human UBIAD1 gene 5′-flanking region was identified to analyse the function of its promoter. The typical TATA box sequence was not found at the promoter site. The TESS program predicted binding sites of several transcription factors, including GATA-1, nuclear factor of activated T-cells, cAMP response element binding, CdxA, specificity protein 1 (SP1), activator protein 1 (AP1), YY1, ETS-1 and upstream stimulatory factor (Fig. 2A).

A series of deletion constructs was fused to a luciferase reporter in pGL4.10, and the resulting constructs were transiently transfected into a variety of cells to determine the regulatory elements

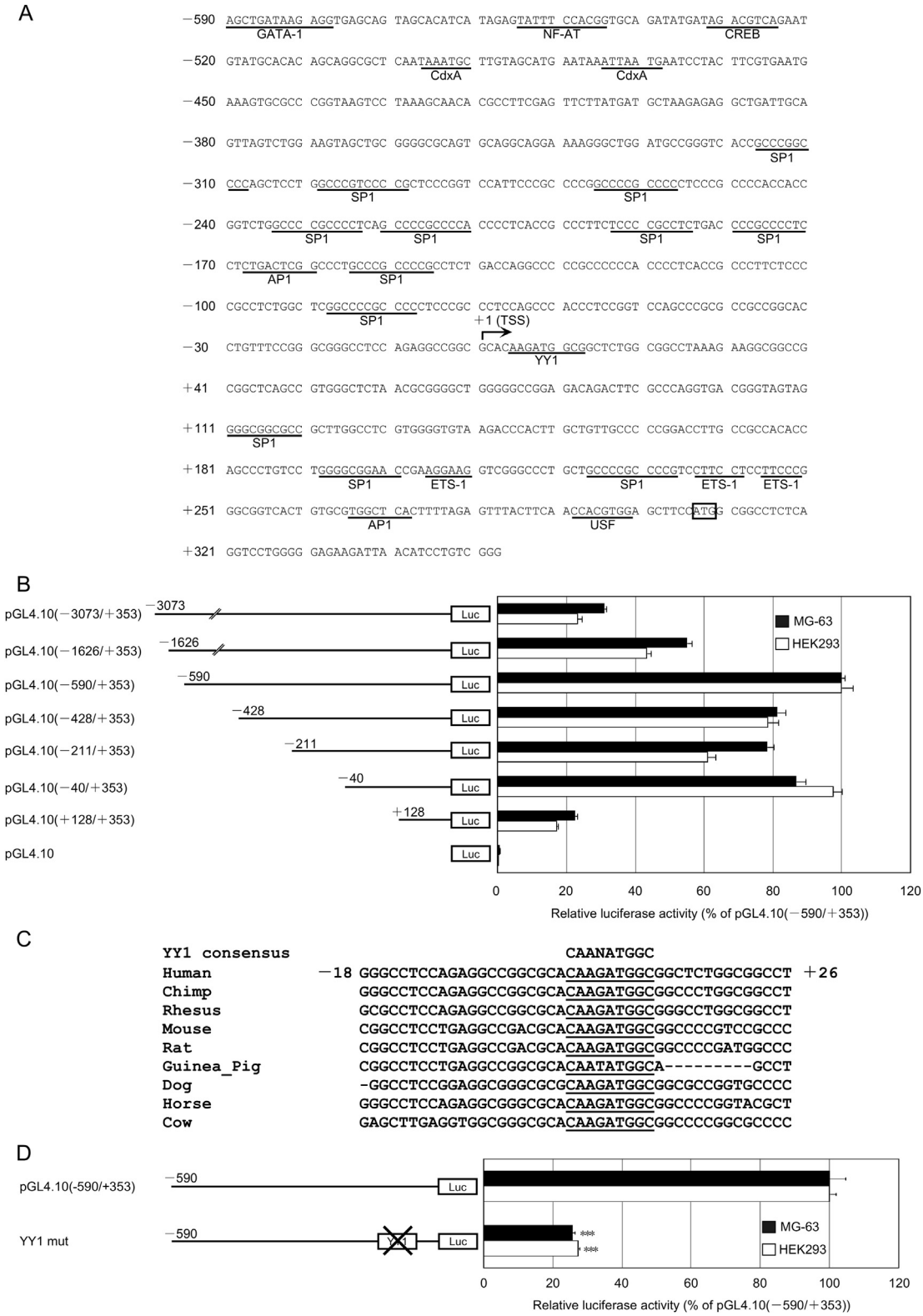

Fig. 2. Deletion and mutation analysis of the human UbiA prenyltransferase domain containing 1 (UBIAD1) promoter. (A) Analysis of the putative transcription factor-binding sites of the human UBIAD1 promoter using the Transcription Element Search System (TESS) website. The putative transcription factor-binding sites are underlined and the names are indicated under the underline. (B) MG-63 and HEK293 cells were transiently cotransfected with luciferase reporter constructs containing the 5′ deletion promoter region of the human UBIAD1 gene and pGL4.74 [hRluc/TK]. Firefly luciferase activity was normalised to *Renilla* luciferase activity. Data represent means ± standard errors of at least three replicates. (C) Alignment of the human UBIAD1 promoter fragments and those of several other species near the transcription start site using the UCSC genome browser. YY1 consensus-binding sequences are underlined. The YY1-binding sites are highly conserved between species. (D) MG-63 and HEK293 cells were transiently co-transfected with luciferase reporter constructs containing the YY1 mutant site promoter region of the human UBIAD1 gene and pGL4.74 [hRluc/TK]. Firefly luciferase activity was normalised to *Renilla* luciferase activity. Data represent means ± standard errors of at least three replicates. Significant differences compared with those of pGL4.10 (−590/+353) were determined using Student's *t*-test and are indicated by asterisks ($^{***}P < 0.001$).

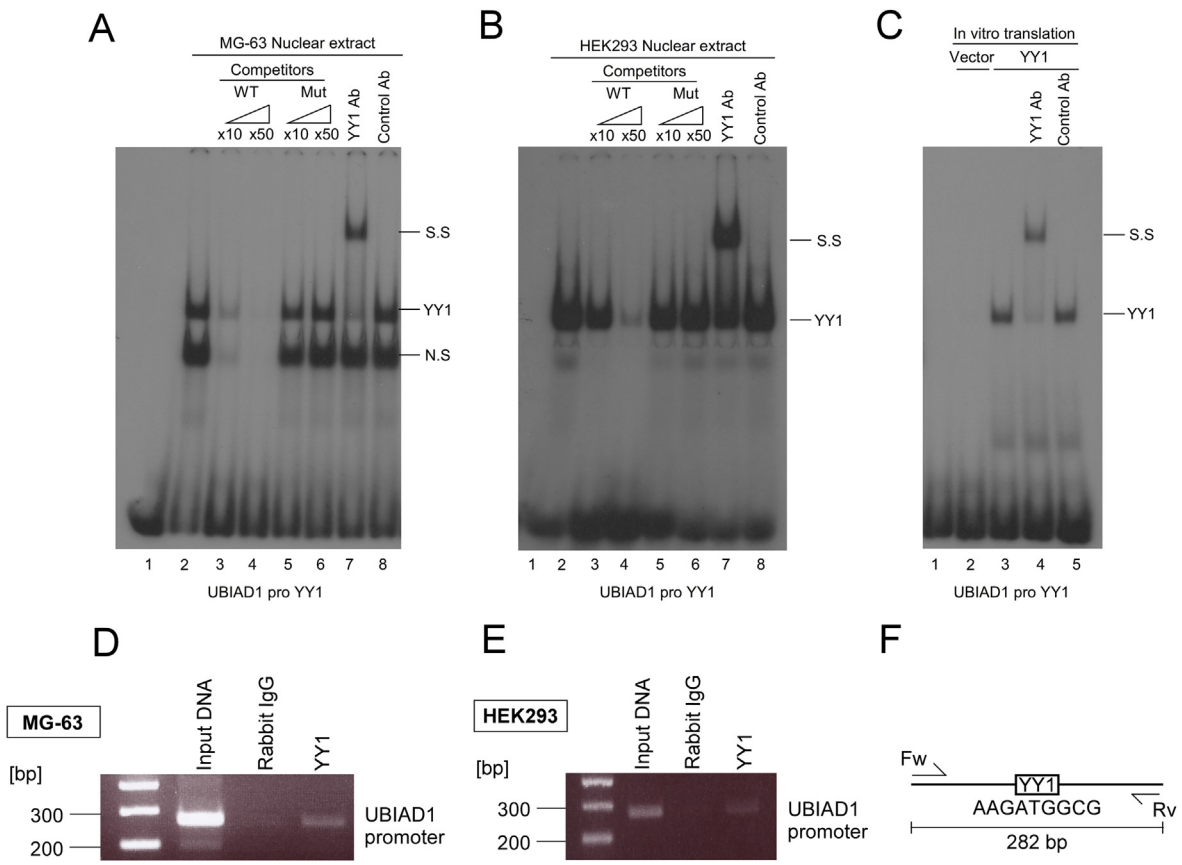

Fig. 3. The YY1 transcription factor directly binds to the human UbiA prenyltransferase domain containing 1 (UBIAD1) promoter. (A) and (B) An electromobility shift assay (EMSA) was performed using MG-63 and HEK293 nuclear extracts and ^{32}P-labelled YY1 probe in the absence (lane 1) or presence of each nuclear extract (lane 2). Competition assays were performed with 10- or 50-fold molar excess unlabelled YY1 probe (WT) (lanes 3 and 4) and 10- or 50-fold molar excess unlabelled mutant YY1 probe (Mut) (lanes 5 and 6). Supershift assays were performed with YY1 antibody (YY1 Ab) and control rabbit IgG (Control Ab) (lanes 7 and 8). S.S, supershift band, YY1, putative DNA–protein complex; N.S., nonspecific band. (C) The EMSA was performed using *in vitro* translated YY1 incubated with ^{32}P-labelled YY1 probe (lane 3). Supershift assays were performed with YY1 Ab and Control Ab (lanes 4 and 5). (D) and (E) Chromatin immunoprecipitation (ChIP) assay for YY1 binding in MG-63 or HEK293 cells. The DNA–protein complexes were immunoprecipitated using an antibody that recognises YY1, and rabbit IgG was used as the negative control. A polymerase chain reaction analysis was performed using the immunoprecipitated DNA fragments and primers shown in Supplementary materials. (F) Schematic representation of the primers used in the ChIP assay. YY1 indicates putative YY1-binding site of the UBIAD1 promoter. Fw and Rv indicate the primer sets used. The PCR product was 282 bp.

within the promoter region. As shown in Fig. 2B, pGL4.10 (−590/+353) demonstrated the highest luciferase activity in both MG-63 and HEK293 cells. Hence we determined that the wild-type UBIAD1 promoter was between −590 and +353 bp. Our serial deletion constructs suggested that activation regions were contained from −590 bp to −428 bp and from −40 bp to +128 bp, respectively. The inhibitory regions were contained from −3073 bp to −1626 bp, from −1626 bp to −590 bp and from −211 bp to −40 bp, respectively. Herein we focused on the most potent activation region from −40 bp to +128 bp.

We searched the highly conserved region between species using the UCSC genome browser to identify the DNA-binding transcription factor mediating potent activator from −40 bp to +128 bp (Fig. 2C) because functional regions are more highly conserved. We found a highly conserved region near the transcription start site, and it included the YY1 transcription factor consensus sequence (5′-CAAGATGGC-3′). Therefore, the YY1-binding site mutant construct was fused to a luciferase reporter in pGL4.10, and the resulting construct was transiently transfected into a variety of cells to evaluate the importance of the putative YY1-binding site in the UBIAD1 promoter. As shown in Fig. 2D, the YY1-binding site mutant caused a 70% reduction in UBIAD1 promoter activity, suggesting that the YY1-binding site contributes to UBIAD1 promoter activity.

3.3. YY1 interacts directly with the UBIAD1 promoter in vitro and in vivo

EMSA was performed to determine whether YY1 binds to the putative YY1-binding site in the UBIAD1 promoter. Incubating the MG-63-cell nuclear extract with a radiolabeled probe spanning the YY1 site resulted in a DNA–protein complex (Fig. 3A, lane 2). This complex was inhibited by adding 10- or 50-fold excess unlabelled wild-type YY1 competitor but was not affected by adding 10- or 50-fold excess unlabelled mutant YY1 competitor (Fig. 3A, lanes 3–6). We performed a supershift assay to determine whether this DNA–protein complex contained YY1. The anti-YY1 antibody produced supershift bands, whereas the control antibody had no effect (Fig. 3A, lanes 7 and 8). EMSA results using HEK293 cells were similar (Fig. 3B). Furthermore, *in vitro*-translated YY1 bound to the YY1 site (Fig. 3C).

We performed the ChIP assay with MG-63 and HEK293 cells to determine whether YY1 binds to the UBIAD1 promoter on cellular chromatin. As shown in Fig. 3D–F, the precipitated YY1-binding site was detected by PCR amplification of the UBIAD1 promoter fragment (nucleotides −172 to +99), which included the YY1-binding site. Taken together, these data confirm that YY1 bound to the YY1-binding site in the UBIAD1 promoter *in vitro* and *in vivo*.

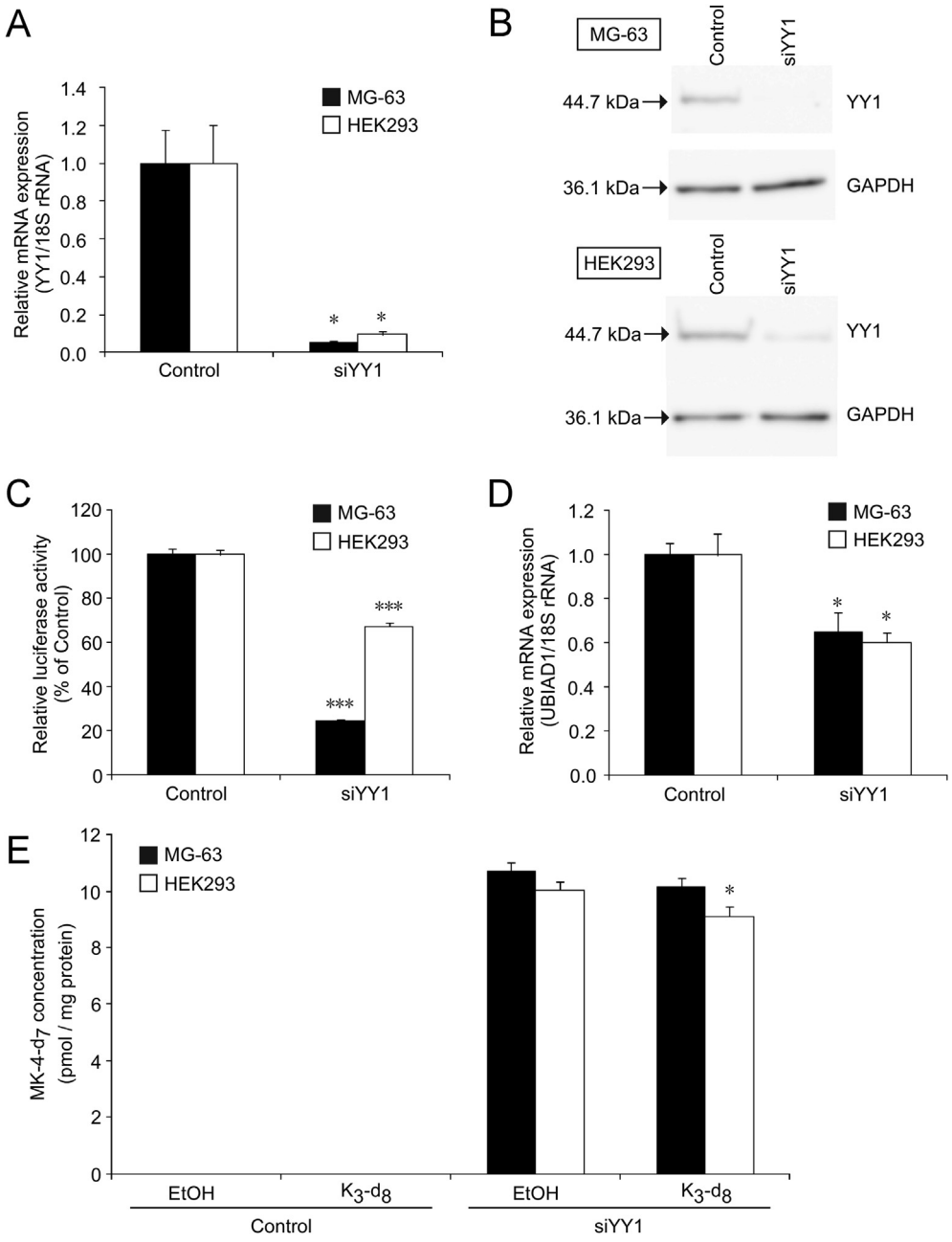

Fig. 4. YY1 is required for full UbiA prenyltransferase domain containing 1 (UBIAD1) expression. (A) MG-63 and HEK293 cells were transiently transfected with Stealth RNAi negative control duplexes (control), or YY1 siRNA (siYY1) and analysed for expression by real-time polymerase chain reaction after 48 h. YY1 mRNA was normalised to 18S rRNA. Data represent means ± standard errors of at least three replicates. Significant differences in the experimental values compared with those of the control were determined using Student's *t*-test and are indicated by asterisks ($^{*}P < 0.05$). (B) MG-63 and HEK293 cells were transiently transfected with control or siYY1 and analysed for expression after 48 h. Cell lysates were analysed by western blot. Western blot analyses were performed with anti-YY1 or anti-GAPDH antibodies. (C) MG-63 and HEK293 cells were transiently transfected with control or siYY1. Furthermore, control or siYY1-treated cells were transiently co-transfected with the pGL4.10 (−590/+353) luciferase reporter construct and pGL4.74 [hRluc/TK]. Firefly luciferase activity was normalised to *Renilla* luciferase activity. Data represent means ± standard errors of at least three replicates. Significant differences in the experimental values compared with those of the control were determined using Student's *t*-test and are indicated by asterisks ($^{***}P < 0.001$). (D) MG-63 and HEK293 cells were transiently transfected with control or siYY1 and analysed for expression after 48 h. UBIAD1 mRNA was normalised to 18S rRNA. Data represent means ± standard errors of at least three replicates. Significant differences in the experimental values compared with those of the control were determined using Student's *t*-test and are indicated by asterisks ($^{*}P < 0.05$). (E) MG-63 and HEK293 cells were transiently transfected with control or siYY1, and conversion of K_3-d_8 to MK-4-d_7 was measured after 48 h. Data represent means ± standard errors of at least three replicates. Significant differences in the experimental values compared with those of the control were determined using Student's *t*-test and are indicated by asterisks ($^{*}P < 0.05$).

3.4. YY1 is necessary for UBIAD1 expression

The mutation construct of the YY1-binding site decreased promoter activity (Fig. 2D), suggesting that YY1 binding to the YY1-binding site contributes to UBIAD1 promoter activity. Thus, the effect of transient YY1 protein siRNA knockdown on endogenous UBIAD1 expression was investigated in MG-63 and HEK293 cells. Total RNA and cell extracts were prepared, and YY1 mRNA and protein levels were investigated by real time RT-PCR and western blot analyses, respectively, 48 h after siRNA treatment. Real time RT-PCR demonstrated that knockdown of endogenous YY1 mRNA using YY1 siRNA decreased mRNA

expression (Fig. 4A). Western blot analysis demonstrated that YY1 protein levels in YY1 knockdown cells markedly decreased (Fig. 4B). Furthermore, we evaluated the change in promoter activity, following YY1 knockdown in MG-63 and HEK293 cells. As shown in Fig. 4C, UBIAD1 promoter activity decreased >20% and 60% compared with that in control MG-63 and HEK293 cells, respectively. Furthermore, YY1 knockdown significantly decreased endogenous UBIAD1 mRNA expression (Fig. 4D). These results suggest that YY1 up-regulates UBIAD1 expression through a proximal promoter. We performed a UBIAD1 conversion assay to determine whether YY1 affects UBIAD1 function. As shown in Fig. 4E, MK-4-d_7 concentration tended to decrease following YY1 knockdown compared with that in control MG-63 cells. MK-4-d_7 concentration decreased significantly following YY1 knockdown compared with that in control HEK293 cells, suggesting that YY regulates the promoter and affects UBIAD1 conversion activity.

4. Discussion

UBIAD1 plays a role converting vitamin K derivatives to MK-4, suppressing tumour growth and accumulating cholesterol. Thus, there are several reported functions of UBIAD1. However, regulation of the human UBIAD1 gene has not been described. Here we isolated and characterised the human UBIAD1 promoter for the first time.

We isolated the UBIAD1 transcription start site using 5′-RACE and found the YY1 consensus sequence near the transcription start site (Fig. 1). As previously reported, the YY1 transcription factor is not only a transcriptional activator and suppressor but also functions similar to the TATA-binding protein [18]. We suggest that the UBIAD1 promoter is a TATA-less promoter (Fig. 2A). Thus, YY1 activated transcription against the UBIAD1 promoter. Furthermore, YY1 directly bound TFIIB and RNA polymerase II and initiated transcription, suggesting that YY1 plays a role in basal UBIAD1 gene transcriptional activity.

A deletion analysis suggested that the activation regulatory element was contained between −590 to −428 and +128 to 0 in the UBIAD1 promoter. As shown in Fig. 2A, several transcription factors including SP1, were predicted to bind between +128 and 0 within the UBIAD1 promoter. A mutation construct of the putative SP1-binding site between +128 and 0 decreased promoter activity (data not shown), suggesting that SP1 contributes to UBIAD1 promoter activity.

Previous reports have demonstrated that UBIAD1 suppresses cell proliferation in prostate carcinoma and bladder tumours [6,19], and that the tumour growth inhibitory mechanism is UBIAD1-induced G1 cell cycle arrest. Moreover, YY1 inhibits tumour cell proliferation and formation of the p21 complex, including cdk4 and cyclin D1 [16]. Furthermore, other groups have reported that YY1 directly activates breast cancer-associated gene 1 expression and inhibits tumour growth [20] by G1 cell cycle arrest. Thus, YY1 regulates several genes that inhibit tumour cell proliferation through G1 arrest. Our data suggest that YY1 regulates UBIAD1 expression and inhibits tumour cell proliferation.

In conclusion, this is the first study to describe the transcription start site and regulatory motifs within the human UBIAD1 promoter. We demonstrated that YY1 positively regulates UBIAD1 expression and UBIAD1 conversion activity through the UBIAD1 promoter.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.018.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.03.018.

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