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Cloning and characterization of human Golgi phosphoprotein 2 gene (*GOLPH2*/*GP73*/*GOLM1*) promoter

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ABSTRACT

Human Golgi phosphoprotein 2 gene (also known as *GOLPH2*, *GP73* or *GOLM1*) encodes an epithelial-specific Golgi membrane protein which can be induced by virus infection. It is also overexpressed in a number of tumors and is currently considered as an early diagnosis marker for hepatocellular carcinoma. However, little is known about how *GOLPH2* is dysregulated in these disease conditions and the functional implications of its overexpression. The aim of this study is to investigate human *GOLPH2* regulation mechanisms. We cloned a 2599 bp promoter fragment of *GOLPH2* and found it maintained epithelial specificity. By deletion analysis, a repressive region (−864 to −734 bp), a positive regulatory region (−734 to −421 bp) and a core promoter region (−421 to −79 bp) were identified. Sequence analysis revealed that *GOLPH2* core promoter was devoid of canonical TATA element and classified as a TATA-less promoter. Adenoviral early region 1A (E1A) was able to activate *GOLPH2* and the CtBP interaction domain of E1A was sufficient but not required for activation. A GC-box motif (−89 to −83 bp) in *GOLPH2* core promoter region partly mediated E1A transactivation. These results delineated regulatory regions and functional element in *GOLPH2* promoter, elucidated adenoviral E1A stimulation mechanisms and provided insight into *GOLPH2* functions.

1. Introduction

GOLPH2, also termed GP73 or GOLM1, is a type II Golgi membrane protein which resides in the *cis*- and *medial*-Golgi cisternae [1,2]. Aberrant expression of *GOLPH2* is associated with a variety of human diseases [3]. It was first isolated as a differentially expressed gene in the liver of patients with adult giant-cell hepatitis and its up-regulation was a general feature of advanced liver diseases including hepatitis B, hepatitis C, autoimmune hepatitis and alcohol-induced liver diseases [4]. Elevated and hyperfucosylated GOLPH2 was detected in serum of patients with hepatocellular carcinoma. Therefore, it was proposed as a novel serum marker for HCC [5–7]. Increased *GOLPH2* expression in hepatocellular carcinoma tissues correlates with tumor aggression but not survival [8]. In addition to liver diseases, *GOLPH2* also overexpressed in lung adenocarcinoma tissues [9], seminomas, Leydig cell tumors [10] and prostate cancer tissues [11]. The mechanisms underlying the up-regulation of *GOLPH2* remain unknown. *GOLPH2* is normally expressed in cells of epithelial lineage, but the mRNA and protein levels vary from tissues to tissues. It is abundant in colonic, bronchial, renal, prostate, and hepatic biliary epithelial cells while absent from heart, muscle and hepatocyte [1]. *GOLPH2* can be elicited by adenovirus infection [12], cytokine stimulation [3] and Golgi stress triggered by alpha 1,6-fucosyltransferase [13], suggesting existence of rapid inducible regulation mechanisms. Uncovering molecular mechanisms of *GOLPH2* regulation will contribute to understanding its functional implications in diseases and evaluating its role as a novel cancer marker.

Adenovirus infection increased *GOLPH2* expression in Hep3B cells through its early region 1A (E1A) gene [12]. E1A proteins prime host cells for viral replication by repressing differentiated cell functions and promoting DNA synthesis cell machinery [14]. The primary E1A transcript is processed by differential splicing to yield five distinct variants, 13S (289R), 12S (243R), 11S (217R), 10S (171R) 9S (55R). They appear at different infection stages and assume distinct functions [15]. According to amino acid sequence homology and splicing patterns among E1A proteins of various adenovirus serotypes, four conserved regions have been identified as CR1, CR2, CR3 and CR4 [16]. Conserved regions interact with different cellular partners to direct viral gene expression,

Abbreviations: GOLPH2, Golgi phosphoprotein 2; HCC, hepatocellular carcinoma; CR, conserved region; CtBP, c-terminal binding protein; CID, CtBP interaction domain; E1A, early region 1A; Sp1, specificity protein 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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deregulate cell cycle and modulate cellular gene transcription. CR1 and CR2 compete retinoblastoma protein (pRb) with E2F and promote cell cycle progression [17]; CR3 corresponds to the sequence unique to 13S (289R) proteins and encodes a zinc finger which stimulates viral gene expression by binding to ATF2 or TFIID [18,19]; the highly conserved amino acid motif PLDLS (also named CID) in CR4 is associated with CtBP family and negatively modulate cell transformation and metastasis [20].

In the present study, we characterized *GOLPH2* promoter and explored molecular basis of E1A activation mechanisms. Our results demonstrated that: (I) the 2.6 kb promoter region displayed epithelial-specific activity and encompassed both positive and negative regulatory regions; (II) the core promoter was characterized by a cluster of GC-boxes and absence of TATA element; (III) E1A transactivation of *GOLPH2* did not depend on any single region and involved CR1, CR3 and CID; (IV) a GC-box was identified and it partly mediated E1A transactivation of *GOLPH2*. These features are fundamental to understanding transcriptional regulation of *GOLPH2*.

2. Materials and methods

2.1. Cloning of human GOLPH2 promoter, deletion mutants and E1A expression plasmids

Genomic DNA was extracted from Hela cells using Genomic DNA Kit (TIANamp) according to the manufacturer's instructions. A 2599 bp genomic fragment (−2618 to −19 bp) was obtained by polymerase chain reaction (PCR) with the primer pair F2618 and R19 (Table 1 in Supplementary data), and inserted into pGL3-basic *Firefly* luciferase reporter vector (Promega). Promoter deletions designated as p-2320/-19, p-2070/-19, p-1441/-19, p-1258/-19, p-1074/-19, p-864/-19, p-734/-19, p-559/-19, p-421/-19, p-302/-19, p-421/-79, p-421/-132 were generated by PCR and subcloned into pGL3-basic vector. Primers used to generate these deletions were listed in Table 1 in Supplementary data.

Three transcripts of adenovirus 5 E1A 289R, 243R and 171R were amplified by PCR using 293T cDNA as templates [21] and ligated to the flag tagged PCR3.1 vector (Invitrogen). △CID was derived from 243R with an internal deletion from residue 225 to residue 238 as described [20]. ExonII corresponds to the amino acid sequence from residue 140 to residue 243 in the 243R background [22]. To prepare PCR3.1-exonII-Flag and PCR3.1-exonII△CID-Flag, DNA inserts were generated by PCR using PCR3.1-243R-Flag and PCR3.1-△CID-Flag as templates respectively and then subcloned into flag tagged PCR3.1. All the primers used were listed in Table 2 in Supplementary data.

2.2. Cell culture, transient transfection and luciferase reporter assays

Human Burkitt's lymphoma cell line Raji (ATCC No.CCL-86) and human meyloma cell line U266 (ATCC No. TIB-196™) were maintained in RPMI 1640 (Gibco) supplemented with 10% FBS (Hyclone). Human cervical carcinoma cell line Hela (ATCC No.CCL-2™), human hepatocellular carcinoma cell lines HepG2 (ATCC No. HB-8065™) and Huh7 (JCRB ID 0403) and human breast adenocarcinoma cell line MCF7 (ATCC No. HTB-22™) were grown in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% FBS.

For luciferase reporter assays, 1×10^4 Hela cells were seeded in 96-well culture plates 24 h before transfection. Promoter plasmids (100 ng/well) were transfected into Hela cells with Lipo2000™ (Invitrogen) together with pRL-TK plasmids (10 ng/well) as an internal control to normalize transfection efficiencies. Cells were harvested 36 h after transfection for luciferase assays using the Dual-glo luciferase assay kit (Promega). All transfections were performed in triplicate and repeated at least three times. Results from one representative experiment were shown. Statistical analysis was performed using a one-way analysis of variance (ANOVA) and $P < 0.05$ was considered as significant. For measuring effect of E1A splicing variants and mutants on GOLPH2 protein level, Hep3B cells were plated into 6-well plate at 80% confluence and E1A expression plasmids (2 μg/well) were transfected. Cells were harvested for western blot 36 h after transfection.

2.3. Western blot and quantitative real time PCR

Hep3B cells were lysed in RIPA buffer (100 mM NaCl, 100 mM Tris, pH 6.8, 1% glycerol, 0.1% SDS, 1% Triton X-100,1% NP40, 100 mM DTT) supplemented with protease inhibitor cocktail (Roche). Equal amounts of proteins were separated by 10% SDS–PAGE. GAPDH was detected with mouse anti-GAPDH monoclonal antibody (Sigma) and GOLPH2 with 5B12 [9].

To quantify *GOLPH2* mRNA in various cell lines, total RNA was extract from cultured cells using Trizol reagent according to the manufacturer's protocol (Sangon). Reverse transcription was performed using a commercially available kit (Takara). Real time PCR was performed with SYBR Green premix Ex Taq (Takara) and β-actin was used as an internal control for standardization. Primers used for *GOLPH2* were: forward, 5′- CGTGG AGCTGAAGAAGAAC-GAG-3′, reverse, 5′-CCTGGTACAGCTTGTTGACGCT-3′. Primers used for β-actin were: forward, 5′-CTCCTCCTGAGCGCAAGTACTC-3′, reverse, 5′ -TCC TGCTTGCTGATCCACATC-3′.

2.4. PCR based site directed mutagenesis and electrophoretic mobility shift assay

Site-specific mutations of two GC-boxes at positions −89/−83 bp and −49/−43 bp were created by a two-step PCR procedure as described [23]. Primers used for GC-boxes mutations were: GC-box Amut forward, 5′-CTCGCTTCTCCGCGCTCGCAACACCGCCT CCTCCCTTCGCGC-3′, GC-box Amut reverse, 5′-GCGCGAAGGGAG-GAGGCGGTGTT GCGAGCGCGGAGAAGCGAG-3′; GC-box Bmut forward, 5′-CGCCGCCGGCGCTGCTC GGGAACAGGGCCTTGCCGCTGCG GA-3′, GC-box Bmut reverse, 5′-TCCGCAGCGGC AAGGCCCTGTT CCCGAGCAGCGCCGGCGGCG-3′. All mutants were verified by sequencing.

For electrophoretic mobility shift assay, nuclear extracts from cultured Hela cells were obtained as described [24]. Biotin labeled double stranded DNA oligonucleotides were purchased from Invitrogen and sequences were as follows: forward 5′-CTCGCTTCTCCG CGCTCGCGGCGCCGCCTCCTCCCTTCGCGC-3′, reverse 5′-GCGCGAAG GGAGGAG GCGGCGCCGCGAGCGCGGAGAAGCGAG-3′. Binding reactions were carried out for 20 min at room temperature in EMSA/Gel-shift binding buffer purchased from Beyotime. DNA–protein complexes were resolved on a 6% (w/v) non-denaturing polyacrylamide gel in 0.5 × TBE running buffer (1 × TBE: 100 mM Tris, pH 8, 90 mM boric acid and 1 mM EDTA). For competition analysis, the conditions were exactly as described above except that 100-fold excess of cold unlabeled oligonucleotides were incubated with reaction mixture.

3. Results

3.1. Identification of GOLPH2 promoter

We previously cloned a 1.3 kb fragment upstream of *GOLPH2* gene which displayed 8-fold of mean fluorescent intensity compared with control in the enhanced green fluorescent protein (EGFP) reporter construct [25]. However, this fragment showed

low transcriptional activity in the luciferase reporter construct (data not shown). We hypothesized that this region did not contain the functional core promoter and extended the initial fragment to 2.6 kb. The elongated fragment exhibited potent promoter activity in transient transfection assays. The transcription start site determined previously by 5′ rapid amplification of cDNA end (5′RACE) was designated as +1 [1] and the active promoter clone ranged from −2618 to −19 bp accordingly. A sequence analysis of the region from 3 kb upstream to 1 kb downstream with CpG Plot program (http://www.ebi.ac.uk/Tools/emboss/cpgplot/) revealed two putative CpG islands locating in −736/−499 bp and −443/+772 bp (Fig. 1A). Since *GOLPH2* is preferentially expressed in cells of epithelial lineage, we examined *GOLPH2* promoter activity in a number of epithelial (Hela, HepG2, Hep3B) and non-epithelial cell lines (Raji and U266). The activities of p-2618/-19 promoter fragment correspondingly reflected levels of endogenous *GOLPH2* mRNA levels (Fig. 1B and C), indicating the presence of regulatory elements which determine the epithelial-specific expression.

3.2. Characterization of GOLPH2 promoter

To map the minimal promoter region and identify potential regulatory regions in *GOLPH2* promoter, we generated a series of progressive deletions and measured their transcriptional activity in Hela cells (Fig. 2). Based on the deletion analysis, the 2.6 kb promoter segment was divided into four regions. Region I extended from −2618 to −864 bp and removal of fragments in this region did not significantly affect the promoter activities. However, deletion of Region II from −864 to −734 bp drastically elevated promoter activities, indicating the presence of suppressive elements. Region III located in between −734 and −421 bp and exerted positive effect on promoter activities. Region IV, also the minimal promoter, spanned from −421 to −79 bp and the GC content was as high as 76%. Sequence analysis revealed that the core promoter of *GOLPH2* was devoid of canonical basal promoter elements such as TATA box but consisted of multiple GC-boxes, which was characteristic of TATA-less promoter.

3.3. Role of conserved regions in E1A transactivation of GOLPH2

Adenovirus E1A has been reported to activate *GOLPH2* expression [12]. To delineate specific domain that is responsible for E1A induction, we generated a series of E1A splice variants and mutants and cotransfected them with p-2618/-19 promoter report plasmids (Fig. 3A). As shown in Fig. 3B, 289R exerted most efficient induction and fold of induction for 243R was attenuated due to the deletion of CR3, suggesting that CR3 was involved in E1A stimulation. Further removal of CR1 in 171R reduced E1A inducibility from 5.4-fold to 3.6-fold, indicative of CR1 involvement in activation. Two to three fold of induction was observed with exonII whereas no significant stimulation was noted with exonII△CID. △CID which was deficient of CID still elevated *GOLPH2* promoter activities three to four fold in the presence of CR1 and CR2. These results suggested that CID in exonII background was sufficient to stimulate *GOLPH2* but loss of CID could be compensated by presence of other conserved regions. The dispensability of CID was verified on the protein level (Fig. 3C). Taken together, E1A transactivation of

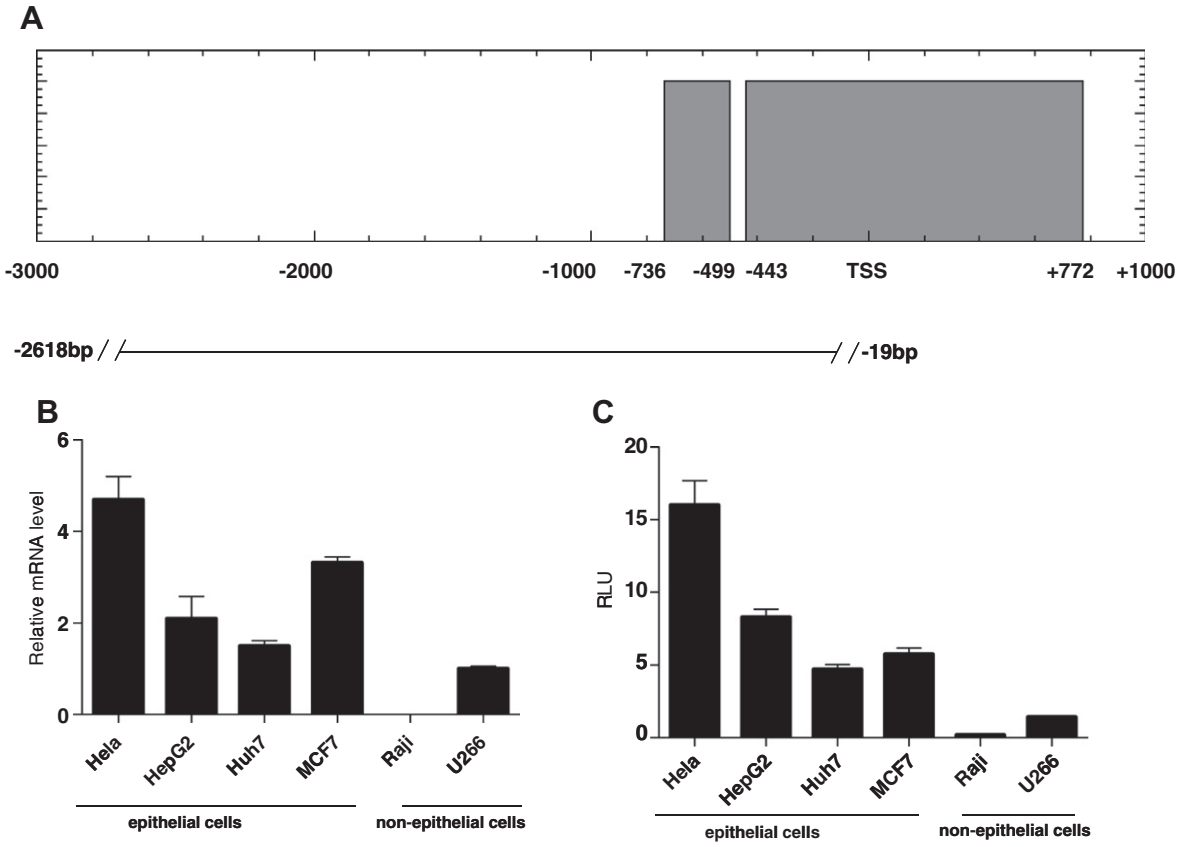

Fig. 1. Analysis of *GOLPH2* promoter and its activities in various cell lines. (A) Two CpG islands were predicted by cpgplot and the one from −443 to +772 bp covered transcription start site (TSS). Putative CpG islands are depicted by gray boxes and numbers represent positions relative to TSS. (B) Endogenous mRNA levels of *GOLPH2* in a series of epithelial and non-epithelial cell lines. Quantitative real time PCR were performed using β-actin as a reference gene and the *error bar* indicates standard deviation. (C) Epithelial-specific expression of *GOLPH2* promoter. Various cell lines were transiently transfected with p-2618/-19 fragment and pRL-TK was co-transfected as an internal control. Luciferase activities were calculated in relation to background activity of pGL3-basic.

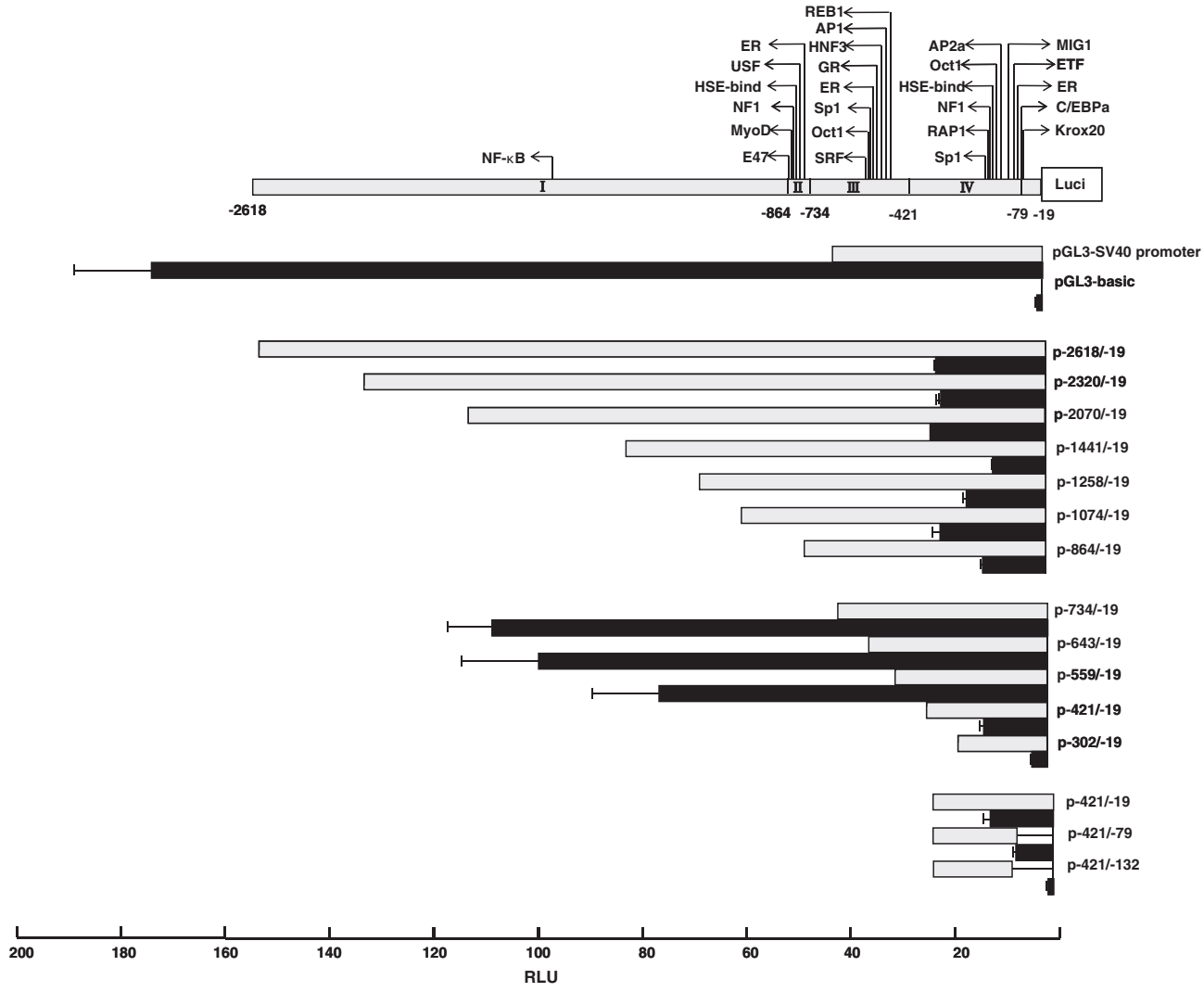

Fig. 2. Deletion analysis of *GOLPH2* promoter. A series of promoter deletions are represented by gray boxes and their luciferase activities are shown relative to pGL3-basic activities by filled bars. The whole promoter fragment above was divided into four regions based on their effect on promoter activities. Putative transcription factor binding sites predicted within each region are annotated by arrows.

GOLPH2 did not depend on any single conserved regions and involved multiple regions including CR1, CR3 and CID.

3.4. Identification of a GC-box as an E1A responsive element in GOLPH2 promoter

To further identify E1A responsive elements, we tested E1A 243R on different promoter deletions and found all fragments showed varied degrees of responsiveness (Fig. 4A), indicating that at least one responsive element located in the core promoter. Since *GOLPH2* core promoter was enriched with GC-boxes which were putative specificity protein 1 (Sp1) binding sites and reported to mediate E1A transactivation [26], we mutated two GC-boxes predicted by bioinformatic analysis at −89/−83 bp and −49/−43 bp respectively. As shown in Fig. 4B, GC-box A mutant did not significantly affect the basal promoter activities but attenuated about 33% stimulatory effect of E1A. Mutation of GC-box B did not result in a measurable change. To confirm that the GC-box A was functional *in vitro*, we carried out electrophoretic mobility shift assay and a specific shifted band was observed. The protein-binding activity increased in a dose dependent manner when different amount of nuclear extracts were added. The specificity of retarded band was verified by competing with unlabeled probes. However, the nature of protein components in the complexes remained unclear.

4. Discussion

Our study investigated basic features of *GOLPH2* promoter and the E1A transactivation mechanisms. By deletion analysis, we demonstrated that core promoter resided between −421 and −19 bp and this explained why the initial 1.3 kb genomic fragment ranging from −1610 to −420 bp was transcriptionally inactive. Sequence analysis revealed that the core promoter was a TATA-less promoter and covered by a CpG island which is commonly found in promoter regions of mammalian housekeeping genes [27]. The identification of the housekeeping gene-like basal promoter structure suggested that *GOLPH2* may assume certain housekeeping functions where it is constitutively expressed. *GOLPH2* promoter was featured by epithelial specificity and disruption of this specificity may account for its overexpression in non-epithelial tissues in viral and non-viral diseases. As a strong suppressive region

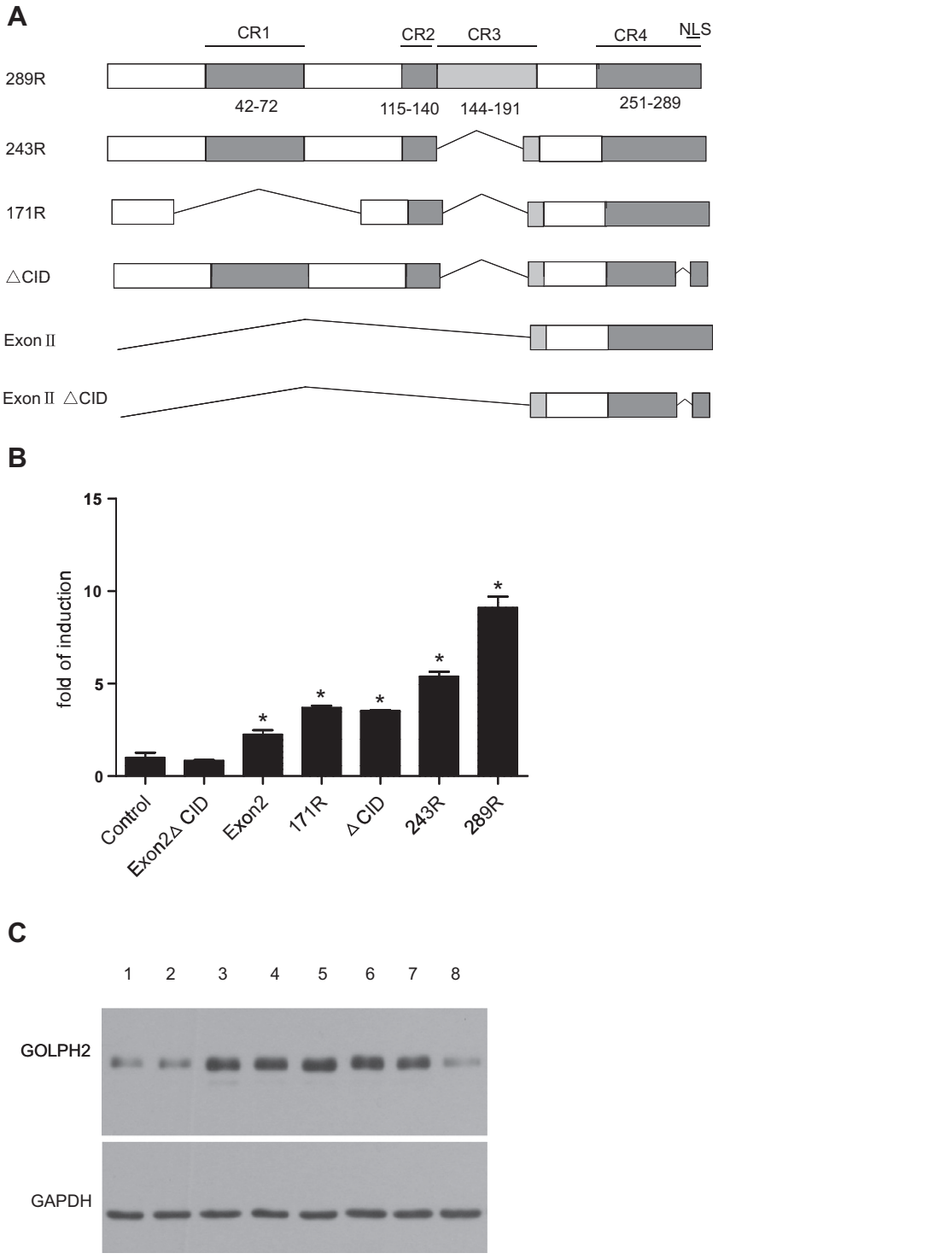

Fig. 3. CID is sufficient but not required for E1A transactivation of *GOLPH2*. (A) Schematic diagram of E1A splicing variants and mutants. CR1, CR2, CR3 and CR4 refer to conserved region 1, 2, 3 and 4 of E1A proteins and NLS refers to nuclear localization signal [38]. Amino acid residues are indicated by numbers according to their positions in 289R proteins. (B) Differential effect of E1A splicing variants and mutants on *GOLPH2* promoter activities in Hep3B cells. (C) Differential effect of E1A variants on endogenous GOLPH2 protein levels in Hep3B cells. Lane 1, null control; Lane 2 empty PCR3.1 vector control; Lane 3, 289R; Lane 4, 243R; Lane 5 171R; Lane 6 △CID; Lane 7 exonII; Lane 8 exonII△CID.

was identified between −864 and −734 bp, we proposed that the aberrant expression of *GOLPH2* was likely attributed to de-depression mechanisms and yet to be determined in future research.

With assistance of the online bioinformatic analysis tool (http://www.gene-regulation.com/pub/programs/alibaba2/index.html), putative binding sites for general expressed transcription factors such as CCAAT-enhancer-binding proteins (C/EBPalp) and activator protein 1 (AP-1) were predicted throughout p-2618/-19 sequence. *Cis*-regulatory sites for inducible transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and heat shock factor (HSF) were also found. These sites could be of interest, because Golgi stress triggered by

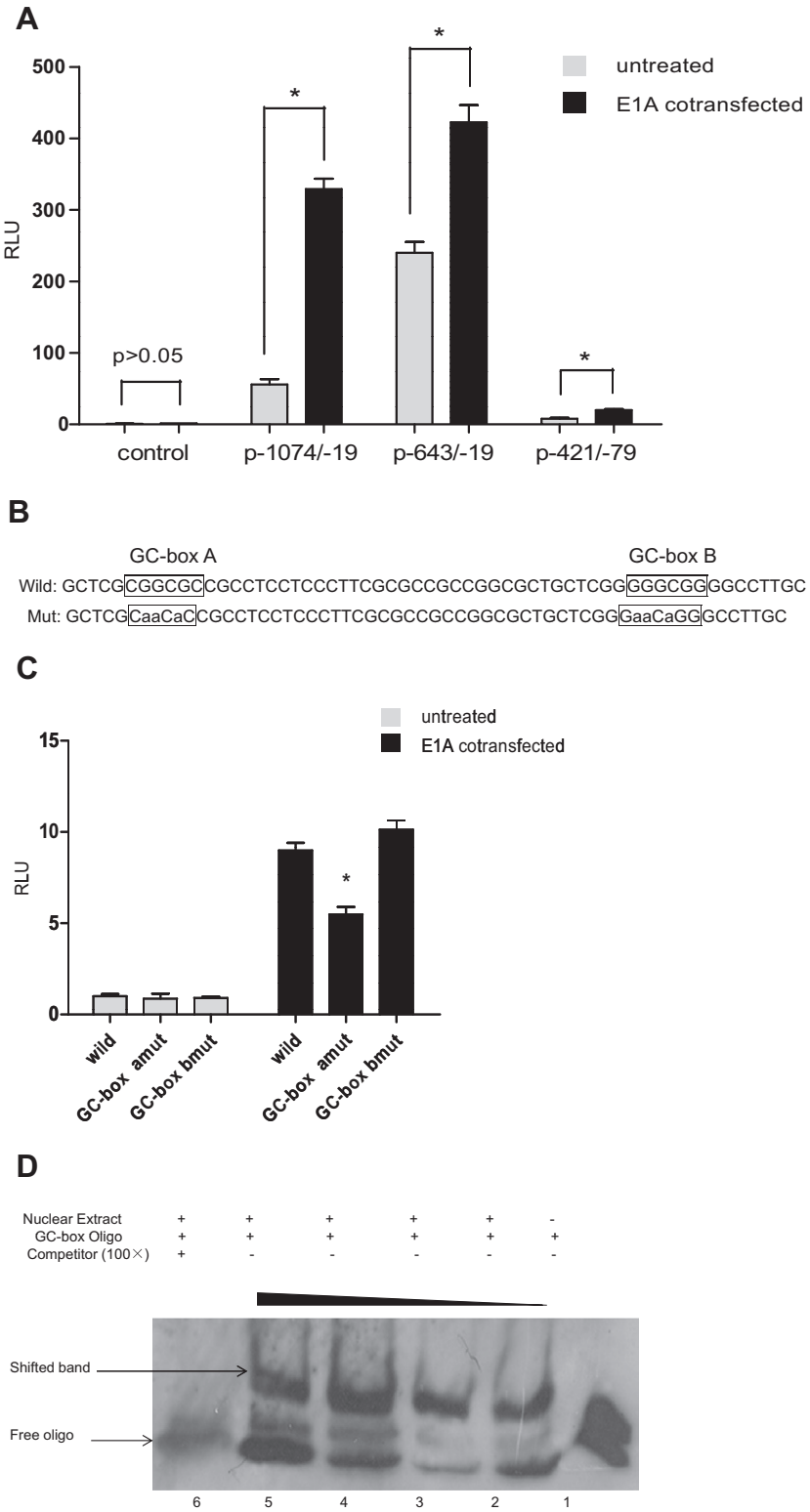

Fig. 4. A GC-box partly mediates E1A transactivation of *GOLPH2* promoter. (A) p-421/-79, p-643/-19 and p-1074/-19 showed varied responsiveness to E1A stimulation. Luciferase activities were normalized to the level of pGL-3 basic control. (B) The sequences for GC-boxes and mutated GC-boxes are framed by square and mutated bases are shown in lower case. (C) Mutation of GC-box A partly decreased stimulatory effect of E1A on *GOLPH2* promoter. (D) Interaction of Hela nuclear proteins with GC-box *in vitro* by electrophoretic mobility shift assay. Lane1, free oligo; Lane 2-lane 5, free oligo incubated with increased amount of Hela nuclear extracts; Lane 6, free oligo incubated with 100-fold excess of unlabeled wild probes.

alpha 1,6-fucosyltransferase and tumor necrosis factor a (TNFa) have been described to regulate *GOLPH2* expression [3,13]. Its interaction with extracellular chaperon clusterin also implies its inducibility to stress stimuli [28]. Further studies are necessary to address whether the HSF and NF-κB binding sites are functional.

It has been reported that CID played a major and essential role for *GOLPH2* activation by E1A [12]. However, our results suggested that CID was sufficient but not required for *GOLPH2* induction as CR1 and CR2 were able to compensate the loss of CID in △CID mutant. In addition to CID, CR1 and CR3 also actively involved in E1A transactivation of *GOLPH2*. The different observation is attributable to experiment systems used in each laboratory. For example, we used promoter luciferase reporter system which was considered more sensitive than western blot. Moreover, Kladney et al. used a panel of mutant adenoviruses to map functional domains while we applied a series of E1A expression constructs. The flexibility of effective domains may result from overlapping functions of different conserved regions: a second CtBP binding site has recently been identified in Ad5 CR3 [29]; either CR1 or CR2 can drive G_0 cells into S phase by binding to pRb [17]. The choice between distinct domains and possibly different mechanisms is suggestive of multiple functions of *GOLPH2* in adenovirus infection. There is evidence that 289R is a short-lived species and only exists in first 30 min after adenoviral infection whereas 243R expression persists throughout adenoviral infection [30]. It is possible to anticipate that the instant stimulation of *GOLPH2* by CR3 of 289R is a byproduct of potent viral genes expression in early virus infection stage while the persistent high levels of GOLPH2 by CR1 of 243R provide a favorable intracellular milieu conducive to viral replication.

We also identified a GC-box in core promoter region and found its mutation reduced E1A inducibility of *GOLPH2* promoter. GC-boxes control transcriptional initiation in TATA-less promoters [31] and mediate virus-induction of cellular genes such as VEGF-A [32], survivin [33] and classical NF-κB subunits (p65 and p105/p50) [34]. Several proteins have been shown to bind GC-boxes: most notably Sp1 [35], Sp family proteins [36] and BTEB [37]. Among them, Sp1 has been described to mediate E1A stimulation of insulin receptor gene [26], we performed electrophoretic mobility shift assay using flag-tagged Sp1 or flag-tagged Sp3 transfected Hela nuclear extracts and found the shifted complexes were not recognized by flag antibodies (data not shown), suggesting that other GC-boxes binding proteins participated in this process. Since the GC-box mutation only partly reduced the stimulatory effect and E1A activated *GOLPH2* through multiple mechanisms as discussed above, we hypothesize that other *cis*-acting elements also involve in E1A activation of *GOLPH2* and remained to be determined in future studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.04.067.

References

[1] R.D. Kladney, G.A. Bulla, L. Guo, A.L. Mason, A.E. Tollefson, D.J. Simon, Z. Koutoubi, C.J. Fimmel, GP73, a novel Golgi-localized protein upregulated by viral infection, Gene 249 (2000) 53–65.

[2] L. Hu, L. Li, H. Xie, Y. Gu, T. Peng, The Golgi localization of GOLPH2 (GP73/GOLM1) is determined by the transmembrane and cytoplasmic sequences, PLoS ONE 6 (2011) e28207.

[3] R.D. Kladney, X. Cui, G.A. Bulla, E.M. Brunt, C.J. Fimmel, Expression of GP73, a resident Golgi membrane protein, in viral and nonviral liver disease, Hepatology 35 (2002) 1431–1440.

[4] A. Maitra, P.J. Thuluvath, GP73 and liver disease: a (Golgi) complex enigma, Am. J. Gastroenterol. 99 (2004) 1096–1098.

[5] C.J. Fimmel, L. Wright, Golgi protein 73 as a biomarker of hepatocellular cancer: development of a quantitative serum assay and expression studies in hepatic and extrahepatic malignancies, Hepatology 49 (2009) 1421–1423.

[6] J.A. Marrero, P.R. Romano, O. Nikolaeva, L. Steel, A. Mehta, C.J. Fimmel, M.A. Comunale, A. D'Amelio, A.S. Lok, T.M. Block, GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma, J. Hepatol. 43 (2005) 1007–1012.

[7] Y. Gu, W. Chen, Y. Zhao, L. Chen, T. Peng, Quantitative analysis of elevated serum Golgi protein-73 expression in patients with liver diseases, Ann. Clin. Biochem. 46 (2009) 38–43.

[8] Y. Sun, H. Yang, Y. Mao, H. Xu, J. Zhang, G. Li, X. Lu, X. Sang, H. Zhao, S. Zhong, J. Huang, H. Zhang, Increased Golgi protein 73 expression in hepatocellular carcinoma tissue correlates with tumor aggression but not survival, J. Gastroenterol. Hepatol. (2011).

[9] F. Zhang, Y. Gu, X. Li, W. Wang, J. He, T. Peng, Up-regulated Golgi phosphoprotein 2 (GOLPH2) expression in lung adenocarcinoma tissue, Clin. Biochem. 43 (2010) 983–991.

[10] F.R. Fritzsche, G. Kristiansen, M.O. Riener, M. Dietel, B. Oelrich, GOLPH2 expression may serve as diagnostic marker in seminomas, BMC Urol. 10 (2010) 4.

[11] S. Varambally, B. Laxman, R. Mehra, Q. Cao, S.M. Dhanasekaran, S.A. Tomlins, J. Granger, A. Vellaichamy, A. Sreekumar, J. Yu, W. Gu, R. Shen, D. Ghosh, L.M. Wright, R.D. Kladney, R. Kuefer, M.A. Rubin, C.J. Fimmel, A.M. Chinnaiyan, Golgi protein GOLM1 is a tissue and urine biomarker of prostate cancer, Neoplasia 10 (2008) 1285–1294.

[12] R.D. Kladney, A.E. Tollefson, W.S. Wold, C.J. Fimmel, Upregulation of the Golgi protein GP73 by adenovirus infection requires the E1A CtBP interaction domain, Virology 301 (2002) 236–246.

[13] S. Kawamoto, K. Moriwaki, T. Nakagawa, M. Terao, S. Shinzaki, N. Yamane-Ohnuki, M. Satoh, A.S. Mehta, T.M. Block, E. Miyoshi, Overexpression of alpha1,6-fucosyltransferase in hepatoma enhances expression of Golgi phosphoprotein 2 in a fucosylation-independent manner, Int. J. Oncol. 39 (2011) 203–208.

[14] A.J. Berk, Adenovirus promoters and E1A transactivation, Annu. Rev. Genet. 20 (1986) 45–79.

[15] S.M. Frisch, J.S. Mymryk, Adenovirus-5 E1A: paradox and paradigm, Nat. Rev. Mol. Cell Biol. 3 (2002) 441–452.

[16] N. Avvakumov, A.E. Kajon, R.C. Hoeben, J.S. Mymryk, Comprehensive sequence analysis of the E1A proteins of human and simian adenoviruses, Virology 329 (2004) 477–492.

[17] M.A. Ikeda, J.R. Nevins, Identification of distinct roles for separate E1A domains in disruption of E2F complexes, Mol. Cell. Biol. 13 (1993) 7029–7035.

[18] F. Liu, M.R. Green, A specific member of the ATF transcription factor family can mediate transcription activation by the adenovirus E1a protein, Cell 61 (1990) 1217–1224.

[19] J.V. Geisberg, J.L. Chen, R.P. Ricciardi, Subregions of the adenovirus E1A transactivation domain target multiple components of the TFIID complex, Mol. Cell. Biol. 15 (1995) 6283–6290.

[20] J.M. Boyd, T. Subramanian, U. Schaeper, M. La Regina, S. Bayley, G. Chinnadurai, A region in the C-terminus of adenovirus 2/5 E1a protein is required for association with a cellular phosphoprotein and important for the negative modulation of T24-ras mediated transformation, tumorigenesis and metastasis, EMBO J. 12 (1993) 469–478.

[21] F.L. Graham, J. Smiley, W.C. Russell, R. Nairn, Characteristics of a human cell line transformed by DNA from human adenovirus type 5, J. Gen. Virol. 36 (1977) 59–74.

[22] J.S. Mymryk, S.T. Bayley, Induction of gene expression by exon 2 of the major E1A proteins of adenovirus type 5, J. Virol. 67 (1993) 6922–6928.

[23] S.N. Ho, H.D. Hunt, R.M. Horton, J.K. Pullen, L.R. Pease, Site-directed mutagenesis by overlap extension using the polymerase chain reaction, Gene 77 (1989) 51–59.

[24] J.D. Dignam, R.M. Lebovitz, R.G. Roeder, Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei, Nucleic Acids Res. 11 (1983) 1475–1489.

[25] H. Xie, Y. Gong, T. Peng, Cloning of the promoter regions of Golgi membrane glycoprotein GP73, Agri. Sci. Technol. 36 (2008) 10359–10361.

[26] H.S. Kim, J.K. Lee, S.Y. Tsai, E1a activation of insulin receptor gene expression is mediated by Sp1-binding sites, Mol. Endocrinol. 9 (1995) 178–186.

[27] T. Juven-Gershon, J.Y. Hsu, J.T. Kadonaga, Perspectives on the RNA polymerase II core promoter, Biochem. Soc. Trans. 34 (2006) 1047–1050.

[28] L.L. Zhou Y, Hu L, Peng T, Golgi phosphoprotein 2 (GOLPH2/GP73/GOLM1) interacts with secretory clusterin, Mol. Biol. Rep. 38 (3) (2011) 1457–1462.

[29] R.K. Bruton, P. Pelka, K.L. Mapp, G.J. Fonseca, J. Torchia, A.S. Turnell, J.S. Mymryk, R.J. Grand, Identification of a second CtBP binding site in adenovirus type 5 E1A conserved region 3, J. Virol. 82 (2008) 8476–8486.

[30] K.R. Spindler, A.J. Berk, Rapid intracellular turnover of adenovirus 5 early region 1A proteins, J. Virol. 52 (1984) 706–710.

[31] M.C. Blake, R.C. Jambou, A.G. Swick, J.W. Kahn, J.C. Azizkhan, Transcriptional initiation is controlled by upstream GC-box interactions in a TATAA-less promoter, Mol. Cell. Biol. 10 (1990) 6632–6641.

[32] T. Wuest, M. Zheng, S. Efstathiou, W.P. Halford, D.J. Carr, The herpes simplex virus-1 transactivator infected cell protein-4 drives VEGF-A dependent neovascularization, PLoS Pathog. 7 (2011) e1002278.

[33] J. Lu, S.C. Verma, M. Murakami, Q. Cai, P. Kumar, B. Xiao, E.S. Robertson, Latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus (KSHV) upregulates survivin expression in KSHV-Associated B-lymphoma cells and contributes to their proliferation, J. Virol. 83 (2009) 7129–7141.
[34] A.D. Yurochko, M.W. Mayo, E.E. Poma, A.S. Baldwin Jr., E.S. Huang, Induction of the transcription factor Sp1 during human cytomegalovirus infection mediates upregulation of the p65 and p105/p50 NF-kappaB promoters, J. Virol. 71 (1997) 4638–4648.
[35] W.S. Dynan, R. Tjian, Control of eukaryotic messenger RNA synthesis by sequence-specific DNA-binding proteins, Nature 316 (1985) 774–778.
[36] L. Lania, B. Majello, P. De Luca, Transcriptional regulation by the Sp family proteins, Int. J. Biochem. Cell. Biol. 29 (1997) 1313–1323.
[37] K. Sogawa, H. Imataka, Y. Yamasaki, H. Kusume, H. Abe, Y. Fujii-Kuriyama, CDNA cloning and transcriptional properties of a novel GC box-binding protein, BTEB2, Nucleic Acids Res. 21 (1993) 1527–1532.
[38] R.H. Lyons, B.Q. Ferguson, M. Rosenberg, Pentapeptide nuclear localization signal in adenovirus E1a, Mol. Cell. Biol. 7 (1987) 2451–2456.