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Regulation of the β1,4-Galactosyltransferase I promoter by E2F1

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Cell surface carbohydrate chains are widely known to contribute to cell migration, recognition and proliferation. β1,4-Galactosyltransferase I (β1,4GalT I) transfers galactose to the terminal *N*-acetylglucosamine of complex-type *N*-glycan, and contributes to cell proliferation, differentiation and migration. Here, we identified β1,4GalT I as a novel target gene of cell cycle regulator E2F1. E2F1 proteins interact with the promoter of the β1,4GalT I gene *in vivo*, and E2F1 over-expression stimulates the activity of β1,4GalT I promoter and the mRNA and protein expression of β1,4GalT I, and augments the level of β1, 4-galactosyltion. Site-specific mutagenesis revealed that this region which contains two E2F1 binding site (nt −215 to −207 and +1 to +6) is necessary for β1,4GalT I activation by E2F1. Furthermore, down-regulation of β1,4GalT I expression attenuates E2F1-induced DNA synthesis and cell cycle progression as well as the expression of cell-cycle regulator Cyclin D1. Thus, β1,4GalT I is an important E2F1 target gene that is required for cell cycle progression in mammalian cells, which elicits a new mechanism of cell growth and a new mechanism of β1,4GalT I transcription.

Keywords: cell cycle/E2F1/β1,4-galactosyltransferase I/promoter/transcriptional regulation.

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; nt, nucleotide; oligo, oligonucleotide; SDS–PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

It is widely known that sugar residues and oligosaccharide chains of glycoproteins play diverse and crucial roles in cell adhesion, metastasis and growth (*1*, *2*). β1,4-Galactosyltransferase I (β1,4GalT I) is one of the key enzymes involved in the biosynthesis of Galβ → 4GlcNAc units in *N*-glycans and core 2 *O*-glycans of glycoproteins (*3*, *4*), including IgG, EGFR, PDX-1, gp120 and serum α-fetoprotein (*5–9*). On the other hand, cell surface β1,4GalT I acts as a recognition molecule and participates in a number of cellular interactions, including neurite extension, cell growth, sperm–egg interaction, cell spreading and migration (*6*, *10*, *11*). Biologically, β1,4GalT I-knockout mice exhibited growth retardation and inflammatory responses suppression, and showed significantly delayed wound healing with reduced re-epithelization, collagen synthesis and angiogenesis (*3*, *12–15*). In contrast with this, accumulated evidence indicated that β1,4GalT I is involved in the ability of tumor cell migration, invasion and growth (*16–19*), indicating the contribution of β1,4GalT I in tumor behavior. Accumulated evidence showed that the mRNA expression of β1,4GalT I is cell cycle regulated in mammalian cells, and β1,4GalT I regulates cell cycle progression (*18*, *20–22*).

The E2F transcription factors are key regulators of cell proliferation, development and differentiation (*23*). For regulating cellular proliferation, they are known to regulate the transcription of a number of genes involved in the G1/S-phase transition and DNA replication in mammalian cells (*23–27*). Among them, E2F1, one of the best characterized members of the E2F transcription factor family, regulates DNA replication and cell cycle progression (*23*, *24*, *26*). Deregulation of E2F1 transcriptional activity by Rb inactivation may be a key event in most human cancers (*28–31*).

To elucidate the molecular mechanism regulating β1,4GalT I expression and the contribution of β1,4GalT I expression to cell proliferation, we investigated the transcriptional regulation of β1,4GalT I and the effect of the inhibition of β1,4GalT I expression on cell cycle progression. Here, we report that β1,4GalT I expression is regulated by the E2F1 transcription factors. In addition, we demonstrate that the inhibition of β1,4GalT I expression impedes E2F1-induced cell cycle progression.

Materials and Methods

Materials

Restriction enzymes, bovine calf serum, Dulbecco's modified Eagle's medium, Trizol Reagent and the mammalian expression plasmid pcDNA3.0 were from Invitrogen. PMSF, aprotinin and pepstatin were from Sigma Chemical Co. γ-^{32}P-dATP and the enhanced chemiluminescence (ECL) assay kits were from Amersham Pharmacia Biotech. Takara RNA PCR Kit (AMV Ver.3.0) and Takara MutanBEST kit were from TaKaRa. Anti-Cyclin D1 antibody, anti-Cyclin D2 antibody, anti-Cyclin D3 antibody, anti-human-E2F1 antibody and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody were from Santa Cruz

Biotechnology. Anti-human-β1,4GalT I antibody was from Sigma Chemical. Anti-mouse-HRP secondary antibody and anti-rabbit-HRP secondary antibody were purchased from New England Biology. Biotinylated RCA-I was purchased from Vector Laboratories. FITC-conjugated streptavidin and HRP-conjugated streptavidin were purchased from Southern Biotechnonlogy Associates. Other reagents were commercially available in China.

Plasmids

Expression constructs for β1,4GalT I shRNA, pRL-CMV, pSilencer-2.0, β1,4GalT I promoter construct pG3L(−1653/+52) and pGL3Basic have been described previously (*18*). pGL3(−215/+52) site-directed mutagenesis constructs were derived from pGL3(−215/+52) by PCR amplification using TakaRa MutanBEST mutagenesis kit. Expression constructs for E2F1 and E2F1 shRNA were generous gifts from Prof. Cress Doug. LacZ shRNA was constructed as previous described (*32*).

Cell lines and cell transfection

Human glioma cell line U87, human hepatoma cell line HepG2, human lung adenocaroinoma cell line A549, human osteosarcoma cell line U2OS and human kidney cell line 293T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 50 μg/ml streptomycin at 37°C in a humidified CO_2 incubator (5% CO_2, 95% air). Cell transfection was performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells was harvested 48–72 h after transfection.

Chromatin immunoprecipitation assay

The association of E2F1 with β1,4GalT I chromatin DNA in U2OS cells was confirmed using a chromatin immunoprecipitation (ChIP) assay kit (Upstate Biotechnology) with anti-E2F1 antibody as described by the manufacturer. Normal anti-mouse IgG was used as a negative control. The β1,4GalT I promoter region (nt −215/+52) was amplified by conventional PCR.

Reverse transcriptase polymerase chain reaction

Total RNA (1 μg) extracted from cells were used as a template for cDNA synthesis. cDNA was prepared by use of a TaKaRa RNA PCR Kit. Primers used for PCR were as follows: β1,4GalT I forward 5′-ATGAGGCTTCGGGAGCCGCTCCTG-3′, reverse 5′-CTAGC TCGGTGTCCCGATGTC-3′. Amplification was carried out for 22–27 cycles under saturation, each at 94°C, 45 s; 60°C, 45 s; 72°C, 1 min in a 50-μl reaction mixture containing 2 μl each cDNA, 0.2 μM each primer, 0.2 mM dNTP and 2.5 units of Taq DNA polymerase. After amplification, 10 μl of each reaction mixture was analysed by 1% agarose gel electrophoresis, and the bands were then visualized by ethidium bromide staining. The PCR products for β1,4GalT I were 1197 bp.

Analysis of cell cycle by FACS, western blot analysis and dual luciferase assay

Cells were harvested and measured by flow cytometry 48 h after transfection, as previously reported (*18*). Western blot and dual luciferase assay were performed as described (*33*, *34*).

Flow cytometry of glycan level of membrane protein and lectin blotting

After washed with PBS, cells were harvested after treatment with EGTA (2 mM) and incubated with biotinylated RCA-I (2 μg/μl) for 45 min at 4°C. Then, the cells were washed with PBS and probed with FITC-conjugated streptavidin (1 : 128) for 30 min at 4°C. Next, the cell samples were subjected to flow cytometry. Lectin blotting assay was performed as in our previous report (*33*, *34*). Cells lysates containing 30 μg of protein were boiled in SDS sample buffer with β-mercaptoethanol, loaded on 8% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) gels, and then transferred onto a PVDF membrane. The membrane was treated with 25 mM H_2SO_4 at 80°C for 60 min to remove sialic acid residues. After being blocked with 5% BSA, the membrane was incubated with 1 : 1000 dilution of biotinylated-RCA I for 2 h at room temperature. After being washed for three times, the membrane was incubated with 1 : 2000 dilution of HRP-conjugated streptavidin for 1 h at room temperature. The blots were washed and developed with the ECL detection system using X-ray film.

Gel shift assay

Gel mobility shift assay was carried out using Gel Shift Assay System (Promega) as follows. The double-stranded oligonucleotide corresponding to human β1,4GalT I promoter sequence nt −215/−185 and −3/+52) and E2F1 consensus oligonucleotide (5′-ATTTAAGTTTCGCGCCCTTTCTCAA-3′) were annealed, end-labelled with ^{32}P using T4 polynucleotide kinase, and purified using Sephadex G-25 quick spin columns (Roche Molecular Biochemicals). Nuclear proteins were pre-incubated for 10 min with 9 μl of electrophoretic mobility shift assay buffer. Then the ^{32}P-end-labelled duplex oligonucleotide (1 μl, 10 fmol) was added, and the reaction was incubated for 20 min on ice. For competition experiments, unlabelled DNA probes were included at 100-fold molar excess over the ^{32}P-labelled DNA probe. DNA–protein complexes were separated on 5% non-denaturing polyacrylamide gels in 0.5× Tris borate/EDTA (pH 8.4) at 4°C and 35 mA. The gels were dried, and the DNA–protein complexes were visualized by autoradiography.

Statistics and presentation of data

All experiments were repeated at least three times. All numerical data are expressed as means ± SDs.

Results

Activation of the β1,4GalT I promoter by E2F1

To determine trans-activating effects of E2F1 on the β1,4GalT I gene, transfection studies using the β1,4GalT I promoter-luciferase reporter pGL3 (−1653/+52) and increasing amounts of E2F1 expression plasmid were performed. The forced expression of E2F1 potently stimulated the β1,4GalT I promoter in a dose-dependent manner in a variable cells, with a maximum activation of 36-fold in U2OS cells (Fig. 1A). To test whether the β1,4GalT I promoter could be regulated by other E2F proteins in addition to E2F1, U2OS cells were co-transfected the β1,4GalT I promoter-luciferase reporter pGL3(−1653/+52) with plasmids that express E2F1, E2F2, E2F3, E2F4, E2F5 or E2F6. Expression of E2F1 significantly stimulated the luciferase reporter gene 20-fold compared with the control vector. Expression of E2F3 and E2F4 also stimulated the luciferase reporter gene 2-fold compared with the control vector, whereas E2F2, E2F5 and E2F6 did not show significant activation of the β1,4GalT I promoter I promoter (Fig. 1B).

To characterize E2F1 transcription factor involvement in the induction of β1,4GalT I mRNA expression, reverse transcriptase polymerase chain reaction (RT–PCR) assays were conducted in U2OS cells transiently transfected with control or E2F1 plasmid. Compared to the control cells, E2F1 overexpression in U2OS cells induced β1,4GalT I mRNA expression (Fig. 1C). Consistent with this, E2F1 overexpression in U2OS cells significantly induced the protein expression of β1,4GalT I and Cyclin D3 (Fig. 1D, left panel), which is a known target gene of E2F1 (*35*). To assess the contribution of E2F1 in the level of β1,4-galactosyltion, lectin blot was performed using RCA-I lectin which interacts with oligosaccharides terminating with the Galβ1 → 4GlcNAc group (*34*). As expected, a significant increase of the binding of total glycoprotein with RCA-I lectin was observed for 34–43 kDa protein bands in the U2OS cells

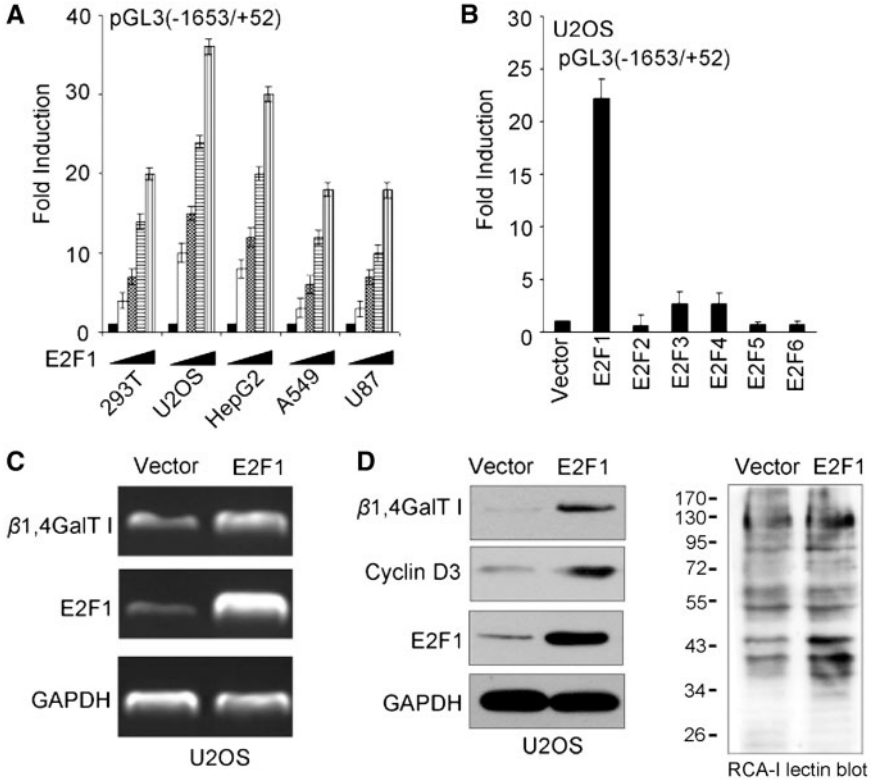

Fig. 1 Activation of the β1,4GalT I promoter by E2F1. (A) Increasing amounts of E2F1 expression plasmids were co-transfected into 293T, U2OS, HepG2, A549 and U87 cells with pGL3(−1653/+52) and pRL-CMV constructs, and the luciferase activity was assayed. Firefly luciferase activities were normalized by Renilla luciferase activities and standardized to the normalized activity from pGL3(−1653/+52) with vector alone. Each value is the mean ± SD of at least three independent experiments. (B) pGL3(−1653/+52) constructs were co-transfected into U2OS cells with and vectors containing E2F1, E2F2, E2F3, E2F4, E2F5, E2F6 or the empty control vector. Firefly luciferase activities were normalized by Renilla luciferase activities and standardized to the normalized activity from pGL3(−1653/+52) with vector alone. Each value is the mean ± SD of at least three independent experiments. (C) RT–PCR analysis of β1,4GalT I mRNA and E2F1 mRNA expression level in U2OS cells transfected with control vector or E2F1 expression construct. The level of GAPDH mRNA expression served as a loading control. (D) Western blot analysis of β1,4GalT I, Cyclin D3 and E2F1 protein expression level in U2OS cells transfected with control vector or E2F1 expression construct (left panel). The proteins were also separated by SDS–PAGE and the binding to RCA-I was analysed by RCA-I-lectin (right panel). The GAPDH western blot served as a loading control.

over-expressing E2F1 as compared to that of control cells (Fig. 1D, right panel). Thus, these results demonstrate that E2F1 transcription factor could mediate regulation of the β1,4GalT I gene.

Identification of the cis*-elements responsible for the effect of E2F1*

Next, deletion analysis was then performed to define functionally important *cis*-elements in this 1705-nt region. Luciferase assays showed that a deletion from nt −215 to +52 resulted in a drastic decrease in the promoter activity (data not shown), and loss of E2F1 activation as compared with that of the pGL3 (−318/+52) construct (Fig. 2A), indicating that the critical E2F1-responsive element resides within a high GC-rich region between nt −215 and +52.

Inspection of this 267-nt region revealed three potential E2F1 protein binding sites (Fig. 2B). We generated point mutations in these three E2F1 protein binding sites, either individually or in combination (Fig. 2B), and tested the activation of the mutant β1,4GalT I promoter by the overexpression of the E2F1 proteins. As shown in Fig. 2C, mutation of the E2F1 binding site at nt −215 to −207 or nt +1 to +6 partially decreases the activation of the β1,4GalT I promoter by E2F1. Simultaneous mutation of both E2F1 sites almost completely abolished the activation of the β1,4GalT promoter by E2F1 protein (Fig. 2C), indicating that both E2F1 sites contribute to the regulation of the β1,4GalT I promoter by E2F1 protein. Furthermore, the activity of luciferase reporter containing simultaneous mutation of both E2F1 sites was decreased to almost the same level as pGL3-basic (Fig. 2D), indicating the essential role of these site in β1,4GalT I transcription.

Endogenous E2F1 proteins bind to the β1, 4GalT I promoter in vivo

Having shown that the β1,4GalT I promoter could be activated by E2F1 proteins in transient transfection assays, we sought to determine whether endogenous E2F1 proteins bind to the β1,4GalT I promoter *in vivo*. For this purpose, we carried out ChIP assays by using antibodies specific for E2F1. To ensure that the E2F1 antibody that we used can specifically immunoprecipitate the DNA fragments known to be bound by E2F1 *in vivo*, we tested the immunoprecipitation of Cyclin D3 promoter sequences by the E2F1 antibody.

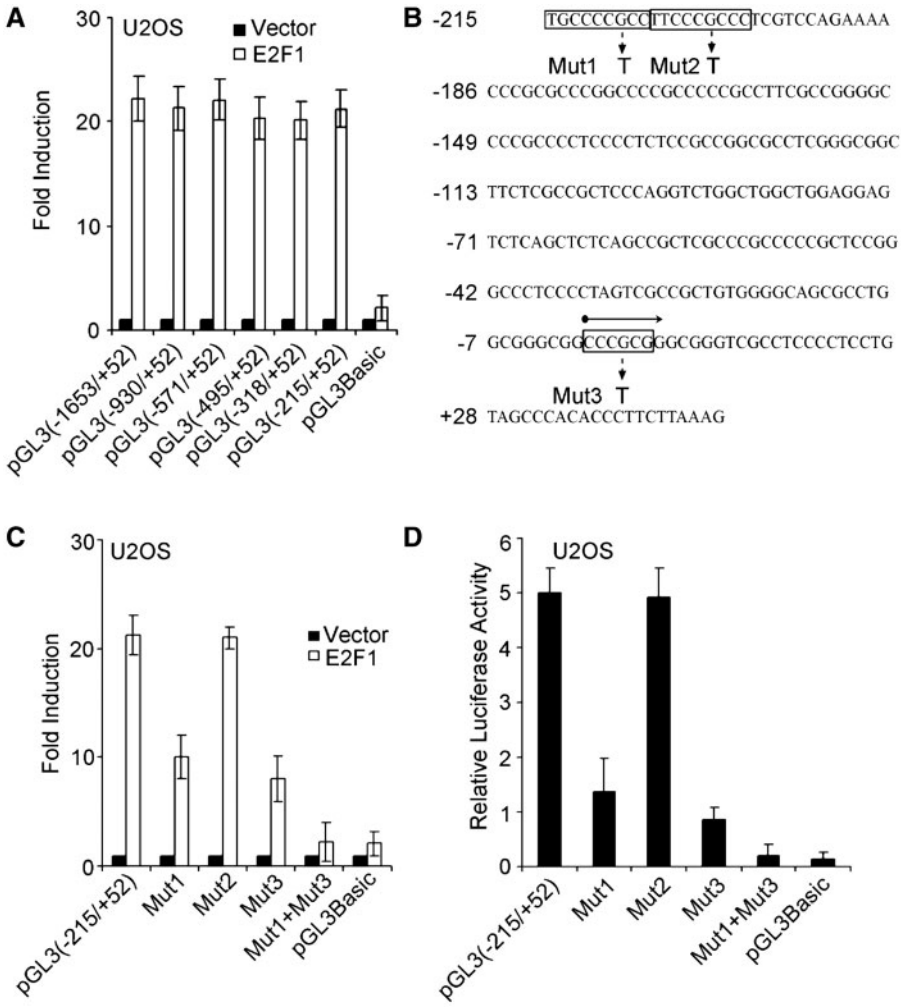

Fig. 2 E2F recognition sites are required for activation of the β1,4GalT I promoter by E2F proteins. (A) Mapping the regions of the β1,4GalT I promoter necessary for E2F1 responsiveness. U2OS cells were transfected with pGL3(−1653/+52) construct or with the truncated β1,4GalT I promoter constructs shown above and with or without E2F1 expression vector. Luciferase activity was normalized to Renilla luciferase activity and standardized to the normalized activity from luciferase reporter construct with vector alone. Each value is the mean ± SD of at least three independent experiments. (B) Nucleotide sequence of β1,4GalT I promoter region between nt −215 and +52. Numbers at the left referred to the transcription start site, which is indicated with an arrow and taken as +1. Potential E2F1 transcription factor binding sites identified by searching TRANSFAC transcription factor data base are labelled. The mutated nucleotides used in (B) are indicated using pane. (C) Responses of wild-type and mutant β1,4GalT I promoters to activation by E2F1 proteins. U2OS cells were transfected with the vector or the indicated E2F1 expressing plasmid together with the indicated luciferase reporter constructs. Firefly luciferase activities were normalized by Renilla luciferase activities and standardized to the normalized activity from luciferase reporter construct with vector alone. Each value is the mean ± SD of at least three independent experiments. (D) U2OS cells were transfected with the indicated luciferase reporter constructs. Firefly luciferase activities were normalized by Renilla luciferase activities. Each value is the mean ± SD of at least three independent experiments.

E2F1 antibodies precipitated promoter fragments from the Cyclin D3 gene in U2OS cells (*35*), but the antibody precipitated negligible amounts of promoter fragments from the β-actin gene, which is not regulated by E2F1. Similar to the results obtained with the Cyclin D3 promoter, the E2F1 antibody also immunoprecipitated a DNA fragment from the β1,4GalT I promoter, while the control antibody failed to precipitate the same β1,4GalT I promoter fragment (Fig. 3A). Next, EMSA was further performed to determine whether E2F1 recognizes the β1,4GalT I promoter. Incubation of the double-stranded oligonucleotide probe between nt −215 to −185 or −3 to +52 of the β1,4GalT I promoter with U2OS nuclear extracts formed specific protein-DNA complex (Fig. 3B, lanes 1 and 2), which had the similar mobility to the complex formed by E2F1-consensus sequence probe and U2OS nuclear extracts (Fig. 3B, lane 3). Furthermore, the complex formed by E2F1-consensus sequence probe and U2OS nuclear extracts was disrupted by treatment with the unlabelled double-strand oligonucleotides spanning the region between nucleotide positions nt −215 to −185 or −3 to +52 of the β1,4GalT I promoter (Fig. 3B, lanes 4 and 5). Thus, these results show that endogenous E2F1 protein interacts with the β1,4GalT I promoter *in vivo*, supporting the idea that β1,4GalT I expression is regulated by E2F1 *in vivo*.

Down-regulation of β1,4GalT I inhibited E2F1-induced cell cycle progression

E2F-1 overexpression induces genes involved in DNA synthesis and thus G_1-S transition (*23*, *26*).

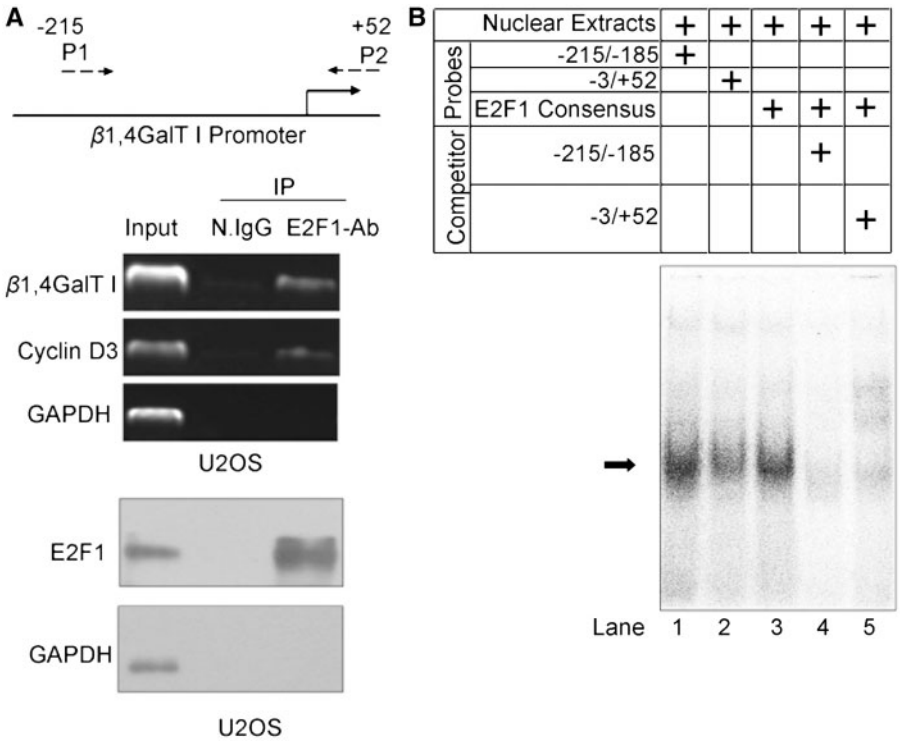

Fig. 3 E2F1 proteins bind the β1,4GalT I promoter *in vivo*. (A) ChIP analysis was performed with U2OS cell extracts by using control IgG or anti-E2F1 antibody. Immunoprecipitated promoter fragments were analysed by PCR to determine the relative presence of E2F1 on the indicated promoters. PCR primers for the β1,4GalT I promoter or the Cyclin D3 promoter was used to detect promoter fragments in immunoprecipitates. The immunoprecipitates were investigated using western blot with anti-E2F1 antibody. (B) EMSA was performed using nuclear proteins of U2OS cells and human β1,4GalT I promoter sequence nt −215/−185 or −3/+52 double-stranded radiolabelled probe and E2F1 consensus oligonucleotides. Competition assays were carried out with a 50-fold excess β1,4GalT I promoter sequence nt −215/−185 or −3/+52 oligonucleotides. The arrowhead indicated the DNA–protein complexes.

To determine whether the β1,4-galactosylation is involved in regulation of the cell cycle, we performed flow cytometry analysis using FITC-conjugated RCA-I to monitor the expression of β1,4-galactosylation in U2OS cells after release from serum starvation. Serum starvation arrested U2OS cells at G_0/G_1 phase, and the addition of serum to the medium stimulated cells started to enter S phase at ~16 h after serum stimulation, and most cells reached S phase by 24 h after stimulation. The binding with RCA-I on the cell surface was low in serum-starved and reached the maximum level when cells when most cells reached S phase by 24 h after stimulation (Fig. 4A). Consistent with this, a gradual increase of the binding of total glycoprotein with RCA-I was observed for 34–45 kDa and 55–72 kDa protein bands in response to serum stimulation (Fig. 4B). These background motivated us to examine the contribution of β1,4GalT I in E2F1-induced cell cycle progression. To address this point, β1,4GalT I shRNA construct was transiently transfected into U2OS cells with control or E2F1 expression construct. Cell cycle analysis showed that U2OS/β1,4GalT I shRNA had a much lower percentage cells in the S phase and a higher percentage cells in the G_0/G_1 phase, compared to U2OS/LacZ shRNA cells. Furthermore, down-regulation of β1,4GalT I obviously inhibited the positive effect of E2F1 on cell cycle progression (Fig. 4C).

To further examine the effect of the inhibition of β1,4GalT I expression on cell cycle progression, we monitored the incorporation of BrdU in U2OS cells co-transfected with β1,4GalT I shRNA and control or E2F1 expression construct. Consistent with previous reporter (*36*), E2F1 overexpression significantly increased the incorporation of BrdU in U2OS and β1,4GalT I down-regulation reduced the incorporation of BrdU in U2OS in presence or absence of E2F1 overexpression (Fig. 4D). Cyclin D members are important regulators of cellular proliferation and are involved in DNA synthesis and thus G_1-S transition (*37*). The alterations of the oligosaccharides structure on glycoprotein regulate the expression of Cyclin D (*38–40*). Thus, we investigated the effect of β1,4GalT I down-regulation on the expression of Cyclin D. Down-regulation of β1,4GalT I inhibited the expression of Cyclin D1 and not obviously changed the expression of Cyclin D3 (Fig. 4E). The expression of Cyclin D2 was faintly expressed in U2OS cells. Thus, β1,4GalT I plays a role in cell cycle progression and contributes to E2F1-induced cell cycle progression.

Discussion

It is a well-known fact that *N*-linked oligosaccharides on glycoprotein are structurally altered during malignant transformation and that these alterations have a

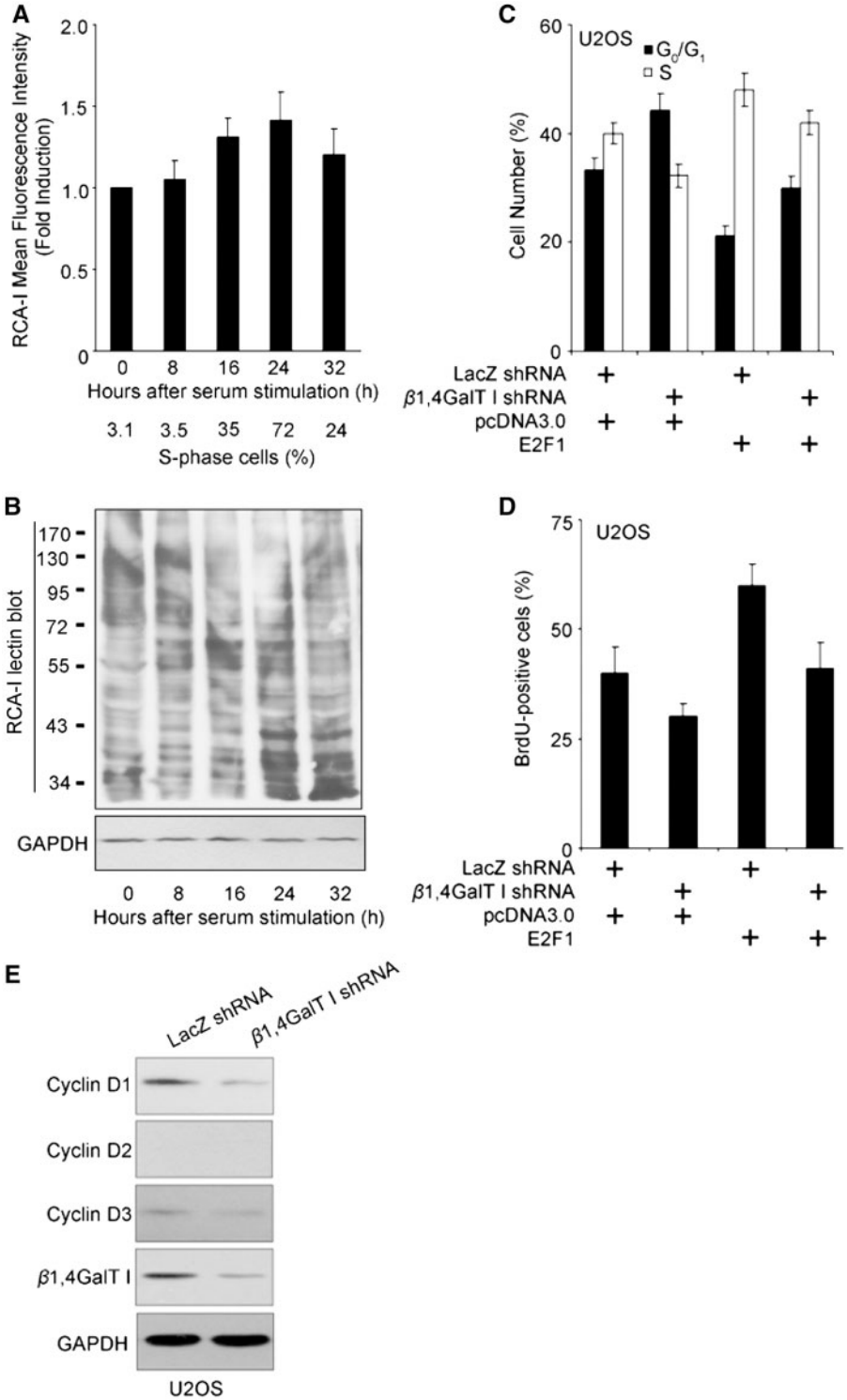

Fig. 4 β1,4GalT I regulates cell cycle progression. (A and B) U2OS cells were serum starved and re-stimulated to enter the cell cycle by serum stimulation. At the indicated times after serum addition, the cell cycle distribution of the cells was analysed by FACS. The ration of S phase cells was indicated. Cells were incubated with biotinylated RCA-I followed by incubation with FITC-conjugated streptavidin. Analysis was performed using FACScan. The RCA-I mean fluorescence intensity was normalized to that of serum-starved cells. Each value is the mean ± SD of at least three independent experiments (A). The proteins were also separated by SDS–PAGE and the binding to RCA-I was analysed by RCA-I-lectin blot. The GAPDH western blot served as a loading control (B). (C) U2OS cells were transiently transfected with control LacZ shRNA, and/or β1,4GalT I RNAi, and/or E2F1 expression construct. After 48 h transfection, cell cycle parameters were determined. Data shown is the means ± SD of at least four independent experiments. (D) U2OS cells transiently transfected with control LacZ shRNA, and/or β1,4GalT I RNAi, and/or E2F1 expression construct were labelled with BrdU. The data represent the means and standard deviations from three independent experiments. More than 300 cells were analysed in each experiment. (E) Western blot analysis of β1,4GalT I, Cyclin D1, Cyclin D2 and Cyclin D3 protein expression level in U2OS cells transfected with control vector or β1,4GalT I shRNA construct. The GAPDH western blot served as a loading control.

biological meaning (*1*, *2*, *41–47*). β1,4GalT I is the enzyme responsible for the biosynthesis of *N*-acetyllactosamine on *N*-glycans by transferring UDP-galactose to the terminal *N*-acetylglusamine (*N*-GlcNAc) residues and also acts as a recognition molecule to participate in a number of cellular interactions, including neurite extension, cell growth, cell spreading and migration (*4*, *6*, *12*, *13*, *17*, *48–50*). Accumulated evidences have shown that β1,4GalT I expression could be regulated by a variety of stimuli. Transfection of p16, a cyclin-dependent-kinase inhibitor, into A549 human lung cancer cells led to down-regulation of β1,4GalT I (*21*). Inhibition of cell cycle related protein TGF-β in SMMC-7721 cells showed the same result (*22*), indicating that β1,4GalT I is regulated at the mRNA level during growth stimulation as well as during the cell division cycle.

The important finding of this study was the characterization of β1,4GalT I as a novel target gene of E2F1 transcription factor. The E2F1 gene, one of the best-characterized members of the E2F1-transcription factor family, is widely known to contribute in the transition from G_0/G_1 to S phase (*51*). Deregulation of E2F1 transcriptional activity by Rb inactivation contributes to the development cancer (*52*, *53*). Several lines of evidence presented in this study indicate that β1,4GalT I is a bona fide E2F1 target gene. First, in transient transfection experiments, the β1,4GalT I promoter is activated by E2F proteins through the E2F1 recognition sequences in the promoter. Second, mutations of the E2F sites impair transcriptional activation of the β1,4GalT I promoter. Third, endogenous E2F proteins associate with the β1,4GalT I promoter *in vivo*, and E2F1 activation induces the expression of β1,4GalT I gene and increases the level of the level of β1,4-galactosyltion. Furthermore, cell biological experiments suggest that β1,4GalT I contributes to E2F1-induced cell cycle progression. Thus, β1,4GalT is an important E2F1 target that is required for cell cycle progression in mammalian cells. The effect of E2F1 on the expression of β1,4GalT II–VII were also analysed in U2OS cells. It was found that the expression of β1,4GalT II–VII was not obviously changed in response to E2F1 activation, though the TRANSFAC search program predicted the E2F putative recognition sites in the promoter of β1,4GalT II–VII (our unpublished data).

E2F activity is negatively regulated by tumour suppressor pRB and related proteins p107 and p130, which in turn are inactivated by cyclin D-dependent kinase Cdk4/Cdk6 (*54*). This pRB/E2F pathway is deregulated in virtually all human cancers (*30*, *43*). In view of our observation that β1,4GalT I is a critical target of E2F and could promote S-phase entry in mammalian cells, it is tempting to speculate that the deregulated expression of β1,4GalT I may also contribute to tumorigenesis in human cancers. β1,4GalT I down-regulation reduces the expression of Cyclin D1. Further studies are needed to explore the molecular mechanism of β1,4GalT I-regulating cell cycle progression.

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Conflict of interest

None declared.

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