Gut-enriched Krüppel-like factor regulates colonic cell growth through APC/β-catenin pathway

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Abstract Studies on colorectal carcinogenesis have suggested a critical role of the adenomatous polyposis coli (*APC*) gene in the development of colorectal cancer. Gut-enriched Krüppel-like factor (GKLF) is a zinc-finger transcription protein with high expression in the colonic epithelium. Our previous studies have shown that GKLF transcript was significantly decreased in colon cancer tissue and suggested that it might play a role in the tumorigenesis of the colon. The signaling pathway of GKLF-regulated cell growth is currently unknown. We sought to determine if the functions of GKLF are mediated through the APC/β-catenin pathway. In a colon cancer cell line (HT29-APC), containing a zinc-inducible *APC* gene, GKLF mRNA levels were significantly increased when wild-type APC protein was induced. No effect on GKLF mRNA concentration was observed in a control cell line (HT29-β-gal), containing an analogous inducible *lacZ* gene. GKLF promoter activity was induced by co-transfection with wild-type *APC* DNA, suggesting that *APC* might be involved in transcriptional activation of the GKLF gene. In HT-29 cells, overexpression of GKLF resulted in decreases in β-catenin protein and mRNA levels and down-regulation of GKLF expression led to increase in β-catenin concentration. Overexpression of GKLF in HT-29 cells inhibited DNA synthesis and this effect was attenuated by co-transfection with wild-type β-catenin, suggesting an essential role of β-catenin in GKLF signaling. Furthermore, co-transfection of GKLF in colon cancer SW 480 cells abrogated the transcriptional activity of a β-catenin-T-cell factor (Tcf) reporter construct in a dose-dependent manner. These findings indicate that the growth-suppressive effect of GKLF may be mediated through the APC/β-catenin pathway. We speculate that when APC is mutated, GKLF gene expression is down-regulated, resulting in increases in β-catenin level and transactivation of growth-promoted genes.

Key words: Colonic cancer; Gut-enriched Krüppel-like factor promoter; Gene expression; Cell growth

1. Introduction

Colorectal cancer is one of the most common and potentially fatal diseases encountered in Western countries. Molecular analysis of colon cancer patients has shown that mutations of the adenomatous polyposis coli (*APC*) gene are present in the majority of sporadic human colorectal carcinomas [1]. The current concept of colonic carcinogenesis begins with inactivation or loss of the APC gene, followed by sequential loss of other tumor suppressor genes or activation of cellular oncogenes. A mutated APC gene produces a truncated protein that lacks the ability to induce degradation of β-catenin. Free β-catenin then translocates to the cell nucleus, interacts with T-cell factor 4 (Tcf-4) transcription factor which then leads to transcriptional activation of several growth-promoting genes including c-Myc and cyclin D1 [2–4].

Recently, a zinc-finger transcription factor GKLF (gut-enriched Krüppel-like factor), also known as Krüppel-like factor 4 (KLF4), was found to be expressed extensively in the colon. Previous studies have shown that GKLF mRNA expression was enhanced in growth-arrested cells and its level decreased prior to the initiation of DNA synthesis suggesting that GKLF might mediate growth arrest [5]. Recently, our laboratory has found that GKLF mRNA levels were significantly reduced in human adenomatous polyps and colon cancer tissue, and that overexpression of GKLF in colon cancer cells significantly inhibited cyclin D1 activity [6,7]. These results suggest that down-regulation of GKLF expression might promote cyclin D1 promoter activity and result in abnormal cell growth in the colon [7]. As stated above, cyclin D1 appeared to be one of the downstream targets of the APC/β-catenin pathway. These data suggest that GKLF and APC may share similar biological properties. In a murine colon carcinogenesis model, GKLF mRNA levels were significantly decreased in multiple intestinal neoplasia (Min) mice possessing mutated APC (APC−/−) protein when compared to wild-type (APC+/+) mice [8]. These results also indicate that the expression of GKLF may be regulated by APC. We therefore hypothesized that the growth-inhibitory effect of GKLF might be mediated through the APC/β-catenin pathway and current studies were undertaken to explore these possibilities.

2. Materials and methods

2.1. Cell culture and creation of stable cell lines

A human colon adenocarcinoma cell line (HT29-APC), containing a zinc-inducible *APC* gene and a control cell line (HT29-β-gal), containing an analogous inducible *lacZ* gene, were kindly provided by Dr. Vogelstein (Johns Hopkins University, Baltimore, MD, USA). When HT29-APC cells were cultured in the presence of 100 μM zinc up to 9 h, the wild-type APC protein was induced [9]. Two additional human colon adenocarcinoma cell lines, HT-29 and SW 48 (APC+/+), were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and SW 480 (APC−/−) was also kindly provided by Dr. Vogelstein. All cells were cultured at 37°C in a 95% air plus 5% CO_2 atmosphere in McCoy's 5A medium (Life

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Technologies, Inc., Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin and 100 U/ml penicillin.

Previous studies from this laboratory have shown that Arg^{390} of human GKLF confers its nucleus localization signal and that mutation of Arg^{390} to Ser (RR/RS) completely abolishes the growth-inhibitory effect of GKLF [10]. To explore the signaling pathways of GKLF-mediated cell growth, HT-29 cells were stably transfected with GKLF or RR/RS mutant DNA using the Lipofectamine method according to the manufacturer's protocol (Gibco, Gaithersburg, MD, USA). To generate stable cell lines, transfected cells were cultured in the presence of G418 (Gibco) to select neomycin-resistant clones. After 2–3 weeks, 20 single independent clones were randomly isolated from each transfection and examined for the expression of the transfected gene by Northern or Western blot analysis.

2.2. Northern blot hybridization analysis

Total RNA from cells was extracted using the acid/phenol method of Chomczynski and Sacchi [11]. The RNA was then electrophoresed on a 1.5% agarose/6% formaldehyde gel and Northern blot hybridization analysis was done using stringent conditions as described previously [12]. Hybridization was performed using human GKLF, β-catenin (cDNAs kindly provided by Dr. Vogelstein), or glyceraldehyde-3-phosphate dehydrogenase (G3PDH) DNA fragment radiolabeled with [^{32}P]dCTP using Klenow DNA polymerase I. The blots were washed under stringent conditions and autoradiograms were developed after exposure to X-ray film at −70°C, using a Cronex intensifying screen (DuPont, Wilmington, DE, USA).

2.3. Western blot analysis

To obtain whole-cell extracts, cells were washed twice with ice-cold phosphate-buffered saline (PBS), scraped, and pelleted by centrifugation (200×*g*). Cell pellets were then lysed in a standard RIPA buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS) containing protease inhibitors. Protein concentration of each extract was determined by Bio-Rad Laboratories (Hercules, CA, USA) assays and cell extract was separated on the SDS–polyacrylamide gel according to the method described by Laemmli [13]. Protein samples were dissolved in loading buffer (60 mM Tris–HCl, pH 6.8, 2% SDS, 100 mM dithiothreitol, and 0.01% bromophenol blue), heated to 100°C for 3 min, and loaded onto the gel in the electrophoresis buffer containing 25 mM Tris–HCl, pH 8.3, 250 mM glycine, and 0.1% SDS. At the completion of electrophoresis, proteins were transferred to a nitrocellulose membrane (Hybond ECL, Amersham Life Science, Piscataway, NJ, USA). The membrane was incubated overnight in the blocking buffer (10 mM Tris, pH 7.5, 100 mM NaCl, 0.1% Tween 20) containing 5% non-fat powdered milk. The membrane was immunoblotted with β-catenin, or actin antiserum (BD Transduction Laboratory, San Diego, CA, USA). To facilitate transfer of high molecular weight APC protein, electrophoresis was performed in a 3% low melting agarose gel, as described [9]. A monoclonal antibody to APC (Ab-1), reacting with full-length and truncated APC proteins, was used to detect APC proteins (Oncogene Science, Boston, MA, USA). Following incubation with the secondary antibody, the membrane was visualized with enhanced chemiluminescence (Amersham Life Science).

2.4. DNA content analysis using bromodeoxyuridine (BrdU) labeling

To evaluate the effects of β-catenin on cell growth, the extent of DNA synthesis was examined in HT-29 cells stably expressing pcDNA3 or GKLF. Cells were transfected with β-catenin (cDNA was kindly provided by Dr. Vogelstein) or control pcDNA3 plasmid using the Lipofectamine PLUS method according to the manufacturer's instruction (Gibco-BRL). Cells were grown in the medium without serum for 48 h and then cultured in the normal medium containing BrdU to a final concentration of 10 μM for 30 min. Cells were washed twice with cold PBS and fixed in 70% ethanol on ice for 30 min. Cells were then pelleted and 1 ml of 2 N HCl/Triton X-100 was added to denature DNA. The acid was neutralized with 0.1 M $Na_2B_4O_7{\cdot}10\ H_2O$, pH 8.5 and cells were resuspended in 0.5% Tween 20/1% PBS, followed by incubation with anti-BrdU FITC (Becton Dickinson, San Jose, CA, USA) at room temperature for 30 min. After washing once in Tween 20/PBS, cells were resuspended in PBS containing 5 μg/ml of propidium iodide and analyzed on a fluorescence-activated flow cytometer (FACScan, Becton Dickinson).

2.5. Luciferase and β-galactosidase measurements

To examine transcriptional regulation of GKLF promoter by the APC gene, the mouse GKLF gene was isolated by screening a liver genomic λ DASH library (Stratagene, La Jolla, CA, USA) using plaque hybridization. Two positive clones containing GKLF gene were identified and subjected to restriction endonuclease digestion and sequencing ([14], GenBank accession number: AY071827). The full-length GKLF promoter construct, containing 2200 bp upstream from the transcriptional initiation site, was ligated to the pGL3-Luc plasmid (pGKLF-2200). Various truncated promoter constructs were created by restriction endonuclease digestion or by polymerase chain reaction using appropriate primers. All constructs were sequenced and ligated to the pGL3-Luc plasmid containing a firefly luciferase reporter gene. To examine the effect of APC on GKLF promoter activity, SW 480 cells were transiently transfected with pCMV gal and pGKLF DNAs in the presence of APC or control pcDNA3 plasmid using the Lipofectamine method. Full-length and truncated APC cDNAs (APC 331, APC 1309, APC 1941, and APC 2644) were kindly provided by Dr. Bert Vogelstein. The APC 331, 1309, 1941, and 2644 proteins were truncated at codons 331, 1309, 1941 and 2644, respectively [2].

To investigate whether GKLF transactivated β-catenin–Tcf complex, we examined the ability of GKLF to inhibit β-catenin–Tcf-regulated transcription (CRT) in APC−/− SW 480 cells. Cells were co-transfected with pCMV-βgal (0.5 μg), a reporter construct (0.5 μg of pTOPFLASH or pFOPFLASH), and the indicated amount of GKLF or APC expression vectors using the Lipofectamine method. The pTOPFLASH reporter contained an optimized Tcf-binding site 5′ of a luciferase reporter gene, whereas the pFOPFLASH plasmid composed of a mutated site that did not bind Tcf [14]. The GKLF (1580) construct is truncated at codon 1580 of the GKLF cDNA sequence where all three zinc-finger-binding regions are deleted. This construct was previously shown to be non-functional and was used for negative control in this study [7].

For luciferase assays, cells were washed twice with PBS 48 h after transfection and then lysed in 500 μl of lysis buffer following the manufacturer's instructions (Analytical Luminescence, San Diego, CA, USA). To assay luciferase activity, 100 μl of the cell lysate was mixed with 100 μl of luciferase substrate solution A (Analytical Luminescence). Using a luminometer with automatic injection, 100 μl of substrate solution B (Analytical Luminescence) was then added, and luciferase activity was measured as the light emission over a 30 s period.

β-Galactosidase activity in 40 μl of the cell lysate was determined after 5–30 min incubation at 37°C with 2 mM chlorophenol red β-galactopyranoside (Boehringer-Mannheim, Indianapolis, IN, USA) in 2 nM $MgCl_2$, 0.1 mM $MnCl_2$, and 45 mM 2-mercaptoethanol, and 100 mM $NaHPO_4$, pH 8.0. The reactions were stopped by adding 500 μl of 0.5 M EDTA, pH 8.0, and the absorbance at 570 nm was measured using a spectrophotometer. With each experiment, luciferase activity was determined in duplicate and normalized to β-galactosidase activity for each dish.

2.6. Statistics

Results were expressed as mean ± S.E.M. Statistical analysis was performed using analysis of variance and Student's *t*-test. A *P*-value less than 0.05 was considered to be statistically significant.

3. Results

3.1. APC regulates GKLF gene expression

The effects of APC on GKLF gene expression were examined in HT29-APC and HT29-β-gal cells at 3, 6, and 9 h after $ZnCl_2$ induction. As illustrated in Fig. 1, full-length wild-type APC protein (FL.APC) first appeared at 3 h and remained detectable at 9 h after $ZnCl_2$ induction in HT29-APC cells (A). In contrast, only mutant APC protein (MT.APC) was detected in control HT29-β-gal cells (B). These results were consistent with those reported by Morin et al. [9]. In HT29-APC cells, the induction of wild-type APC protein is associated with the increases of GKLF mRNA concentration (C), whereas no significant change in GKLF mRNA levels was observed in HT29-β-gal cells (D). The expression of

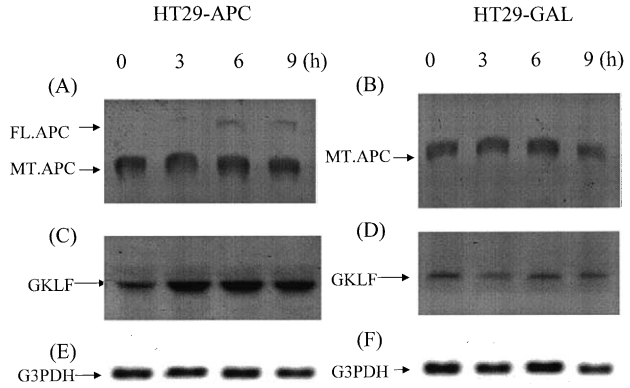

Fig. 1. Detection of APC protein (A, B), GKLF (C, D), or G3PDH (E, F) mRNA by Western or Northern blot analysis in HT29-APC and HT29-Gal cells incubated with 100 μM $ZnCl_2$ for 0, 3, 6, and 9 h. Total protein lysates (80 μg) were assayed by Western blot with monoclonal anti-APC antibody, and 20 μg of total RNA extracted from cells was subjected to Northern blot analysis using human GKLF or G3PDH cDNA probe as described in Section 2. FL.APC: full-length APC protein; MT.APC: mutant truncated APC protein.

G3PDH mRNA transcript was identical in both HT29-APC and HT29-β-gal cells (E, F), indicating the specificity of APC-induced GKLF mRNA expression.

3.2. APC transactivates GKLF promoter

To further examine the role of APC in GKLF gene expression, GKLF promoter constructs were co-transfected with APC or control pcDNA3 DNA into SW 480 cells. The pGKLF-2200, pGKLF-515, and pGKLF-250 constructs contained GKLF promoter sequence −2200, −515, and −250 bp, respectively, upstream of the transcriptional initiation site. As demonstrated in Fig. 2, co-transfection with APC DNA significantly increased GKLF promoter activities in the pGKLF-2200 but not in the pGKLF-515 or pGKLF-250 construct.

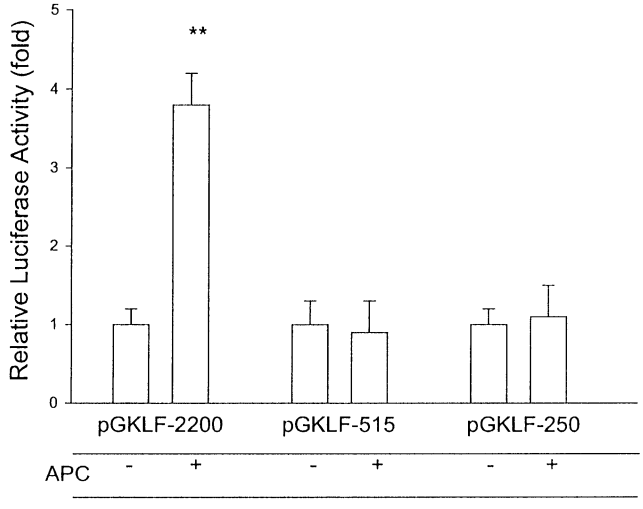

Fig. 2. Induction of GKLF promoter activity by APC. SW 480 cells were co-transfected with GKLF promoter construct (0.5 μg) and 0.5 μg wild-type APC (APC+) or pcDNA3 (APC−; as a control). Luciferase activity was determined 48 h later. pCMV-βgal (0.1 μg) was also co-transfected with each construct to correct for differences in transfection efficiency. Data are expressed as means ± S.E.M. of four separate experiments. $*P < 0.05$, compared to pcDNA3-transfected cells in each individual construct.

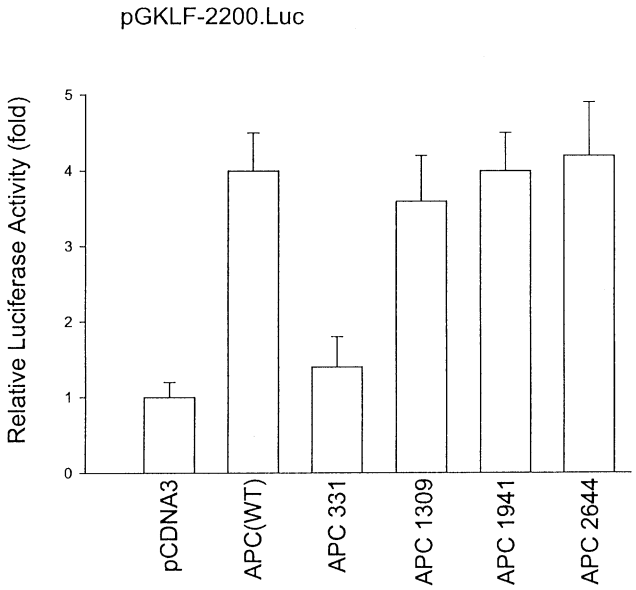

Fig. 3. Effects of wild-type APC (WT) or truncated APC 331, 1309, 1941, or 2644 DNA on pGKLF-2200 promoter activity in SW 480 cells. The pGKLF-2200 reporter plasmid (0.5 μg) was co-transfected with pCMV-βgal (0.1 μg), and 0.5 μg APC (wild-type or truncated) cDNA or pcDNA3 (as control). Luciferase activity was determined 48 h later. Data represent the mean ± S.E.M. of five separate experiments.

These data suggested that APC enhanced GKLF transcriptional activity and that the region between −2200 and −515 of GKLF promoter is important for APC interaction.

The effect of APC on GKLF promoter activity was evaluated by co-transfection with wild-type or truncated APC DNA [2]. As illustrated in Fig. 3, the stimulatory effect of APC on GKLF promoter was observed in wild-type (WT) APC and in truncated APC 1309, APC 1941, or APC 2644 but not in APC 331 constructs, suggesting that the region between codons 331 and 1309 on the APC DNA was important for its function on the GKLF promoter.

3.3. GKLF down-regulated β-catenin expression

The levels of β-catenin protein and mRNA transcript were examined in wild-type HT-29 and HT-29 cells overexpressing GKLF or RR/RS GKLF mutant. Previous studies from this laboratory have shown that a nucleus localization-deficient GKLF mutant (RR/RS) functions as a dominant-negative inhibitor of the GKLF function [10]. As illustrated in Fig. 4, β-catenin protein and mRNA levels were significantly decreased in GKLF-expressed HT-29 cells (GKLF). Conversely, β-catenin protein and mRNA concentrations were significantly induced in cells expressing dominant-negative GKLF mutant DNA (RR/RS). The concentrations of actin protein and G3PDH mRNA were identical in these cells indicating that GKLF may specifically down-regulate β-catenin gene expression.

3.4. β-Catenin attenuates GKLF-mediated growth inhibition

To further explore the role of β-catenin in GKLF-mediated growth arrest, DNA synthesis was examined in HT29-pcDNA3 and HT29-GKLF cells transfected with β-catenin or control pcDNA3 DNA. Flow cytometric analysis of transfected cells was illustrated on Fig. 5A. As shown in Fig. 5B,

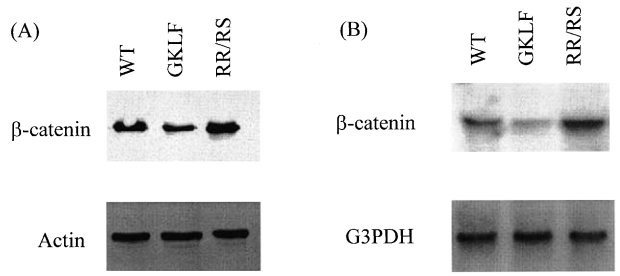

Fig. 4. Expression of (A) β-catenin or actin protein, and (B) β-catenin or G3PDH mRNA in wild-type (WT) HT-29, HT29-GKLF, or HT29-RR/RS cells. Total protein lysates (80 μg) were assayed by Western blot with anti-β-catenin or actin antibody, and 20 μg of total RNA extracted from cells was subjected to Northern blot analysis using human β-catenin or G3PDH cDNA probe as described in Section 2.

compared with control cells (HT29-pcDNA3), overexpression of GKLF (HT29-GKLF) resulted in a significant decrease in cells in S-phase, consistent with the growth-inhibitory property of GKLF. The decrease in DNA synthesis in HT29-GKLF cells was attenuated by co-transfection with wild-type β-catenin DNA, suggesting that this inhibitory effect was probably mediated through β-catenin.

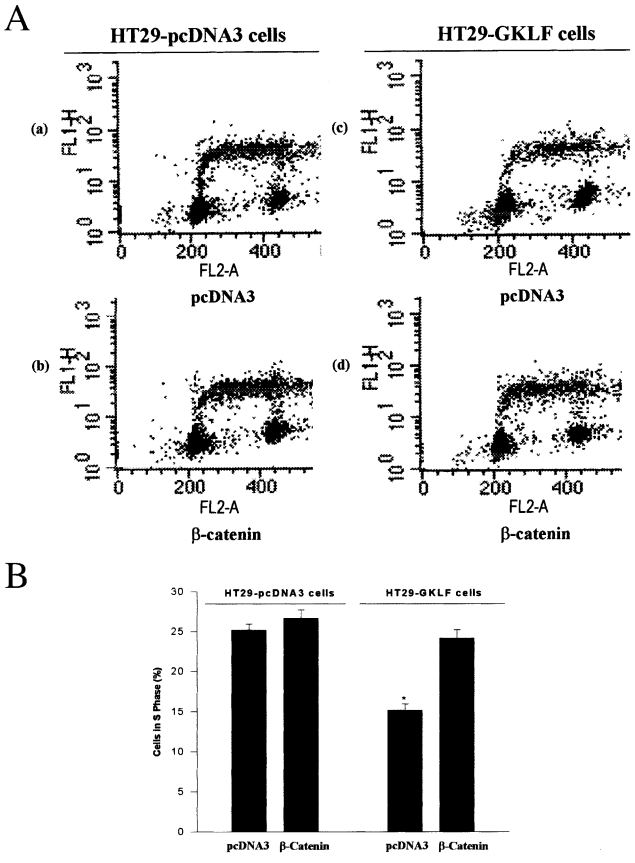

Fig. 5. A: Representative graphs of flow cytometric analysis of HT29-pcDNA3 transfected with control (a) or β-catenin plasmid (b), and HT29-GKLF cells transfected with control (c) or β-catenin (d) cDNA. B: Percent of cells in the S-phase of HT29-pcDNA3 and HT29-GKLF cells transfected with pcDNA3 or wild-type β-catenin. Each data point represents the mean ± S.E.M. of four separate experiments. *$P < 0.05$ compared to HT29-pcDNA3 transfected with control plasmid.

3.5. Suppression of β-catenin–Tcf pathway by GKLF

The effect of GKLF on β-catenin–Tcf function was further evaluated by examining its inhibition on CRT in SW 480 cells. As shown in Fig. 6, wild-type APC significantly inhibited CRT reporter activity in a dose-dependent manner but a truncated mutant (APC 331) failed to do so. These data were consistent with previous reports from Morin et al. showing the suppressive effect of APC on the β-catenin–Tcf pathway [2]. Similarly, co-transfection of GKLF with pTOPFLASH reporter significantly inhibited CRT activity and the inhibition was also dose-dependent (Fig. 6). Moreover, GKLF-mediated inhibition of CRT was not seen in cells transfected with the truncated non-functional GKLF construct (GKLF-1580) or with pFOPFLASH reporter plasmid demonstrating the specificity of the repressive effects of GKLF on β-catenin–Tcf signaling (Fig. 6).

4. Discussion

GKLF is a member of the Krüppel family of transcription factors characterized by the presence of highly conserved multiple zinc-finger motifs at the carboxy-terminus, which represent the DNA-binding domain [15,16]. The amino-terminus contains numerous proline and serine residues, which are transcriptional activation domains [4]. GKLF exhibits a highly restricted pattern of expression in tissues, and in situ hybridization and Northern blot analyses have shown that GKLF is expressed extensively in the gastrointestinal tract but its level is most abundant in the colonic epithelium [17]. As stated above, our laboratory has recently shown that

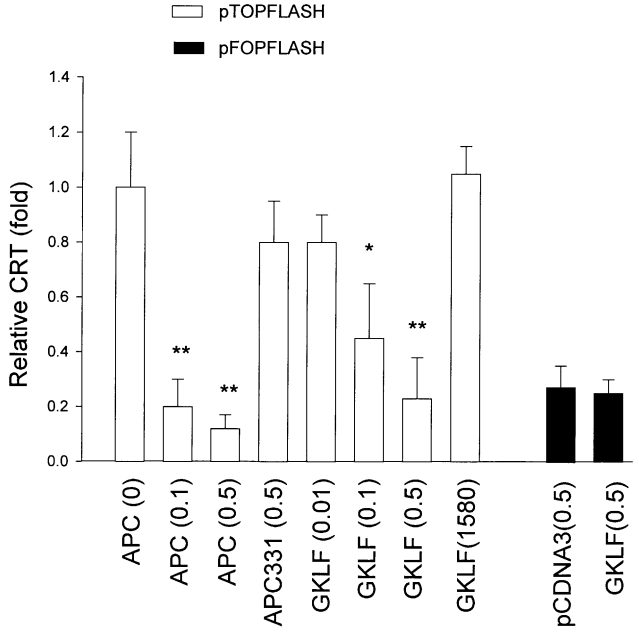

Fig. 6. Effects of APC or GKLF on transactivation of β-catenin–Tcf reporter (CRT). SW 480 cells were transfected with 0.5 μg pTOPFLASH (open bar) or pFOPFLASH (black bar) reporter plasmid and a different concentration (μg) of wild-type APC, truncated APC (APC 331), GKLF, truncated GKLF (1580) or control pCDNA3 DNA, as indicated. CRT reporter activities are expressed relative to the assay containing no APC or GKLF and are expressed as the means ± S.E.M. of four separate experiments. *$P < 0.05$, **$P < 0.01$ compared to control APC (0).

GKLF mRNA levels were significantly decreased in the dysplastic epithelium of the colon, and overexpression of GKLF in HT-29 cells resulted in cell cycle arrest at the G1/S-phase [6]. These data suggest that GKLF may function as a tumor suppressor gene that regulates normal cell growth in the colon.

The findings from the current study support the hypothesis that GKLF exhibits its growth-suppressive effect through the APC/β-catenin pathway. In colon cancer cells, the levels of GKLF mRNA transcript were significantly increased when wild-type APC protein was induced. Furthermore, co-transfection with wild-type APC DNA significantly stimulated GKLF promoter activity, indicating that APC protein played an important role in the transactivation of the GKLF gene. These results also suggest that the expression of GKLF in colonic mucosa may be tightly regulated by APC. These findings were supported by reports from Ton-That et al. [8]. In their study, the expression of GKLF in Min mice, a model that bears a mutation in the murine homologue of the APC gene, was examined. In Min mice, GKLF expression in the intestinal epithelium was down-regulated and GKLF mRNA levels were significantly decreased at the time of tumor development [8]. Together, these findings suggest that an intact wild-type APC gene is necessary for the expression of GKLF gene. When the APC gene was mutated, as commonly seen during the early stage of colonic tumorigenesis, GKLF expression was suppressed, resulting in hyperproliferation of colonic epithelium. Because APC protein is not a transcription factor, it is likely that APC modulates GKLF promoter activity through the interaction with other protein(s) and this hypothesis warrants further investigation. In this report, we have shown that the APC 331 mutant, one of the most common mutations observed in human colorectal carcinoma [18], failed to transactivate the GKLF promoter. Because of the complexity of APC function and its interaction with other proteins, the regulation of GKLF gene expression by APC needs to be further explored.

The involvement of APC/β-catenin–Tcf complex in the regulation of cell growth has recently been extensively investigated. The product of the APC gene is a large protein containing multiple domains that interact with many intracellular proteins including β-catenin, γ-catenin, axin, and glycogen synthase kinase (GSK)-3β [19]. Binding of GSK-3β, APC, axin and β-catenin leads to phosphorylation and subsequent degradation of β-catenin [20]. When APC protein is mutated, the formation of a GSK-3β, APC, axin and β-catenin complex is disrupted and results in increased cytoplasmic β-catenin levels. Free β-catenin is then translocated to the nucleus and interacts with Tcf-4. This interaction induces transcriptional activation of many oncogenes including c-Myc and cyclin D1 [3,4]. In the current report, we showed that β-catenin protein and mRNA concentrations were significantly decreased in cells expressing GKLF and increased in cells possessing dominant-negative GKLF mutant DNA indicating that GKLF might exhibit its growth-inhibitory effect through β-catenin. These conclusions were further supported by our data showing that β-catenin transfection abolished the growth-inhibitory effect of GKLF in HT-29 cells. Interestingly, transfection with β-catenin has no effect on DNA synthesis in HT29-pcDNA3 cells. The lack of β-catenin effect is likely due to a high level of intrinsic β-catenin expression inherited in these cells (data from ATCC, Rockville, MD, USA). Furthermore, GKLF inhibited transcriptional activity of the pTOPFLASH reporter in a dose-dependent manner, a similar property observed with APC protein. Previously, our laboratory has shown that GKLF inhibited cyclin D1 gene expression, and cyclin D1 has been demonstrated to be one of the downstream targets of β-catenin signaling. These data indicate that GKLF may exert its function through the expression of β-catenin in controlling proliferation and malignant transformation of colonic epithelium. Additional studies will be necessary to determine whether GKLF affects the degradation and/or nuclear translocation of β-catenin protein.

In summary, results from this study suggest that the growth-inhibitory property of GKLF appears to be mediated, at least in part, through the APC/β-catenin pathway. Based upon the data presented here, we speculate that APC may regulate GKLF expression, which, in turn, modifies β-catenin levels and transcriptional activation of the downstream target genes, including cyclin D1. When the APC gene is mutated, the GKLF mRNA level is decreased, resulting in accumulation of β-catenin and hyperproliferation of colonic epithelium.

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