[CANCER RESEARCH 61, 6345–6349, September 1, 2001]

Advances in Brief

Identification of *AF17* As a Downstream Gene of the β-catenin/T-Cell Factor Pathway and Its Involvement in Colorectal Carcinogenesis[1]

Yu-Min Lin, Kenji Ono, Seiji Satoh, Hideyuki Ishiguro, Manabu Fujita, Nobutomo Miwa, Toshihiro Tanaka, Tatsuhiko Tsunoda, Kou-Ching Yang, Yusuke Nakamura, and Yoichi Furukawa[2]

Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan [Y-M. L., K. O., S. S., H. I., M. F., N. M., Y. N., Y. F.]; SNP Research Center, Riken (Institute of Physical and Chemical Research), 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan [T. Ta., T. Ts.]; and Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan [Y-M. L., K-C. Y.]

Abstract

To elucidate the molecular mechanism of colorectal carcinogenesis, we have been attempting to isolate genes involved in the β-catenin/T-cell factor pathway. In the experiments reported here, analysis by cDNA microarray indicated that *AF17*, a fusion partner of the *MLL* gene in acute leukemias with t(11;17)(q23;q21), was transactivated according to accumulation of β-catenin. Expression of *AF17* was significantly enhanced in 8 of the 12 colorectal cancer tissues examined. Introduction of a plasmid designed to express AF17 stimulated growth of NIH3T3 cells, and fluorescence-activated cell sorter analysis indicated that the AF17 regulation of cell-cycle progression was occurring mainly at the G_2-M transition. Our results suggest that the *AF17* gene product is likely to be involved in the β-catenin-T-cell factor/lymphoid enhancer factor signaling pathway and to function as a growth-promoting, oncogenic protein. These findings should aid development of new strategies for diagnosis, treatment, and prevention of colon cancers and acute leukemias by clarifying the pathogenesis of these conditions.

Introduction

APC[3] was isolated as a gene responsible for familial adenomatous polyposis of the colon and was subsequently shown to be involved also in sporadic colon tumors (1). In association with β-catenin, APC acts as a negative regulator of the Wnt signaling pathway by modulating cytoplasmic and nuclear levels of β-catenin. APC facilitates the phosphorylation of β-catenin by glycogen synthase kinase 3β. With a scaffold protein, axin/conductin, APC leads to degradation of β-catenin via the ubiquitin-proteosome pathway (2–5). Abnormal accumulation of β-catenin as a consequence of mutation(s) in *APC*, *β-catenin*, or *AXIN1* genes has been observed in a variety of human cancers including colorectal and hepatocellular carcinomas (6, 7). Accumulated β-catenin interacts with the Tcf/LEF transcription complex, translocates to the nucleus, and transactivates target genes such as *c-myc*, *cyclin D1*, *matrilysin*, *WISP*, *c-jun*, *fra-1*, *NBL4*, and *MDR1* (8, 9). However, the precise mechanisms remain unknown.

Various cytogenetic abnormalities play critical roles in leukemogenesis. For example, translocations involving chromosomal band 11q23, *e.g.*, t(4;11), t(6;11), t(9;11), and t(11;19), are observed in 10% of patients with acute lymphoblastic leukemia and more than 5% of myeloid leukemias (10). The *MLL* (*ALL-1*, *HRX*, *TRX*) gene, located at the breakpoint on band 11q23, is cleaved by these translocations, and its fusion to specific genes on partner chromosomes results in production of chimeric proteins. The *AF17* gene at chromosome 17q21 is a fusion partner of a less frequent translocation of the *MLL* gene, t(11;17)(q23;q21). The predicted amino acid sequence of AF17 contains three zinc-finger domains at the NH_2 terminus, and a leucine-zipper dimerization motif located 3′ of the fusion point. Although AF17 is thought to function as a transcriptional regulator (10), its role in leukemogenesis remains to be explained.

In this study, we report identification of *AF17* as a possible downstream target of the β-catenin-Tcf/LEF transcriptional complex. We also document its involvement in cell-cycle regulation and discuss its possible role in the mechanisms of colorectal carcinogenesis.

Materials and Methods

Cell Lines and Primary Tumor Samples. Human fibroblasts (NHDF), mouse fibroblasts (NIH3T3), monkey fibroblasts (COS7), and human colon-cancer cell lines SW480 and DLD1 were obtained from the American Type Culture Collection (Rockville, MD). All of the cell lines were grown in appropriate media and maintained at 37°C in a humidified atmosphere with 5% CO_2 (for NHDF, NIH3T3, COS7, and DLD1) or without CO_2 (for SW480). Cancerous tissues and corresponding noncancerous mucosae were excised during surgery from 12 patients with colon cancer after informed consent had been obtained.

cDNA Microarray and Selection of Target Genes. Fabrication of the cDNA microarray slides and construction of recombinant adenovirus have been described elsewhere (8, 11). Duplicate sets of cDNA microarray slides containing 9216 cDNA spots in all were used for each analysis of expression profiles to reduce experimental fluctuation. Briefly, SW480 cells were infected with adenovirus expressing *APC*, Ad-APC, or control virus, Ad-LacZ. Total RNAs were extracted 72 h after infection, mRNA was purified from the samples, and T7-based RNA amplification was carried out. Aliquots (5-μg) of amplified RNA from SW480/Ad-APC and SW480/Ad-LacZ were labeled with Cy5-dCTP and Cy3-dCTP, respectively (Amersham Pharmacia Biotech, Piscataway, NJ). Hybridization, washing, and detection were carried out as described previously (11). Genes were excluded from further investigation when the intensities of both Cy3 and Cy5 were below 250,000 fluorescence units. Those with Cy3/Cy5 signal ratios greater than 2.0 were selected for further evaluation.

RT-PCR. We carried out semiquantitative RT-PCR using cDNA reversely transcribed from 0.2 μg of total RNA from each cell line. The PCR exponential phase was determined on 20–32 cycles to allow comparison among cDNAs developed from identical reactions. *GAPDH* served as an internal control. Primers for human *AF17* were H-AF17F (5′-GGAGACCTCTGAGAGCAGC-3′), H-AF17R (5′-GGAGTACTTGTCCTCCTCTG-3′); for human *GAPDH*, H-GAPDH3F (5′-ACAACAGCCTCAAGATCATCAG-3′), H-GAPDH3R (5′-GGTCCACCACTGACACGTTG-3′); and for mouse homologue of *AF17*, M-Af17F (5′-GAGGTGCCCACTAGGACAG-3′), M-Af17R (5′-GCACAA-TTCCAGGCTTGGAG-3′). All of the reactions were carried out in 25-μl volumes and amplified for 3 min at 94°C for initial denaturation, followed by

Received 3/21/01; accepted 7/18/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by Research for the Future Program Grant #96L00102 from the Japan Society for the Promotion of Science.

[2] To whom requests for reprints should addressed, at Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. Phone: 81-3-5449-5373; Fax: 81-3-5449-5406; E-mail: furukawa@ims.u-tokyo.ac.jp.

[3] The abbreviations used are: APC, adenomatous polyposis coli; MLL, myeloid/lymphoid or mixed lineage leukemia; FACS, fluorescence-activated cell sorter analysis; Tcf, T-cell factor; LEF, lymphoid enhancer factor; RT-PCR, reverse transcription-PCR; GAPDH, glyceraldehydes-3-phosphate dehydrogenase.

Table 1 *Candidate genes downstream of β-catenin/Tcf pathway*

Cy3/cy5	Accession No.	Gene
>10.00	AA477929	FLJ10767
8.75	M21389	KRT5
8.20	AI027554	DKFZP586J1624
6.20	L32976	MAP3K11
6.04	X04325	GJB1
5.58	D16431	HDGF
5.37	Z29630	SYK
4.90	U35113	MTA1
4.85	AB000520	APS
4.62	U04241	AES
4.19	AF016903	Agrin precursor
4.13	D26512	MMP14
4.08	U23803	HNRPAO
3.98	L05628	ABCC1
3.95	AI356637	ESTs
3.90	AA430643	SEPW1
3.79	M77640	L1CAM
3.71	M55153	TGM2
3.64	AA234962	PKP3
3.57	M16462	DIA1
3.52	AI149705	FLJ21929
3.48	AI081175	IFITM1
3.36	U07550	HSPE1
3.32	X63564	POLR2A
3.22	J03075	PRKCSH
3.18	JO4046	CALM3
3.18	U41745	PDAP1
3.17	M73554	PRAD1
3.17	AI040181	ESTs
3.17	M15796	PCNA
3.05	AF037261	SCAM
2.99	AA434038	GJB2
2.98	Z31696	DXS1357E
2.98	D88153	HYA22
2.95	AI096393	CPSF4
2.93	AA459728	GLTSCR2
2.85	J03853	ADRA2C
2.80	X66503	ADSS
2.79	AI149639	FIBROSIN
2.76	X03212	KRT7
2.74	R73352	LOC51210
2.73	Z18950	S100A4
2.73	M33518	D6S51E
2.71	X04654	SNRP70
2.71	J03191	PFN1
2.70	AA479010	E1B-AP5
2.67	U42376	LY6E
2.66	AA993406	EVA1
2.61	D30758	ACAP1
2.61	X85237	SF3A1
2.59	M16279	MIC2
2.59	X69550	ARHGDIA
2.58	AI312094	ESTs
2.57	AA493324	ESTs
2.53	M13452	LMNA
2.50	M77235	SCN5A
2.50	AF035299	DOK1
2.45	BF971926	LMNA
2.43	AF072836	HMG20B
2.42	L05096	Ribosomal protein
2.42	X04366	CAPN1
2.41	M23419	EIF5A
2.40	AA195512	HSEC61
2.38	M58028	UBE1
2.35	AA291909	LOC51608
2.33	AB014583	KIAA0683
2.31	D83782	SCAP
2.31	J04101	ETS1
2.31	X07868	IGF2
2.30	AI189382	FBI1
2.30	**AA812940**	**AF17**
2.25	U48734	ACTN4
2.23	U53347	SLC1A5
2.22	Y09022	NOT56L
2.18	R13691	PCDH9
2.17	AA608804	D6S51E
2.16	AA634326	ESTs
2.15	M60922	FLOT2
2.15	U66914	HDAC3
2.15	R99311	ESTs
2.11	AF028824	C19ORF3
2.08	D38251	POLR2E
2.05	U18920	ATP6N1A
2.03	AA770408	IMPDH2

20–32 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min on a GeneAmp PCR system 9700 (PE Applied Biosystems, Foster City, CA).

Real-time quantitative RT-PCR (TaqMan PCR; Perkin-Elmer) was carried out using 7700 Sequence Detector (Perkin-Elmer) according to the manufacturer's recommendations. Quantification and equalization of the amount of cDNA was achieved by amplifying *GAPDH* as an internal control (TaqMan GAPDH Control Reagents). Primers and the probe for *AF17* were AF17F (5′-TCGCTTGGCAACAACACAAG-3′), AF17R (5′-TGGTCTGGGCAGTGAGGACT-3′), and AF17-Probe (5′-Fam-CAGCAGTAGCAGCAGCAGGCGGA-Tamra-3′).

Construction of Plasmids. The entire coding region of *AF17* was amplified by RT-PCR with primers AF17-forward (5′-AGGAATTCCATGGGAGTATGAAGGAGATGGTAG-3′) and AF17-reverse (5′-TACTCGAGGATATAGCCTTTTTCCTGGTTGGCTG-3′). The product was digested with *Eco*RI and *Xho*I and cloned into the appropriate sites of pcDNA3.1(+) (Invitrogen, Carlsbad, CA) and pFlag-CMV-5a (Sigma Chemical Co., St. Louis, MO).

Immunocytochemical Staining. NHDF cells were transfected with pFlag-CMV-5a/AF17 using FuGENE 6 (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions and fixed with PBS containing 4% paraformaldehyde. Fixed cells were incubated with a mouse

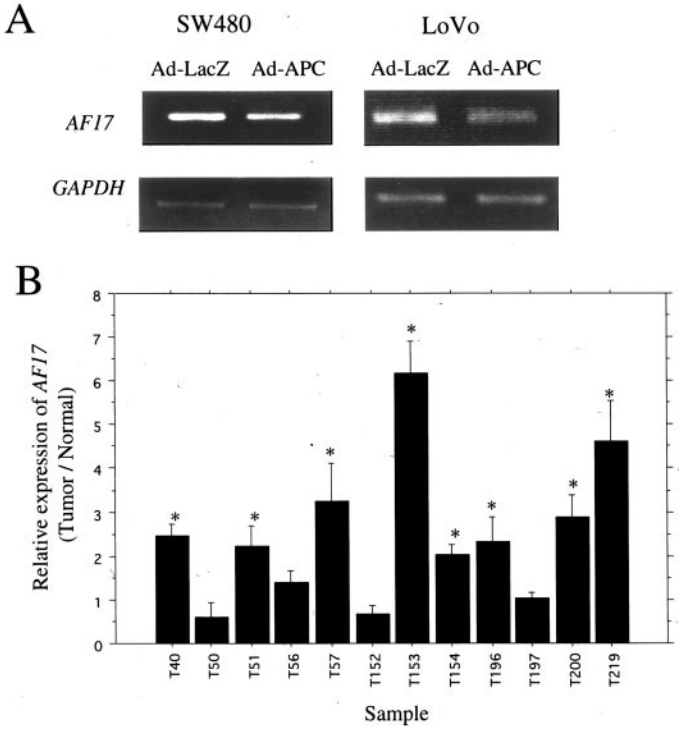


Fig. 1. *A*, expression of *AF17* in SW480 and LoVo cells infected with either Ad-LacZ or Ad-APC, as assessed by semiquantitative RT-PCR. Expression of *GAPDH* served as a control. *B*, relative expression ratios of *AF17* in 12 colon cancer tissues to corresponding noncancerous mucosae are presented as mean ± SD of quadruplicate experiment. *, the expression ratio (tumor:normal) is greater than 2.

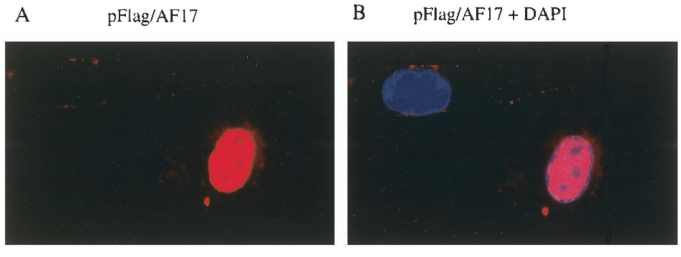


Fig. 2. Subcellular localization of AF17. *A*, NHDF cells transfected with pFlag/AF17, stained with anti-Flag monoclonal antibody, and visualized by rhodamine-conjugated antimouse secondary antibody. *B*, 4′,6′-diamidine-2′-phenylindole dihydrochloride staining indicates cell nuclei.

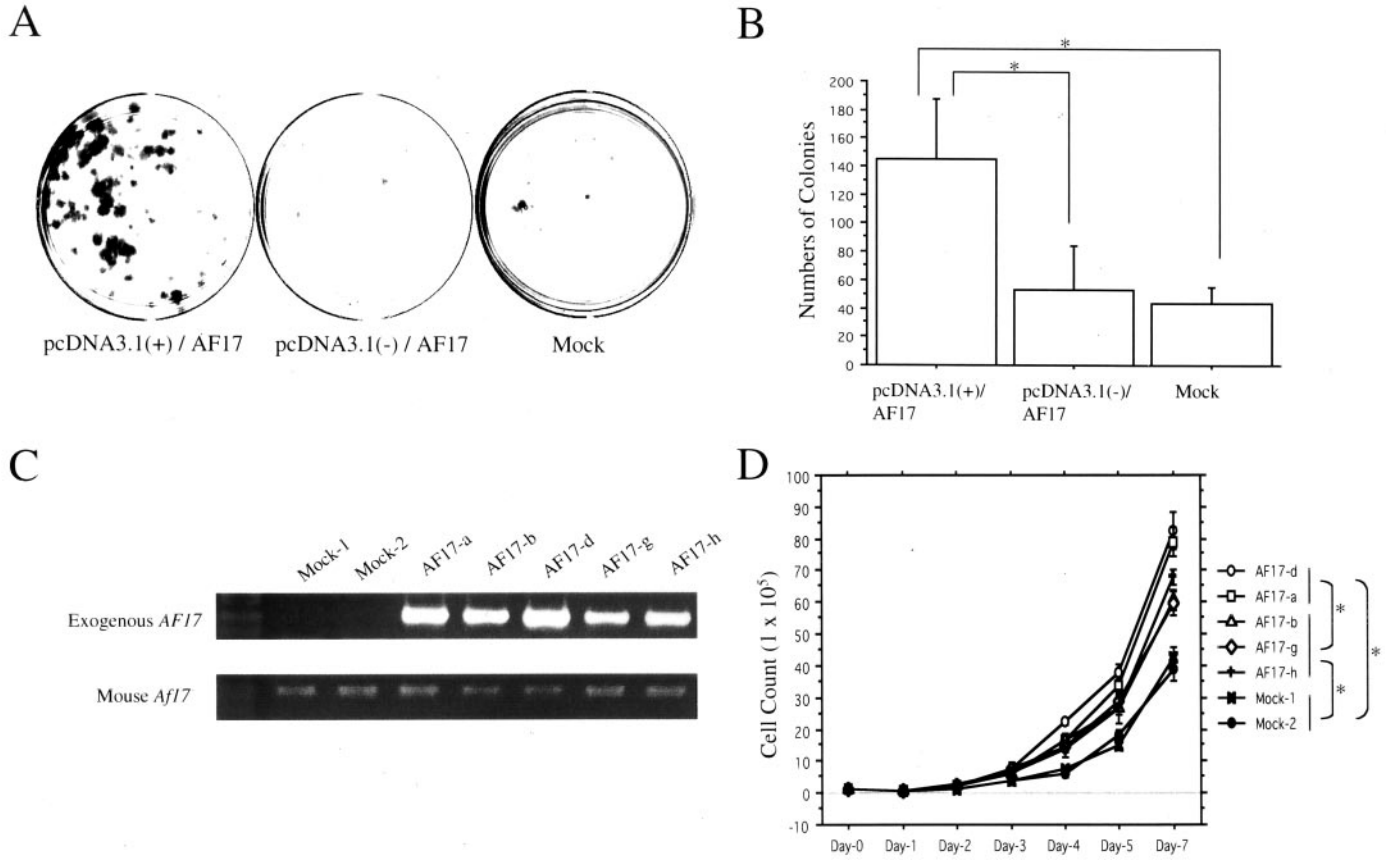


Fig. 3. Effect of AF17 on cell growth. *A*, colony-formation assay of NIH3T3 cells. Sizes and numbers of colonies derived from cells transfected with pcDNA3.1(+)/AF17 were significantly greater than those achieved with pcDNA3.1(-)/AF17 or mock vector. *B*, colonies larger than 3 mm were counted and presented as mean ± SD of triplicate plates. *C*, expression of *AF17* in stable transformants of NIH3T3 cells. Semiquantitative RT-PCR showed expression of exogenous *AF17* was significantly higher than that of endogenous *Af17*. *D*, effect of AF17 on growth of NIH3T3 cells. Five transformants expressing high levels of AF17 (*AF17-a*, *-b*, *-d*, *-g*, and *-h*) and controls (*Mock-1* and *Mock-2*) were cultured in triplicate. Cell numbers are presented as mean ± SD of triplicate plates. ∗, a significant difference ($P < 0.05$) as determined by a Fisher's protected least significant difference test.

anti-Flag antibody (Sigma Chemical Co.) and stained by a rhodamine-conjugated antimouse secondary antibody (ICN Biomedicals, Inc., Costa Mesa, CA). After nuclei were stained with 4′,6′-diamidine-2′-phenylindole dihydrochloride (Boehringer Mannheim), the preparations were observed under a Nikon Eclipse E800 fluorescence microscope.

Colony-formation Assay (Anchorage-dependent Growth Assay). We transiently transfected either pcDNA3.1(+)/AF17, expressing AF17, or empty vector into NIH3T3 cells and treated the cultures with G418 (0.8 mg/ml) for 2 weeks. Cells that survived were fixed with 100% methanol and stained by Giemsa's solution (Merck, Darmstadt, Germany).

Growth Analysis. NIH3T3 and DLD1 cells stably expressing AF17 were established by transfecting NIH3T3 and DLD1 cells with pcDNA3.1(+)/AF17 plasmid using FuGENE 6. Control and AF17-expressing NIH3T3 and DLD1 cells were seeded on 6-cm plates (1×10^5 cells/plate) and counted with a hemacytometer every day.

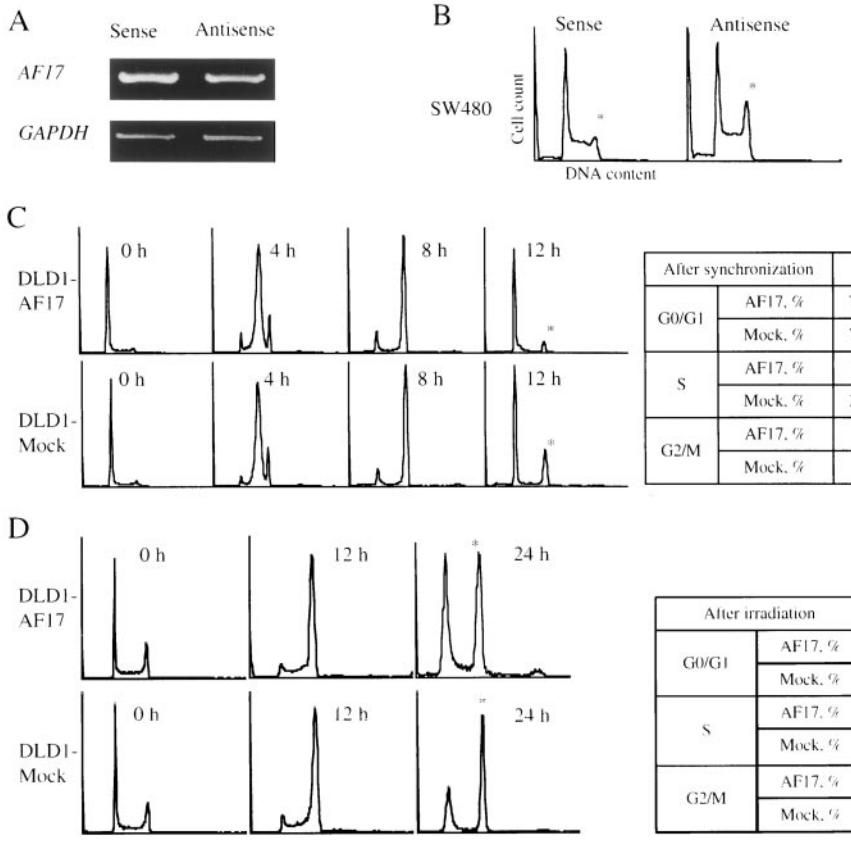
Cell-cycle Analysis. To examine whether AF17 has a role in cell-cycle progression, SW480 cells expressing abundant amounts of AF17 were transfected with sense (5′-ATGAAGGAGATGGTAG-3′) or antisense (5′-CTACCATCTCCTTCAT-3′) *S*-oligonucleotide, the latter being designed to suppress expression of AF17, by Lipofectin (Life Technologies, Inc., Rockville, MD) according to the manufacturer's recommendations and maintained for an additional 24 h. RT-PCR and FACS were performed on a FACScan flow cytometer using CycleTEST reagents and the manufacturer's protocol (Becton Dickinson, San Jose, CA). To examine the role of AF17 further, DLD1 cells expressing low levels of AF17 were transfected with pcDNA3.1(+)/AF17, and we selected stable clones (DLD1-AF17-1 and DLD-AF17-2) expressing high levels of AF17. These cells, together with DLD1-vector (mock) cells as controls, were growth-arrested in G_1 phase by incubation with 5 μg/ml aphidicolin (Sigma Chemical Co.) for 36 h and released from G_1 by removal of aphidicolin. FACS was performed 0, 4, 8, and 12 h later. We also treated those cells with γ-irradiation (10 Gy) and analyzed the population at each phase of the cell cycle by FACS 12 h and 24 h after irradiation.

Statistics. Assessment of statistical differences for Figs. 3 and 4 were determined by Fisher's Protected Least Significant Difference test and Student's *t* test, respectively. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using StatView software.

Results

Identification of AF17. We reported earlier that adenovirus-mediated gene transfer of wild-type *APC* reduced the accumulation of nuclear β-catenin and decreased the transactivational activity of Tcf/LEF signaling in (constitutionally APC-deficient) SW480 cells (8). Among the 9216 cDNAs spotted on our microarray slides, we identified 84 candidate genes (Table 1), the expression of which correlated with accumulation of β-catenin. From among those 84, we focused on *AF17* because it was known to be involved in the pathogenesis of acute leukemia. By semiquantitative RT-PCR, we confirmed significant down-regulation of *AF17* by infection with Ad-APC as compared with that with Ad-LacZ in SW480 and LoVo cells (Fig. 1*A*).

Because accumulation of β-catenin is frequently observed in colorectal cancers as a result of mutations in either *APC* or *β-catenin*, we examined expression of *AF17* in 12 colorectal cancer tissues and their corresponding normal mucosae by real-time quantitative RT-PCR. These experiments detected increased expression of *AF17* in eight (66.7%) of the cancer tissues examined (Fig. 1*B*). This result was in line with the view that *AF17* is up-regulated in response to activation of the Tcf/LEF transcriptional complex.



	Sense	Antisense
G0/G1, %	42.3 ± 0.6	31.5 ± 2.4*
S, %	51.1 ± 0.4	52.2 ± 2.0
G2/M, %	**6.7 ± 0.4**	**16.3 ± 2.1***

After synchronization		0 h	4 h	8 h	12 h
G0/G1	AF17, %	77.9 ± 1.7	4.6± 1.1	8.4 ± 0.9	70.2 ± 3.6*
	Mock, %	76.3 ± 1.8	3.7 ± 0.9	7.3 ± 0.6	58.6 ±1.3
S	AF17, %	19.3 ± 0.5	84.6 ± 1.8	12.6 ± 0.5	17.5 ± 1.2
	Mock, %	20.5 ± 0.4	83.7 ± 2.7	12.2 ± 1.1	15.5 ± 0.8
G2/M	AF17, %	2.9 ± 0.4	11.5 ± 0.9	79.2 ± 3.4	**12.9 ± 1.2***
	Mock, %	3.9 ± 0.5	13.7 ± 1.2	80.5 ± 2.6	**26.6 ± 1.6**

After irradiation		0 h	12 h	24 h
G0/G1	AF17, %	44.8 ± 2.9	4.0 ± 0.8	33.1 ± 6.9
	Mock, %	49.3 ± 3.5	7.5 ± 1.1	20.8 ± 4.0
S	AF17, %	36.3 ± 2.1	27.5 ± 1.6	22.9 ± 1.6*
	Mock, %	33.5 ± 2.5	22.3 ± 1.1	14.9 ± 1.4
G2/M	AF17, %	18.9 ± 1.1	68.5 ± 2.0	**43.9 ± 6.1***
	Mock, %	17.1 ± 2.0	70.3 ± 1.9	**64.2 ± 4.5**

Fig. 4. Cell-cycle analysis by FACS. *A*, effect of synthetic antisense *S*-oligonucleotides on expression of *AF17* in SW480 cells. *B*, suppression of AF17 significantly increased the number of cells in G_2-M phase. *C* and *D*, acceleration of G_2-M transition by AF17. DLD-AF17 cells overexpressing AF17 and control DLD1 cells were synchronized in G_1 phase by aphidicolin (*C*) or in G_2-M phase by irradiation (*D*). Overexpression of AF17 significantly promotes G_2-M transition. Values are mean ± SD of triplicate determinations. *, a significant difference ($P < 0.05$) from control cells as determined by a Student's *t* test.

Localization of AF17 in Mammalian Cells. Flag-tagged AF17 protein was detected in the nuclei of transfected cells by immunocytochemical staining (Fig. 2). Similar results were obtained when we transfected the plasmid into COS7 and LoVo cells (data not shown).

Effects of AF17 on Growth of NIH3T3 Cells. We performed colony-formation assays after transfecting pcDNA3.1(+)/AF17, expressing wild-type *AF17*, into NIH3T3 cells. The number of colonies that developed from transfected cells was significantly greater than the numbers achieved on plates of control cells transfected with the mock vector, pcDNA3.1(+) (Fig. 3, *A* and *B*). We isolated five clones expressing high levels of AF17 compared with the expression of endogenous homologue of AF17 in mouse (Fig. 3*C*) and found that exogenously expressed AF17 significantly accelerated growth of NIH3T3 cells (Fig. 3*D*). This growth advantage was closely associated with the expression level of exogenous AF17. These results were confirmed in two independent experiments in triplicate plates.

Cell-cycle Regulation. We performed FACS using SW480 cells transfected with an antisense *S*-oligonucleotide designed to suppress expression of AF17 (Fig. 4*A*). A larger proportion of SW480 cells were arrested at the G_2-M phase after this treatment in comparison with cells treated with control *S*-oligonucleotide (16.2% *versus* 6.7%; $P < 0.05$; Fig. 4*B*).

To confirm a role of AF17 in G_2-M progression, we cloned DLD1 cells that stably expressed high levels of AF17 (DLD1-AF17 cells) and synchronized them at the G_1 phase by aphidicolin treatment. Overexpression of AF17 significantly shortened the G_2-M transition time as shown in Fig. 4*C* (12.9% *versus* 26.6%; $P < 0.05$). In addition, 24 h after cells were exposed to γ-irradiation (10 Gy), about 20% more of the DLD1-AF17 cells arrested at the G_2-M phase had progressed to G_0/G_1 than had DLD1-mock cells (43.9% remaining in G_2-M *versus* 64.2%; $P < 0.05$; Fig. 4*D*). These results indicated that AF17 accelerated cell-cycle progression by promoting G_2-M transition. All of the results were confirmed in at least two independent experiments in triplicate plates.

Discussion

To identify downstream genes of the β-catenin/Tcf complex, we compared expression profiles between SW480/Ad-APC cells and SW480/Ad-LacZ cells using cDNA microarray slides prepared in our laboratory. An approximately 3.17-fold higher level of expression of *cyclin D1*, a known target of this complex (12), was observed in SW480/Ad-LacZ cells compared with SW480/Ad-APC cells. Expression of *AF17* was also down-regulated in SW480 cells in response to a decrease of Tcf/LEF-dependent transcription. Expression of *AF17* is frequently elevated in colorectal cancer tissues where β-catenin is augmented in the cytoplasm or nucleus (2). Therefore, *AF17* appears to be a target of the β-catenin/Tcf complex and likely to be involved in colorectal carcinogenesis.

Because molecules of the Tcf/LEF family interact with the consensus sequence 5′-CTTTGWW-3′, we searched for this binding motif in the 5′ flanking region of *AF17* and found one located about 523–529 bp upstream of the transcription-initiation site. Although we carried out reporter assays using plasmids containing a DNA fragment encompassing the motif and various deletion mutants of it, we were unable to detect any significant difference in the reporter activities among them. Thus, the putative binding element did not affect the elevated transcription of *AF17*, a result that suggested two possibili-

ties: either AF17 may be regulated only indirectly by the β-catenin-Tcf complex or β-catenin may regulate AF17 through other binding motifs coupled with a different transcriptional factor. The latter notion is consistent with a recent report (13) that the *WISP1* gene, a known target gene of β-catenin, is transactivated by cyclic AMP-responsive element binding protein through its binding site but not through putative Tcf/LEF-binding elements.

The *AF17* gene was initially isolated as a fusion partner of the *MLL* gene in t(11;17)(q23;q21) translocations present in some acute myeloid leukemias (10). Although chromosomal translocations are usually associated with overexpression or activation of oncogenes, Prasad *et al.* (10) proposed a model in which *MLL* rearrangements would result in loss of function of that gene because most of the partner genes encode unrelated proteins except for similarities between *AF9* and *ENL* and between *AF10* and *AF17* (14, 15). However, reciprocal translocations can produce two chimeric proteins. Thus, in addition to inactivation of or interference with MLL, t(11;17) translocations might confer oncogenic activity through abnormal activation of AF17 and produce a malignant phenotype in leukemic cells. AF10 bears significant homology to AF17 within their respective cysteine-rich domains at the NH_2 termini and leucine zipper domains toward the COOH termini, although they diverge outside those regions (15). The first part of the cysteine-rich region in each case contains conserved zinc-finger domains known as LAP/PHD-finger. The remainder contains a cluster of 12 conserved cysteines and histidines. These cysteine-rich domains show similarity with part of BR140, a bromodomain- and PHD finger-containing protein that is homologous to the TAF250 subunit of transcription factor TFIID. Therefore, AF17 and AF10 may both function as transcriptional repressors or activators (16).

Our experiments have supported a view that AF17, like c-myc and cyclin D1, is involved in cell-cycle progression and is regulated by β-catenin (12, 17). Although the mechanisms by which AF17 regulates the cell cycle are not clear at present, accelerated progression of the G_2-M boundary could result from abrogation of a checkpoint. If that is the case, cells that overexpress AF17 may accumulate genetic alterations in addition to conferring a growth advantage. Additional investigations of its functions and isolation of its target molecules will help to clarify the role of AF17 in colorectal carcinogenesis. Such studies may also provide clues for identifying predictive and prognostic markers for diagnosis and for developing more effective therapeutic strategies for specific cancers.

References

1. Nagase, H., and Nakamura, Y. Mutations of the *APC* (*adenomatous polyposis coli*) gene. Hum. Mutat., *2:* 425–434, 1993.
2. Bienz, M., and Clevers, H. Linking colorectal cancer to Wnt signaling. Cell, *103:* 311–320, 2000.
3. Rosin-Arbesfeld, R., Townsley, F., and Bienz, M. The APC tumour suppressor has a nuclear export function. Nature (Lond.), *406:* 1009–1012, 2000.
4. Ikeda, S., Kishida, S., Yamamoto, H., Murai, H., Koyama, S., and Kikuchi, A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3β and β-catenin and promotes GSK-3β-dependent phosphorylation of β-catenin. EMBO J., *17:* 1371–1384, 1998.
5. Aberle, H., Bauer, A., Stappert, J., Kispert, A., and Kemler, R. β-catenin is a target for the ubiquitin-proteasome pathway. EMBO J., *16:* 3797–3804, 1997.
6. Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clever, H., Vogelstein, B., and Kinzler, K. W. Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. Science (Wash. DC), *275:* 1787–1790, 1997.
7. Satoh, S., Daigo, Y., Furukawa, Y., Kato, T., Miwa, N., Nishiwaki, T., Kawasoe, T., Ishiguro, H., Fujita, M., Tokino, T., Sasaki, Y., Imaoka, S., Murata, M., Shimano, T., Yamaoka, Y., and Nakamura, Y. *AXIN1* mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. Nat. Genet., *24:* 245–250, 2000.
8. Kawasoe, T., Furukawa, Y., Daigo, Y., Nishiwaki, T., Ishiguro, H., Fujita, M., Satoh, S., Miwa, N., Nagasawa, Y., Miyoshi, Y., Ogawa, M., and Nakamura, Y. Isolation and characterization of a novel human gene, *DRCTNNB1A*, the expression of which is down-regulated by β-catenin. Cancer Res., *60:* 3354–3358, 2000.
9. Yamada, T., Takaoka, A. S., Naishiro, Y., Hayashi, R., Maruyama, K., Maesawa, C., Ochiai, A., and Hirohashi, S. Transactivation of the multidrug resistance 1 gene by T-cell factor 4/β-catenin complex in early colorectal carcinogenesis. Cancer Res., *60:* 4761–4766, 2000.
10. Prasad, R., Leshkowitz, D., Gu, Y., Alder, H., Nakamura, T., Saito, H., Huebner, K., Berger, R., Croce, C. M., and Canaani, E. Leucine-zipper dimerization motif encoded by the *AF17* gene fused to *ALL-1 (MLL)* in acute leukemia. Proc. Natl. Acad. Sci. USA, *91:* 8107–8111, 1994.
11. Okabe, H., Satoh. S., Kato, T., Kitahara, O., Yanagawa, R., Yamaoka, Y., Tsunoda, T., Furukawa, Y., and Nakamura, Y. Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression. Cancer Res., *61:* 2129–2137, 2001.
12. Shtutman, M., Zhurinsky, J., Simcha, I., Albanese, C., D'Amico, M., Pestell, R., and Ben-Ze'ev, A. The *cyclin D1* gene is a target of β-catenin/LEF-1 pathway. Proc. Natl. Acad. Sci. USA, *96:* 5522–5527, 1999.
13. Xu, L., Corcoran, R. B., Welsh, J. W., Pennica, D., and Levine, A. J. *WISP-1* is a Wnt-1- and β-catenin-responsive oncogene. Genes Dev., *14:* 585–595, 2000.
14. Rubnitz, J. E., Morrissey, J., Savage, P. A., and Cleary, M. L. *ENL*, the gene fused with *HRX* in t(11;19) leukemias, encodes a nuclear protein with transcriptional activation potential in lymphoid and myeloid cells. Blood, *84:* 1747–1752, 1994.
15. Chaplin, T., Ayton, P., Bernard, O. A., Saha, V., Della Valle, V., Hillion, J., Gregorini, A., Lillington, D., Berger, R., and Yang, B. D. A novel class of zinc finger/leucine zipper genes identified from the molecular cloning of the t(10;11) translocation in acute leukemia. Blood, *85:* 1435–1441, 1995.
16. Thompson, K. A., Wang, B., Argraves, W. S., Giancotti, F. G., Schranck, D. P., and Ruoslahti, E. BR140, a novel zinc-finger protein with homology to the TAF250 subunit of TFIID. Biochem. Biophys. Res. Commun., *198:* 1143–1152, 1994.
17. He, T. C., Sparks, A. B., Rago, C, Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. Identification of c-MYC as a target of the APC pathway. Science (Wash. DC), *281:* 1509–1512, 1998.