# Modulation of Oncogenic Transcription and Alternative Splicing by β-Catenin and an RNA Aptamer in Colon Cancer Cells

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## Abstract

**Activated β-catenin regulates the transcription of oncogenic target genes and is critical for tumorigenesis. Because nuclear functions are frequently coupled, we investigated whether it also has a role in alternative splicing of oncogenic genes. We showed that stabilized β-catenin caused alternative splicing of estrogen receptor-β pre-mRNA in colon cancer cells. To establish a direct role of β-catenin in regulated splicing, we selected a high-affinity RNA aptamer that associated with β-catenin *in vivo*. Nuclear localized aptamer inhibited β-catenin-dependent transcription of cyclin D1 and c-*myc* in colon cancer cells; thus, cells stably expressing the aptamer exhibited cell cycle arrest and reduced tumor forming potential. Most significantly, the aptamer prevented the alternative splicing induced by stabilized β-catenin. Taken together, our results establish that β-catenin has an important role in both transcription and splicing, and that its action can be modulated by a high-affinity RNA aptamer. The RNA aptamer could be further developed as a specific inhibitor for cancer therapeutics.** (Cancer Res 2006; 66(21): 10560-6)

## Introduction

β-Catenin is a multifunctional protein that plays critical roles in cell adhesion and signaling (1, 2). Although it was first identified as a component of a cell adhesion complex, it is now recognized as a central component of the developmentally important Wnt pathway (3). The scaffolding proteins adenomatous polyposis coli (APC) and Axin/Axin2 interact with β-catenin, and glycogen synthase kinase-3β (GSK-3β) subsequently phosphorylates β-catenin, leading to its proteasomal degradation (4, 5). Mutations of APC or β-catenin are frequently found in various types of cancer cells, especially in colon cancers (6, 7). As β-catenin levels increase, it accumulates in the nucleus, where it interacts with DNA-bound TCF family proteins to activate the transcription of various oncogenic target genes, such as *cyclin D1* and *c-myc* (8–13). Although β-catenin regulates target gene expression mostly at the transcriptional level, this effect is not enough to explain its full oncogenic effect.

Posttranscriptional regulation of mRNA, such as alternative splicing, may contribute to cancer development by generating rare but oncogenic transcripts. Defects in mRNA splicing are known to be an important cause of disease, and aberrantly spliced alternative RNA isoforms are found in many cancers (14–16). Because transcription is frequently coupled with RNA processing (17), it is also possible that mRNA splicing is affected by activated β-catenin. In fact, it was previously suggested that β-catenin may regulate pre-mRNA splicing (18). We explored the possible role of β-catenin in regulated splicing and found that several transcripts were alternatively spliced in colon cancer cells as well as in tumor tissues.

Because β-catenin is an excellent target for cancer therapeutic, diverse antagonists have been developed (19–21). To elucidate the mechanism of β-catenin-induced transcription and splicing, we generated an RNA aptamer against β-catenin using the SELEX method and expressed it as a nuclear localized aptamer (22–24). The RNA aptamer inhibited the transcription of β-catenin target genes, as expected. Most significantly, it reversed the expression of a splicing variant induced by the overexpressed β-catenin in HCT116 cells. Our results suggest that the RNA aptamer could be a novel tool providing further insight into the functions of nuclear β-catenin.

## Materials and Methods

**Plasmids and reagents.** A retroviral vector expressing human S37A/β-catenin was kindly provided by Dr. Jang-Soo Chun (Gwangju Institute of Science and Technology; ref. 25). Bacterial expression vectors for recombinant β-catenin and HuR proteins were obtained from Dr. Tsutomu Nakamura (University of Tokyo) and Dr. Yoshikuni Nagamine (Friedrich Miescher Institute), respectively. Bacterial expression vector for Arm 1-12 was constructed by inserting the armadillo repeats sequence into the *Bam*HI-*Xho*I sites of the vector pGEX-5X (Amersham Biosciences, Piscataway, NJ). The nRNA expression vector designated U6-Vector was a gift of Dr. David Engelke (University of Michigan), and the TCF-responsive luciferase reporters pGL3-OT (wild-type) and pGL3-OF (mutant) were from Dr. Shivdasani (Dana-Farber Cancer Institute). Wild-type and three TCF site −1745 cyclin D1 promoter reporters were kindly donated by Dr. Pestell (Albert Einstein College of Medicine). Luciferase activity was determined with a luciferase assay system (Promega, Madison, WI) and measured with a Turner Luminometer TD-20/20. Anti-TCF-4 polyclonal (H-125) and anti-cyclin D1 monoclonal (HD11) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA), and the anti-β-catenin monoclonal antibody was from BD Biosciences (San Jose, CA).

**Cell culture, patient tissues, and transfection.** Human 293T embryonic kidney cells, human HCT116 colorectal carcinoma cells, and SW480 adenocarcinoma cells (American Type Culture Collection, Rockville, MD) were cultured in DMEM, with 10% fetal bovine serum (FBS). Human colorectal adenocarcinoma LoVo cells were cultured in RPMI 1640 with 10% FBS. Cells were transfected with DNA using LipofectAMINE (Invitrogen, San Diego, CA). Normal colorectal and tumor tissues were obtained from the same individuals by biopsy (Dr. Seong-Eun Kim, Ewha Women's University Hospital).

**Retrovirus infection.** To produce retrovirus, retroviral expression vectors (human S37A-β-catenin and empty vector) were transfected into pLinX cells. Supernatants were collected 72 hours after transfection, and cell debris was removed by centrifugation at 500 × *g* for 5 minutes and with a syringe filter. To infect HCT116 cells, three fourths of a viral supernatant was mixed with one-fourth fresh medium and 10 μg/mL of polybrene, and

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the mixture was applied to freshly seeded cells. Stable cell lines were selected with geneticin (500 μg/mL).

***In vitro* RNA selection, aptamer construction, and RNA-binding assays.** An RNA library of random 50-nucleotide sequences ($1 \times 10^{14}$ different molecules, IDT) was used as starting material for the SELEX procedure (26). In each cycle, RNA was preincubated with glutathione *S*-transferase (GST) protein in binding buffer [25 mmol/L HEPES (pH 7.5), 150 mmol/L NaCl, 1 mmol/L $MgCl_2$, 2 mmol/L DTT, and 40 units RNase inhibitor]. GST-bound RNA was discarded, and RNA bound to GST-Arm 1-12 was selected with glutathione-Sepharose beads. After four and eight cycles of selection, the selected sequences were cloned into pUC19 (pUC19-Aptamer) and sequenced. To construct the aptamer, the DNA was amplified from the pUC19-Aptamer with primers U6-F1 (5′-TGATGTCGACTAGG-GACGCGTGGT-3′) and U6-R1 (5′-GACTCTAGAGGATCCCCG-3′). The PCR product was digested with *Sal*I and *Xba*I and cloned into the same sites of the U6-Vector, generating pU6-Aptamer. To test for RNA binding, the RNA was radiolabeled as described previously (26), incubated with GST-fusion protein, and assayed by GST pull-down or RNA-electrophoretic mobility shift assay (EMSA).

**Reverse transcription-PCR analysis.** Total cellular RNA was isolated with TRIzol (Invitrogen), reverse transcribed with M-MuLV reverse transcriptase, and used in the PCR reactions. The following PCR primers were used: cyclin D1, 5′-CTGGCCATGAACTACCTGGA-3′ (forward) and 5′-GTCACACTTGATCACTCTGG-3′ (reverse); β-catenin, 5′-CGGGATCCA-CAAGAAACGGCTTTCA-3′ (forward) and 5′-GAGAATTCCAGGTCAGTATCA-AACCA-3′ (reverse); *c-myc*, 5′-CTTCTGCTGGAGGCCACAGCAAACCTCCTC-3′ (forward) and 5′-CCAACTCCGGGATCTGGTCACGCAGGG-3′ (reverse). For alternative splicing analysis, reverse transcription was carried out using Superscript II reverse transcriptase (Stratagene, La Jolla, CA). cDNA was subjected to standard PCR. Reverse transcription-PCR (RT-PCR) analysis of estrogen receptor-β (ER-β) Δ5-6 and prostate-specific antigen–linked molecule (PSA-LM) was done according to the previously described method (18, 27). PCR products were analyzed on 1.5% agarose gel followed by ethidium bromide staining.

**RNA coimmunoprecipitation assay.** HCT116 cells were transiently transfected with the various U6 RNA expression plasmids and then treated with 1% formaldehyde (28). Sonicated lysates were immunoprecipitated with normal IgG, anti-γ-catenin antibody, or anti-β-catenin antibody. Pellets and supernatants were subsequently incubated at 70°C for 1 hour to reverse the cross-links, and the RNA was purified with TRIzol and subjected to RT-PCR.

**Selection of stable aptamer transfectants.** HCT116 cells were cotransfected with pU6-Aptamer, pU6-NC, or pU6 vector, in the presence of pTK-Hyg (Clontech, Mountain View, CA). Stably transfected clones were selected with hygromycin B (Invitrogen). After 2 weeks, hygromycin-resistant clones were tested for expression of the RNA aptamer by RT-PCR.

**Cell cycle analysis and soft agar colony formation.** For flow cytometry, aptamer transfectants (10,000 cells per sample) were fixed in 70% ethanol, stained with propidium iodide (10 μg/mL), and incubated for 30 minutes at 37°C. Cell cycle profiles were analyzed on a FACSCalibur with Cellquest Software (Becton Dickinson, San Jose, CA). For soft agar colony formation assays, 5,000 cells were seeded in six-well plates with 0.7% agar. After 10 days, colonies formed were fixed in 70% ethanol and stained with 0.005% crystal violet.

## Results

**Induction of ER-β Δ5-6 and PSA-LM isoforms by β-catenin.** To test whether nuclear β-catenin has any role in alternative RNA splicing, we established the HCT116 colorectal cancer cell line that produces stabilized S37A β-catenin. The stable cell line expressed high levels of β-catenin (Fig. 1*A*) with a preferential nuclear localization, in contrast to the mainly membrane localization of β-catenin with minor staining in cytoplasm and nucleus in the parental HCT116 cells (Fig. 1*B*). This nuclear localization was also observed after transient transfection of S37A β-catenin cDNA.

We tested whether the nuclear β-catenin modulated the regulated splicing of ER-β because it was previously reported that many isoforms of ER-β were present in colon cancer cells (29). We were especially curious about whether the ER-β Δ5 and Δ5-6 isoforms were generated because they encode dominant-negative proteins lacking the ligand-binding domain and have been implicated in tumorigenesis (18, 30). As shown in Fig. 1*C* and *D*, RT-PCR analysis revealed that stabilized β-catenin induced the alternatively spliced ER-β Δ5-6 isoform. We further characterized the expression pattern of the ER-β isoform in human colorectal tissue from cancer patients (Fig. 2). The ER-β Δ5-6 isoform was present in most of the human colon tumor samples. Strikingly, in tumor tissue where the level of β-catenin protein was high, expression of the ER-β Δ5-6 mRNA isoform was also elevated compared with the corresponding normal tissues.

We also found that the stabilized β-catenin induced the expression of an unusual mRNA splice variant of kallikrein 3 mRNA, which encodes PSA (Fig. 1*C* and *D*). This variant mRNA is generated by including intronic sequences adjacent to the first exon of PSA and has been designated PSA-LM (27). These observations show that nuclear localized β-catenin has a role in the induction of aberrant alternative splicing of ER-β and PSA in colon cancer cells.

**Selection of an RNA aptamer for β-catenin.** Because we detected the production of alternatively spliced mRNA by

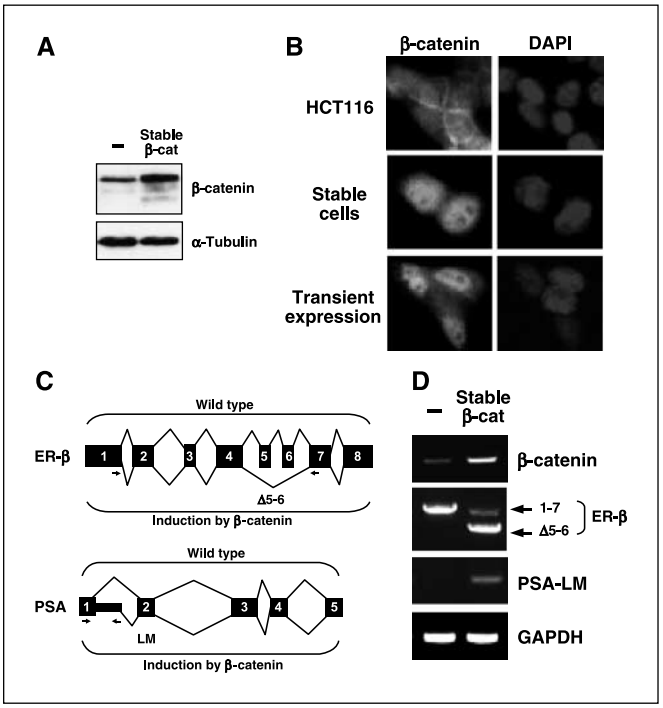


**Figure 1.** Regulation of alternative splicing by β-catenin. *A,* HCT116 cells were infected with retrovirus expressing S37A-β-catenin. Stable cells were selected with geneticin and tested for expression of the stable S37A-β-catenin by Western blotting. α-Tubulin is shown as a control. *B,* subcellular localization of β-catenin. HCT116 cells were stained by indirect immunofluorescence using FITC-labeled mouse monoclonal anti-β-catenin antibody. Nuclei were stained with 4′,6-diamidino-2-phenylindole (*DAPI*). *C,* schematic representation of ER-β and PSA mRNAs. Exons (*filled boxes*) and introns (*connecting lines*) are not drawn to scale. Arrows indicate the positions and directions of the PCR primers used to detect alternative splicing. *D,* RT-PCR analysis of ER-β Δ5-6 and PSA-LM in the parental HCT116 cells and cells stably expressing S37A β-catenin. *GAPDH,* glyceraldehyde-3-phosphate dehydrogenase.

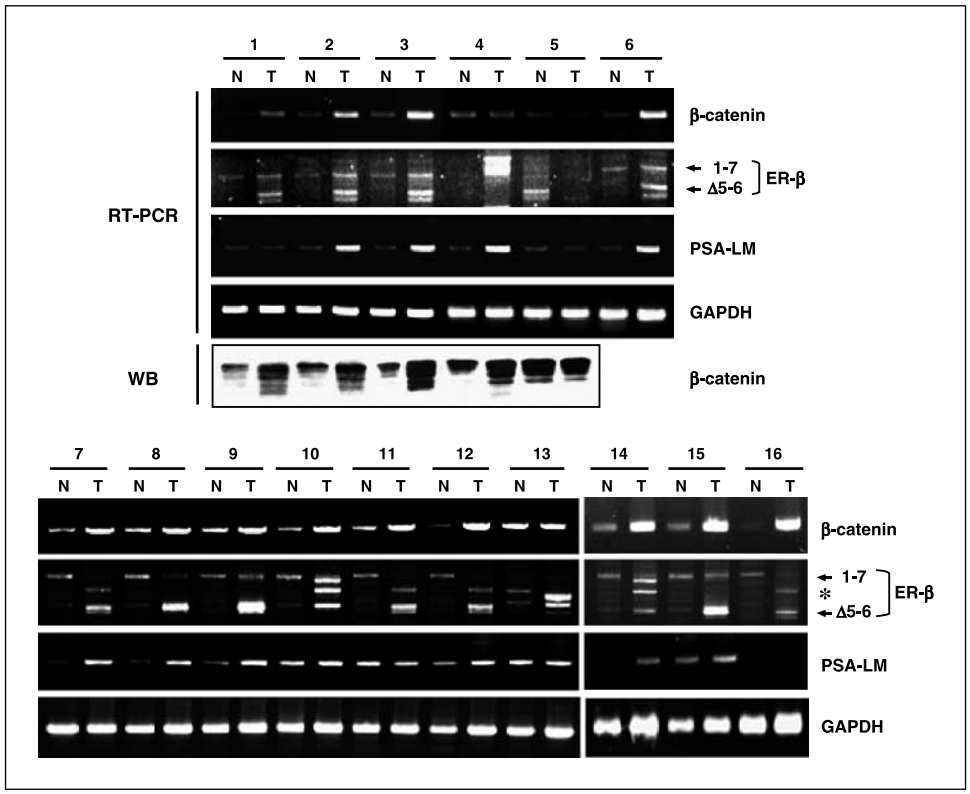


**Figure 2.** Expression of ER-β Δ5-6, PSA-LM and β-catenin in normal colon (*N*) and tumor (*T*) tissue. Total RNA from 16 patients (tissues from patients 1-6, *top*; tissues from patients 7-16, *bottom*) were analyzed by RT-PCR and Western blot assay (*WB*). The numbers indicate individual patients. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) is shown as a control. Asterisk indicates an unidentified band.

β-catenin, we developed an RNA aptamer that could specifically modulate nuclear β-catenin functions. First, we isolated β-catenin binding RNA aptamers that bound to the armadillo repeats 1-12 (Arm 1-12) of β-catenin because they are the interaction motifs for diverse proteins, including the transcription factor TCF (8–10). The selected RNA bound to β-catenin after four cycles of selection (SE-4; Fig. 3*A*), whereas the original RNA library (SE-0) did not bind to a detectable extent. A similar level of binding was observed after eight cycles of selection (SE-8). Individual RNA molecules were isolated from the SE-8 RNA pools, and 40 independent clones were sequenced. Surprisingly, all 40 clones had the same sequence. This sequence is shown in Fig. 3*B*. RNA-EMSAs showed that the RNA aptamer not only bound to the Arm 1-12 but also to β-catenin (Fig. 3*C*). RNA-EMSAs showed that the binding affinity ($K_d$) of the RNA aptamer for β-catenin was about 5 nmol/L. The RNA aptamer did not bind to other proteins such as the RNA-binding protein HuR. The specificity of binding was also confirmed by competitive RNA-EMSAs (data not shown). We conclude that the RNA aptamer binds specifically to a part of Arm 1-12 of β-catenin, suggesting that it may represent a useful approach to controlling the activity or affinity of interacting partners of β-catenin.

**Inhibition of the β-catenin/ER-β mRNA complex by the RNA aptamer.** Because the RNA aptamer exhibited high affinity and specificity for β-catenin *in vitro*, we tested its effect in mammalian cells. We expressed it in cells using the U6 promoter-driven vector, which expresses nuclear localized RNA (31, 32). We confirmed a high level of nuclear expression and prolonged stability of the RNA aptamer by Northern blotting and real-time PCR analysis (data not shown).

Aptamer/β-catenin complex within cells was demonstrated in Fig. 4*A*. We performed RNA immunoprecipitation with anti-β-catenin antibody followed by RT-PCR analysis. Only the RNA aptamer (U6-Apt) was coimmunoprecipitated with anti-β-catenin antibody, whereas a control RNA aptamer for NC protein (nucleocapsid protein of HIV-1, U6-NC; ref. 26) or vector RNA was not. Similarly, immunoprecipitation with normal rabbit IgG or highly homologous anti-γ-catenin antibody did not immunoprecipitate the RNA aptamer (Fig. 4*A*).

Because we observed that β-catenin induced alternative splicing of ER-β, we reasoned that β-catenin might directly or indirectly bind to the ER-β transcript *in vivo*, and we might be able to capture the β-catenin/ER-β mRNA complex by RNA coimmunoprecipitation. As shown in Fig. 4*C*, the ER-β transcript was indeed coimmunoprecipitated with anti-β-catenin antibody in HCT116 cells expressing stabilized β-catenin. This suggests that β-catenin is able to interact with the ER-β transcript, which, in turn, is likely to be responsible for the aberrant splicing of ER-β mRNA. In addition, the RNA aptamer (U6-Aptamer) significantly reduced the level of the β-catenin/ER-β mRNA complex in the stable cell line (Fig. 4*B*).

**Suppression of β-catenin-induced alternative splicing by the RNA aptamer.** Because the data in Fig. 4*B* raised the interesting possibility that the RNA aptamer might affect the β-catenin-induced formation of ER-β Δ5-6 and PSA-LM, we tested whether the alternative splicing pattern could be changed by cotransfecting the RNA aptamer. As shown in Fig. 5*A*, transient transfection of S37A β-catenin induced the expression of ER-β Δ5-6 as in the stable HCT116 cells (Fig. 1*D*). Strikingly, the RNA aptamer reversed the β-catenin-induced ER-β Δ5-6 alternative splicing, whereas control U6-vector and U6-NC did not. We also tested whether β-catenin actually regulates the endogenous alternative spliced isoforms, using LoVo cells, which have high levels of ER-β Δ5-6 (18). The RNA aptamer clearly reduced the expression of ER-β Δ5-6 (Fig. 5*B*), and similar results were obtained for the aberrant alternative splicing of PSA-LM (Fig. 5*C*). Together, these results

show that the RNA aptamer can suppress the alternative splicing of pre-mRNA induced by stabilized nuclear β-catenin in colon cancer cells.

**Inhibition of target gene expression in colon cancer cell lines.** Because the main role of β-catenin is the activation of TCF-dependent transcription, we tested whether the RNA aptamer inhibited β-catenin/TCF–mediated transcription using TCF-responsive wild-type (OT) and mutant (OF) luciferase reporters. Dose-dependent inhibition of the OT luciferase activity was clearly correlated with the expression of U6-aptamer (Fig. 6*A*). We further showed that β-catenin/TCF–responsive cyclin D1 promoter activity was also reduced by U6-aptamer (Fig. 6*B*). These inhibitory effects were sequence-specific because the RNA aptamer for U6-NC had no effect.

We next tested whether the RNA aptamer could, in fact, inhibit the expression of endogenous target genes in HCT116 (Fig. 6*C*) and SW480 (Fig. 6*D*) colon cancer cell lines. Interestingly, it reduced the expression of cyclin D1 and c-myc mRNAs in both cell lines. This finding confirms the idea that the RNA aptamer can modulate the transcription of β-catenin target genes in colon cancer cells.

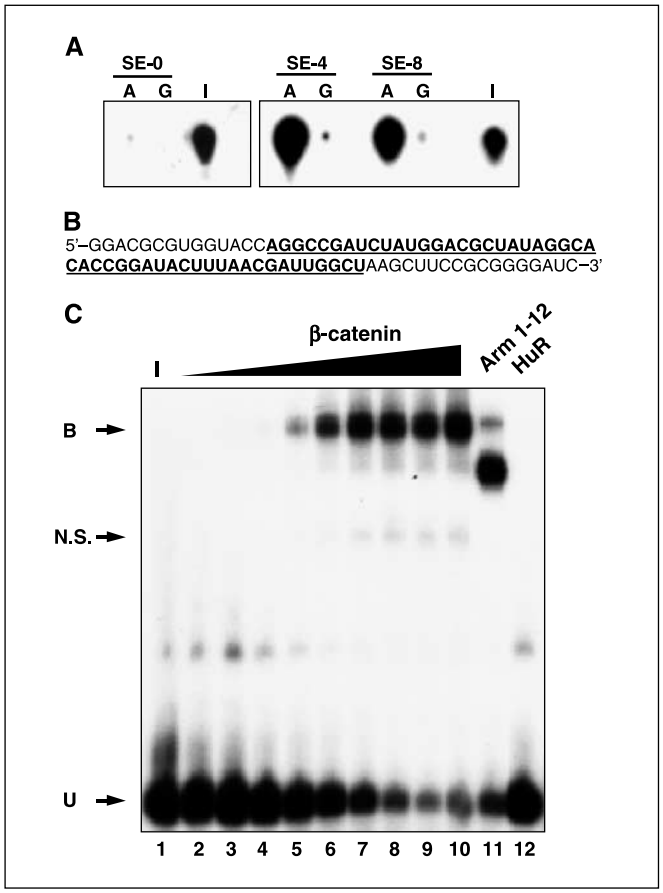


**Figure 3.** Selection of the RNA aptamer. *A,* GST pull-down assay after various numbers of selection cycles (SE-0, cycle 0; SE-4, cycle 4; SE-8, cycle 8). [$^{32}$P]UTP-labeled RNA (180 pmol/L) was incubated with 200 nmol/L of GST (*G*) or Arm 1-12 (*A*). One tenth of the RNA was loaded as input (*I*). Complexes were precipitated with glutathione-Sepharose beads, and bound RNA was eluted and subjected to 6% polyacrylamide/7 mol/L urea gel electrophoresis. *B,* sequence of the selected RNA aptamer. The 5′ and 3′ ends are of defined sequence, and the selected sequence is underlined and in bold letters (16-65 nucleotides). *C,* RNA-EMSAs. [$^{32}$P]UTP-labeled RNA aptamer (80 pmol/L) was incubated with increasing concentrations of recombinant β-catenin. *Lane 1,* labeled RNA only; *lanes 2* to *10,* β-catenin (0.25, 1.25, 2.5, 5, 12.5, 18.7, 25, 37.5, and 50 nmol/L); *lane 11,* Arm 1-12 (200 nmol/L); *lane 12,* HuR (200 nmol/L). *N.S.,* nonspecific band.

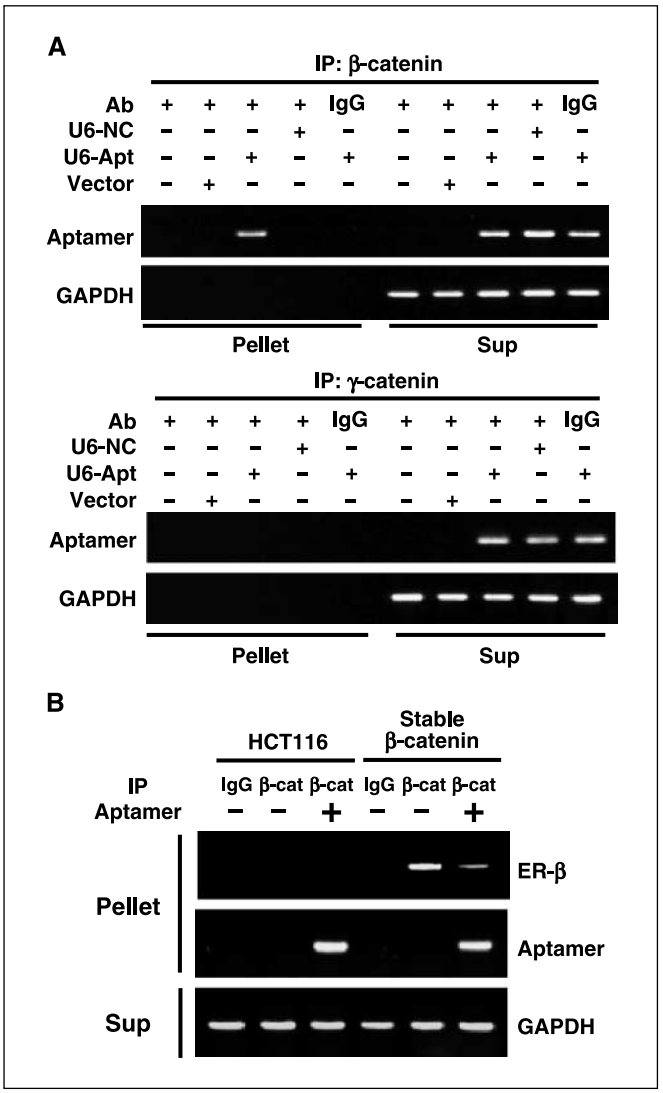


**Figure 4.** Specific association of RNA aptamer with cellular β-catenin. *A,* RNA coimmunoprecipitation assay. 293T cells were transfected with U6-NC, U6-Aptamer (*U6-Apt*), or U6 (*Vector*). Whole-cell extracts were immunoprecipitated with anti-β-catenin antibody followed by RT-PCR analysis as indicated. Similar immunoprecipitation was done with anti-γ-catenin antibody. *B,* interaction of ER-β mRNA with β-catenin *in vivo*. Parental HCT116 cells and cells stably expressing S37A β-catenin were transfected with either U6-Aptamer (+) or U6 vector (−). After formaldehyde fixation, sonicated lysates were immunoprecipitated with either anti-β-catenin antibody or mouse normal IgG. RNA was extracted and analyzed by RT-PCR.

**Inhibition of tumorigenesis by the RNA aptamer.** Finally, to test whether the RNA aptamer can suppress the transforming potential of β-catenin, we selected stable HCT116 cells expressing the RNA aptamer. Because cyclin D1 expression was reduced in the stable RNA aptamer cell lines (Fig. 7*A*), these should tend to be arrested in the $G_1$-S transition of the cell cycle. As shown by the flow cytometric analysis in Fig. 7*B*, the percentage of S phase was reduced form 31.5% to 15% in the cells stably expressing the RNA aptamer, pointing to arrest in $G_1$. We also assayed soft agar colony formation (Fig. 7*C*) to test whether the aptamer lowered tumor-forming potential. The number and size of colonies were reduced

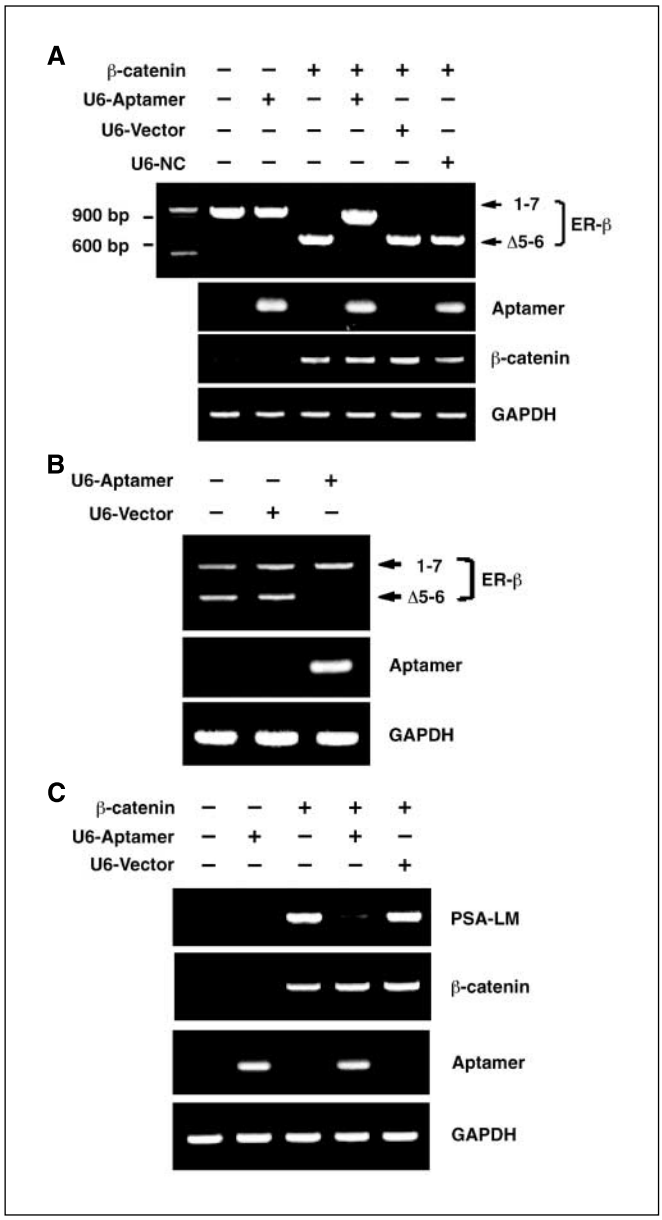


**Figure 5.** Effects of RNA aptamer on the β-catenin–induced alternative splicing. *A,* RT-PCR analysis to detect ER-β alternative splicing. HCT116 cells were cotransfected with S37A β-catenin cDNA in addition to U6-Vector, U6-NC, or U6-aptamer as indicated. *B,* LoVo cells, expressing ER-β and ER-β Δ5-6, were transfected with U6-Vector or U6-aptamer as indicated. RT-PCR analysis was done to detect the ER-β Δ5-6 expression. *C,* RT-PCR analysis to detect PSA alternative splicing. HCT116 cells were cotransfected with the DNA as described in (*A*).

in the cells stably expressing the RNA aptamer. Taken together, these results strongly suggest that the β-catenin-binding RNA aptamer is effective in arresting the cell cycle and ultimately reducing the tumorigenesis of colon cells.

## Discussion

It is becoming clear that a single protein can be involved in more than one step of gene expression, such as transcription, posttranscriptional processing, and translation. In this respect, although β-catenin is a nuclear transcriptional activator, it can also act as a posttranscriptional modulator involved in regulated splicing (18, 33). We showed here that stabilized S37A β-catenin induced rare alternative splicing variants of ER-β and PSA in HCT116 colon cancer cells. This is consistent with the recent report by Sato et al. in cervical cancer HeLa cells (18). In our study, stable and transient expression of S37A β-catenin caused dramatic induction of the ER-β and PSA alternatively spliced variants, which are not present in control HCT116 cells. These findings are strengthened by previous reports that diverse isoforms of ER-β could be found in colon cancers, and hence that the expression of the dominant-negative ER-β Δ5-6 was likely to contribute to the neoplastic transformation of intestinal epithelial cells (29, 30).

Nuclear localization of β-catenin may be critical not only for the transcription of oncogenic genes but also for the alternative splicing of pre-mRNA. Therefore, a molecule that specifically targeted nuclear β-catenin could be useful in antitumor therapy (34, 35). Here, we report the first characterization of a β-catenin-binding RNA aptamer that meets these criteria. We employed the SELEX procedure and a nuclear U6 RNA expression vector to target the nuclear functions of β-catenin. Approaches involving restricted localization of the RNA aptamer may have advantages because they could disrupt protein-protein interaction in specific cellular compartments (21, 22). The RNA aptamer significantly inhibited the generation of β-catenin-mediated splicing variants of ER-β and PSA, suggesting that it could inhibit β-catenin from interacting with other splicing regulators. The use of the RNA aptamer may shed new light on the mechanism of β-catenin-mediated splicing.

Intracellular aptamers have many uses (36, 37). First, their exceptional specificity and high level of expression make it possible

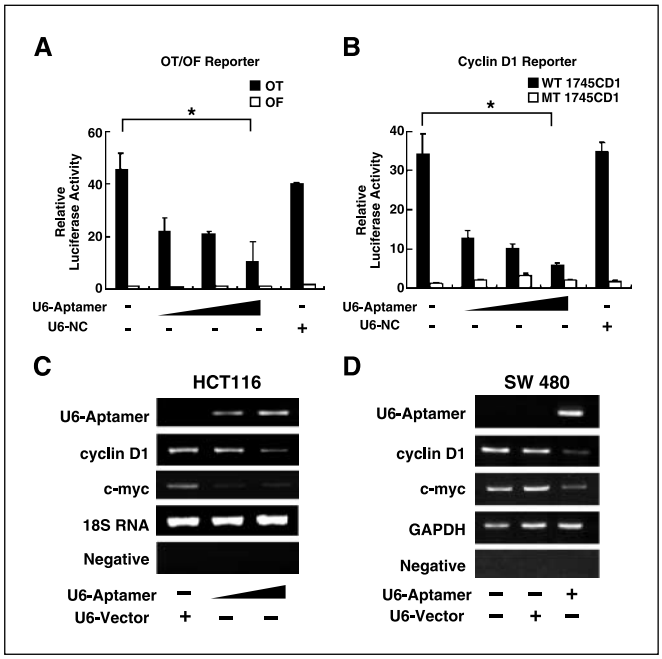


**Figure 6.** Inhibition of β-catenin transcriptional activity by the RNA aptamer. *A,* HCT116 cells were cotransfected with OT or OF luciferase reporters together with either U6 vector or U6-aptamer (0.2, 0.5, and 0.7 μg). Five independent experiments were done. *, $P < 0.001$. *B,* HCT116 cells were transfected with -1745 cyclin D1 promoter luciferase reporters (*WT*, wild-type; *MT*, mutant TCF sites) together with U6, U6-NC, or the U6-aptamer (0.2, 0.5, 0.7 μg). Three independent experiments were performed. *, $P < 0.001$. The expression of β-catenin target genes was detected by RT-PCR in HCT116 (*C*) and SW 480 (*D*) colon cancer cells. Negative is RT control.

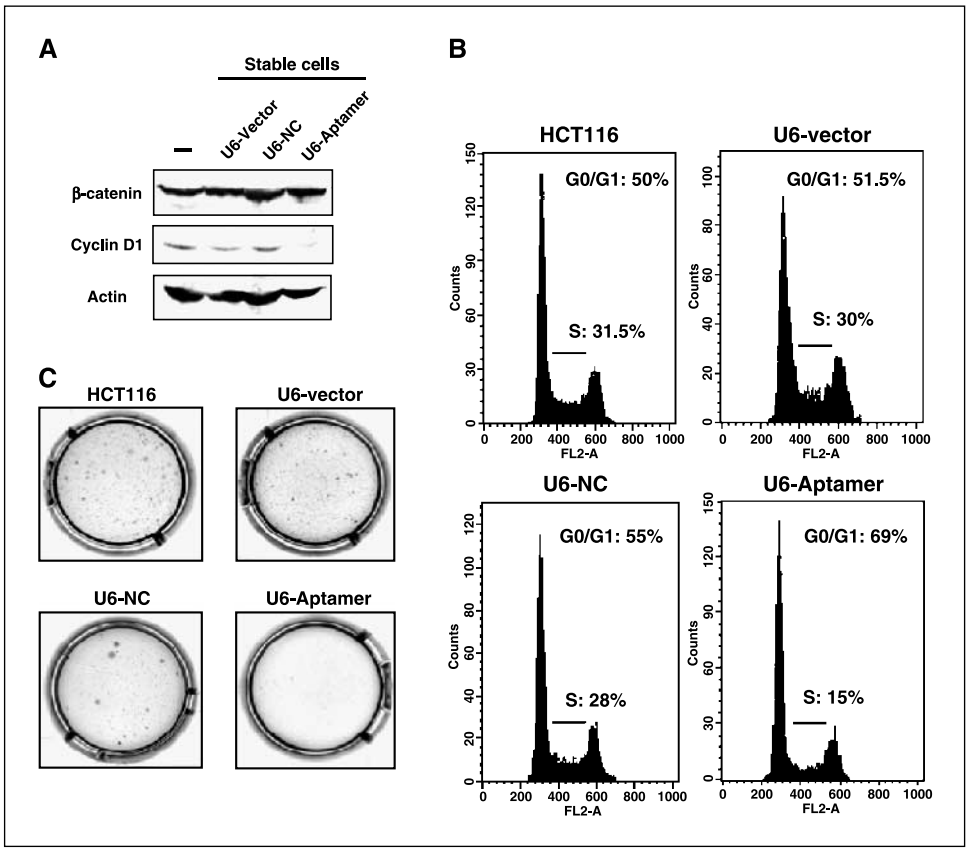


**Figure 7.** Cell cycle arrest and inhibition of colony formation in stable aptamer cell lines. *A,* Western blot analysis of HCT116 cells stably transfected with RNA aptamer. The blot was probed sequentially with anti-cyclin D1, anti-β-catenin, and anti-actin antibodies. *B,* flow cytometric analysis of the U6 stable cell lines. The cells were stained with propidium iodide to measure DNA content. Percentages of cells in $G_0$-$G_1$ and S phase are indicated. *C,* soft agar colony forming assay of the U6 stable cell lines. The cells were cultured in soft agar. After 10 days, colonies were stained with 0.005% crystal violet.

to distinguish between similar cellular proteins and to differentially modulate the functions of these latter. Second, they not only alter the known functions of their targets but can also affect previously unidentified functions, thereby providing insight into novel protein functions, as shown in this study. This aptamer, thus, fulfils the criteria for a specific inhibitor of the β-catenin/TCF transcription complex as well as of the alternative splicing of diverse RNAs in tumor cells and could be further developed as a therapeutic agent for β-catenin-mediated tumorigenesis in conjunction with a TCF-binding RNA aptamer (37–39).

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