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Selective Targeting to the Hyperactive β-Catenin/T-Cell Factor Pathway in Colon Cancer Cells[1]

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ABSTRACT

Many colon cancers suffer mutations in either the adenomatous polyposis coli or β-catenin genes that lead to stabilization of β-catenin and activation of downstream T-cell factor (Tcf) target genes. We have developed a novel approach targeting colon cancer cells based on their aberrant β-catenin/Tcf signaling pathway. A recombinant adenovirus, in which an apoptosis gene *fadd* is under the control of the promoter containing Tcf-responsive elements, selectively and efficiently kills colon cancer cells in which the β-catenin/Tcf pathway is hyperactivated. Our data therefore provide a conceptual proof that aberrantly activated Wnt/β-catenin/Tcf pathways can be used to selectively target colon cancers.

INTRODUCTION

β-Catenin is a multifunctional molecule involved in cell-cell adhesion and Wnt signaling during development. Regulation of membrane, cytoplasmic, and nuclear pools of β-catenin is crucial for modulating its adhesion and signaling functions (1). Normally, β-catenin is localized in cell-cell junctions with very low levels of β-catenin in the cytoplasm and nucleus. Excess β-catenin is targeted to proteasome-mediated degradation by complexes containing GSK-3β,[4] Axin, the APC, and other components (2–8). β-catenin was initially predicted to be a component of the mammalian Wnt pathway based on the fact that its homologue, *armadillo*, is a segment polarity gene in the wingless pathway in *Drosophila* (9). Wnt signaling results in the inactivation of GSK-3β, which in turn leads to the stabilization of cytoplasmic β-catenin. The β-catenin protein then translocates into the nucleus where it interacts with the transcriptional factor Tcf/Lef to activate transcription of Wnt responsive genes (10–12).

Regulation of the signaling activity of β-catenin is important for tumorigenesis. *wnt-1* was mapped adjacent to the retroviral insertion sites of the mouse mammary tumor virus and contributes to mammary tumorigenesis in mice (13). The APC tumor suppressor is mutated in ~80% of both the familial adenomatous polyposis syndrome and sporadic colon cancers. Mutant APC proteins lose their ability to down-regulate β-catenin, which results in activation of β-catenin/Tcf-mediated transcription. Loss of APC is believed to be one of the early initiating events in multistage colorectal tumorigenesis (14). In addition, activating β-catenin mutations in the putative GSK-3β phosphorylation sites have been identified in 50% of colon cancers that retain the wild-type APC, which render the β-catenin protein resistant to the proteasome-mediated destruction (15–17). Taken together, the primary lesion in most colon cancer cells is a defect in the down-regulation of β-catenin, causing abnormal activation of genes containing Tcf/Lef-responsive elements. Both *c-myc* and *cyclin D1* have been identified as transcriptional targets of β-catenin (18, 19). Activation of these genes may cause deregulation of cell cycle progression, and hence unscheduled proliferation. Furthermore, activating β-catenin mutations have also been identified in a variety of tumors, such as melanomas, hepatocellular carcinomas, skin cancers (pilomatricomas), brain tumors (medulloblastomas), ovarian cancers, and prostate cancers (20–26).

In this report, we designed a strategy to exploit the differential transcription potential of β-catenin to selectively target tumor cells with elevated β-catenin signaling activity. We show that introduction of the cell death gene *fadd* under the control of the promoter containing wild-type Tcf/Lef-binding sites resulted in preferential killing of colon cancer cells with hyperactive β-catenin/Tcf activity. We believe this approach may be used to develop therapies to target tumor cells that have defects in the Wnt/β-catenin/Tcf signal transduction pathway.

MATERIALS AND METHODS

Cell Culture. All media and supplements were purchased from Life Technologies, Inc. (Rockville, MD) and all cells were purchased from American Type Culture Collection (Manassas, VA) or otherwise indicated. 293 and HeLa cells were cultured in DMEM supplemented with 10% of fetal bovine serum and 1% of penicillin/streptomycin. SW480 and SW48 colorectal cancer cell lines were cultured in Leibovitz's L-15 media supplemented with 10% of fetal bovine serum and 1% of penicillin/streptomycin; the HCT116 colorectal cancer cell line was cultured in McCoy's 5A medium with similar supplements. A549 lung carcinoma cells were cultured in Ham's F-12K medium with similar supplements. NCM460 normal human colon epithelial cells were generously provided by Dr. M. Moyer (INCELL Corp., San Antonio, TX) and cultured in M3:10 complete medium (27). HRCE cells were purchased from Clonetics (San Diego, CA) and cultured in renal epithelial cell growth medium (Clonetics).

Construction of Plasmids and Adenoviral Vectors. The blunted *Kpn*I-*Bam*HI *fadd* fragment from pcDNA3-Fadd (a generous gift from Dr. V. Dixit, Genentech Inc., South San Francisco, CA) was used to replace the *Nco*I-*Xba*I Luc fragment in the pGL3-Basic vector (Promega, Madison, WI) to create O-Fd. TOP-TK-Luc, and FOP-TK-Luc (generous gifts from Dr. H. Clevers, Utrecht University, Utrecht, The Netherlands) (28) were used as templates for the PCR fragments containing the TK basal promoter plus either the wild-type or mutant Tcf/Lef binding sites. These PCR fragments were then cloned into the *Bgl*II site in O-Fd to give rise to Wt-Fd or Mut-Fd, respectively. The AdEasy system for adenovirus construction was a generous gift from Drs. B. Vogelstein and K. W. Kinzler (Johns Hopkins Oncology Center, Baltimore, MD) (29). Briefly, a *Xho*I cassette containing various promoters and the *fadd* coding sequence from O-Fd, Wt-Fd, or Mut-Fd was cloned into the adenoviral shuttle vector pAd-Track, which contains a GFP tracking tag. The *Kpn*I-*Xho*I *fadd* fragment from pcDNA3-Fadd was cloned into the adenoviral shuttle vector pAd-Track-CMV. The resulting shuttle vectors were then linearized with *Pme*I and cotransformed with E1-deleted adenoviral backbone AdEasy-1 into the competent bacterial strain BJ5183, which allows efficient recombination to occur. After screening, a panel of recombinants for adenoviruses AdCMV-Fd, AdWt-Fd, AdMut-Fd, and AdO-Fd were generated.

Adenovirus Production and Titering. To produce viruses, 4 μg of *Pac*I-linearized adenoviral DNA was transfected into 50–70% confluent 293 cells in T-25 flasks by LipofectAMINE (Life Technologies, Inc.). Five to 7 days

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[4] The abbreviations used are: GSK-3β, glycogen synthase kinase-3β; APC, adenomatous polyposis coli; β-gal, β-galactosidase; CMV, cytomegalovirus; GFP, green fluorescence protein; Lef, lymphoid enhancer factor; Tcf, T-cell factor; HRCE, human renal cortical epithelial; FADD/Fd, Fas-associated death domain; TK, thymidine kinase; Luc, luciferase; Wt, wild type; moi, multiplicity of infection; Parp, poly(ADP-ribose) polymerase.

posttransfection, plaques marked by GFP were observed under a fluorescent microscope and cells were harvested and lysed in 2 ml of PBS by four cycles of freeze/thaw/vortex. The supernatant was collected and half of the supernatant was used to reinfect 50–70% confluent 293 cells. Viruses were collected 3 days postinfection when cytopathic effect became evident. Further amplification of virus stocks was achieved through a few rounds of infections.

To titer the viruses, 50–70% confluent 293 cells in 6-well dishes were infected with serial dilutions of the virus stocks. GFP plaques were counted 5 days postinfection.

Transfections, β-Gal Assay, and Luc Assay. Cells were seeded in 6-well dishes for β-gal assays and 12-well dishes for Luc assays. Transfections were performed using LipofectAMINE or FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer's instructions.

For β-gal assay, 2×10^5 SW480 or 1×10^5 HeLa cells were seeded and cotransfected with CMV-β-gal and different combinations of plasmids. Cells were then stained 48 h later for β-gal activity using a β-gal staining kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. Live blue cells were counted and the relative number of blue cells represents the survival rate of transfected cells.

For Luc assays, $1–2 \times 10^5$ SW480, HCT116, or SW48 cells were seeded in 12-well dishes. The next day cells were about 50% confluent and cotransfected with 0.8 μg of either vector or pcDNAI-ΔN-hTcf4 (generous gifts from Dr. H. Clevers, Utrecht University) plus 0.01 μg of pRL-TK (Promega) and 0.2 μg of TOP-TK-Luc or FOP-TK-Luc. Luc assays were performed 48 h posttransfection. Briefly, cells were washed once with PBS and then lysed in 200 μl of lysis buffer for 15 min at room temperature. The lysates were clarified by centrifugation at 14,000 rpm for 10 min and 50 μl of each lysate were used to measure Luc reporter gene expression (Luc assay kit; Promega). The Luc activity was normalized to *Renilla* Luc activity from cotransfected internal control pRL-TK. All experiments were performed in triplicate at least twice.

Cell Viability Assay. Different cells were plated as follows in 96-well dishes: 10,000 cells in 100 μl of media were plated for NCM460, 8,000 cells for SW480, 5,000 cells for SW48 and HCT116, 2,500 cells for HRCE and A549, and 2,000 cells for HeLa. The next day 8 wells were infected with each adenovirus at a moi of 50. Cell viability in each well was determined using a cell proliferation assay kit according to the manufacturer's instruction (Promega). Twenty-five microliters of the assay reagent were added to each well of cultured cells and incubated at 37°C for 1–4 h. The absorbance difference of 490 nm *versus* 650 nm was recorded using the SpectraMax340 96-well plate reader (Molecular Devices, Menlo Park, CA). Cell viability is expressed as a percentage of the absorbance difference relative to mock-infected cells. The average of at least two independent experiments with eight replicates was shown.

Gel Electrophoresis and Western Blot Analysis. Transfected or infected cells were harvested and protein concentrations were measured using Bio-Rad protein assay kits (Bio-Rad, Hercules, CA). An equal amount of protein from each lysate was analyzed by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were blocked in 2% BSA, 1× Tris-buffered saline, and 0.05% Tween 20, incubated with primary antibodies for 1 h, and then probed with horseradish peroxidase-conjugated secondary antibodies. Enhanced chemiluminescence reagents (Amersham Pharmacia Biotech, Piscataway, NJ) were used to visualize the protein bands on the membranes. Antibodies against β-catenin and Parp were purchased from Transduction Laboratories (Lexington, KY). Antibodies against Fadd, actin, and GFP were purchased from Upstate Biotechnology (Lake Placid, NY), Sigma (St. Louis, MO), and Santa Cruz Biotechnology (Santa Cruz, CA), respectively.

RESULTS

Elevated β-Catenin/Tcf-dependent Transactivation in Colon Cancer Cell Lines. Mutations in APC and β-catenin contribute to elevation of β-catenin/Tcf transactivation in colorectal and melanoma cell lines (15, 20). A number of colorectal cell lines had increased cytosolic β-catenin levels (data not shown). Among them, cell lines SW480 (with a truncation in APC), SW48 (with a S33Y substitution in β-catenin), and HCT116 (with a deletion at residue S45 in β-catenin) (15, 17) were further examined for the β-catenin/Tcf-dependent transactivation. As shown in Fig. 1, upon transfection of TOP-TK-Luc, a reporter plasmid containing the wild-type Tcf/Lef binding sites and a basal TK promoter upstream of a Luc gene, all three cell lines expressed high levels of Luc activity. In contrast, upon transfection of FOP-TK-Luc, a reporter containing the mutant Tcf/Lef binding sites, only basal level Luc activity was detected, indicating that the elevated activity of TOP-TK-Luc in those cell lines was Tcf/Lef dependent. The β-catenin/Tcf-specific transactivation was further supported by the fact that ΔN-Tcf4, a dominant negative form of Tcf4 that lost its β-catenin-binding activity, was capable of suppressing the reporter expression. In conclusion, these data strengthened the notion that the β-catenin/Tcf transcriptional potential is abnormally up-regulated in the colon cancer cells, and this activity is Tcf/Lef dependent regardless of their genetic lesions.

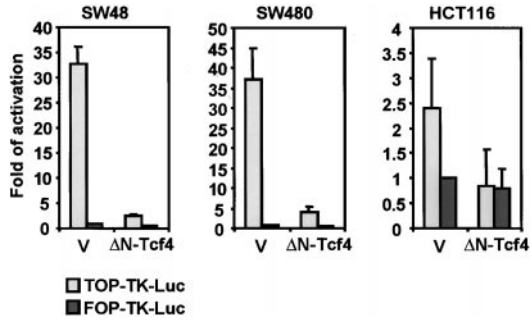

Fig. 1. β-Catenin/Tcf-mediated Luc reporter activity in colon cancer cell lines. SW48, SW480, and HCT116 cells were transfected with TOP-TK-Luc (the Wt Tcf/Lef Luc reporter) or FOP-TK-Luc (the mutant reporter) plus either vector (V) or pcDNAI-ΔN-Tcf4 (ΔN-Tcf4). Luc activities were assayed 48 h later and plotted as fold of activation of TOP-TK-Luc relative to that of FOP-TK-Luc (set at 1). The mean value of absolute Luc activity for activation of TOP-TK-Luc in SW48, SW480, or HCT116 is 59, 74, or 4.3, respectively, and that for activation of FOP-TK-Luc in all three cell lines is between 1.5 and 2.1.

Wt-Fd Induces Cell Death in SW480 Cells. We devised a strategy that makes use of the differential transcriptional activation of β-catenin between normal and cancer cells to kill cancer cells preferentially. A panel of constructs were made in which the cell death gene, *fadd*, was placed downstream of the constitutive CMV promoter (CMV-Fd), the promoter containing either the Wt (Wt-Fd) or mutant (Mut-Fd) Tcf/Lef binding sites, or no promoter (O-Fd; Fig. 2*A*). Fadd is a death domain protein that interacts with Fas and mediates Fas-induced apoptosis and overexpression of Fadd induces apoptosis in various cell types (30). We anticipated that the expression of Fadd would be controlled by the activity of the β-catenin-Tcf complex and so would Fadd-induced cell death. We cotransfected each of these constructs along with a CMV-β-gal plasmid (an indicator of transfected cells) into SW480 or HeLa cells. Forty-eight hours later, cells were stained for β-gal expression and live blue cells were counted. As shown in Fig. 2*B*, Wt-Fd eliminated SW480 cells in a dose-dependent manner and was as efficient as CMV-Fd, whereas Mut-Fd did not cause significant death of SW480 cells. In HeLa cells, which have no detectable β-catenin/Tcf transactivation activity (data not shown), neither Wt-Fd nor Mut-Fd caused any cell death although CMV-Fd killed 90% of the transfected cells (Fig. 2*C*). In addition, β-catenin was able to sensitize HeLa cells to the killing only by Wt-Fd but not by Mut-Fd (Fig. 2*D*). Altogether, these data suggest that Wt-Fd is capable of inducing death selectively in cells that have elevated β-catenin/Tcf transcriptional activity.

β-Catenin Activates Expression of Fadd from Wild-Type Tcf/Lef-responsive Elements. To demonstrate that the observed cell death was due to the controlled expression of Fadd, we probed Fadd expression by Western blot analysis. In HeLa cells, high levels of Fadd expression were detected from CMV-Fd, but not from Wt-Fd or Mut-Fd (Fig. 3*A*). In SW480 cells, however, Fadd expression from Wt-Fd reached a similar

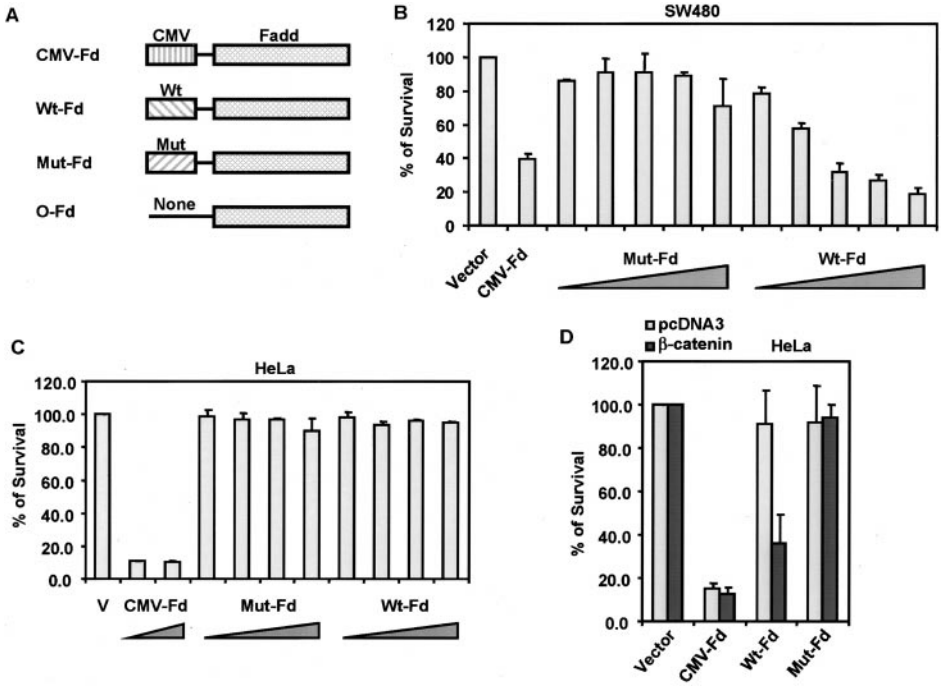

Fig. 2. SW480 but not HeLa cells are killed by Wt-Fd in a dose-dependent manner. *A*, schematic representation of different Fd constructs. CMV, the constitutive CMV promoter; Wt, the promoter containing the Wt Tcf/Lef binding sites; Mut, the promoter containing the mutant Tcf/Lef binding sites; O, no promoter in front of *fadd*. *B*, SW480 cells were cotransfected with 2 μg of CMV-β-gal (indicator of transfected cells) plus 1 μg of empty vector or CMV-Fd, or increasing amounts of Mut-Fd or Wt-Fd (0.05–1 μg). *C*, HeLa cells were cotransfected with 1 μg of CMV-β-gal plus 1 μg of vector (V) or increasing amounts of CMV-Fd (0.5–1 μg), Mut-Fd, or Wt-Fd (0.125–1 μg). *D*, HeLa cells were cotransfected with 0.5 μg of CMV-β-gal plus 1.5 μg of different Fadd constructs as indicated and 1 μg of either pcDNA3 or pCAN-β-catenin (β-catenin). Cells were stained for β-gal 48 h posttransfection and relative numbers of live blue cells to the vector control (percentage of survival) were graphed.

level as that from CMV-Fd. Neither O-Fd nor Mut-Fd expressed significant amounts of Fadd, as expected (Fig. 3*B*). We wondered whether the differential expression of Fadd from Wt-Fd truly reflected the differential transcriptional activity of β-catenin in these cells. We tested the dependence of Fadd expression on β-catenin in 293 cells since the adenovirus E1B 19K protein in 293 cells protects them from Fadd-induced apoptosis (31), which allows easier detection of Fadd. We cotransfected various Fadd constructs along with vector or ΔN-β-catenin, a nondegradable form of β-catenin lacking the N-terminal degradation signal, into 293 cells. As shown in Fig. 3*C*, the Fadd protein was detected from transfections with as little as 10 ng of CMV-Fd. Nevertheless, Fadd expression was barely above background levels in transfections with up to 2 μg of Wt-Fd or Mut-Fd. In cells cotransfected with ΔN-β-catenin, Tcf/Lef-dependent transcription was activated (data not shown) and concomitantly Fadd expression from Wt-Fd was increased selectively. In addition, Fadd expression from Wt-Fd was also preferentially elevated in 293-Wnt-1 cells and in lithium-treated 293 cells (data not shown), likely due to Wnt-1 or lithium-induced inhibition of GSK-3β and subsequent activation of β-catenin/Tcf-dependent transcription (32–35). These observations demonstrate that the expression of Fadd from the Tcf/Lef-responsive elements is tightly controlled by the activity of the Wnt/β-catenin/Tcf pathway.

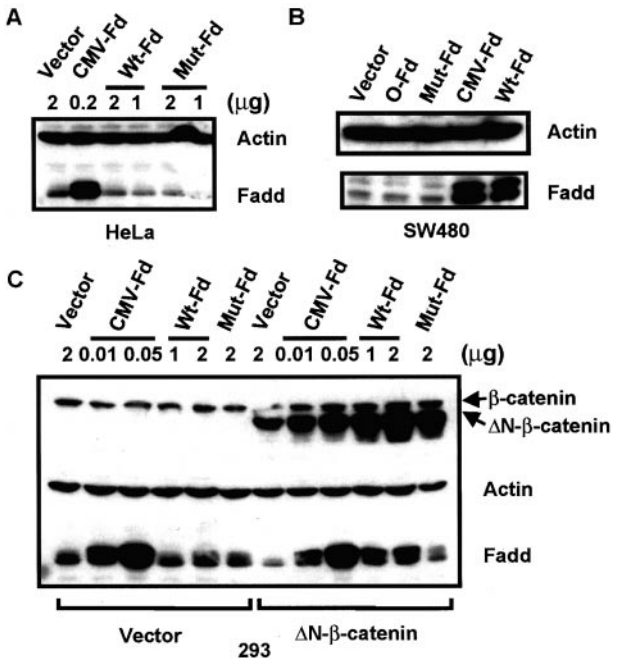

Fig. 3. Activated β-catenin/Tcf signaling specifically induces Fadd expression from Wt-Fd. *A*, HeLa cells were transfected with indicated amounts of different constructs. *B*, SW480 cells were transfected with 2 μg of indicated constructs. C, 293 cells were cotransfected with 2 μg of either vector or pCAN-ΔN-β-catenin (ΔN-β-catenin) plus indicated amounts of different constructs. Cells were harvested 48 h later and the lysates, normalized to protein concentration, were analyzed by Western blot using Fadd, actin, and β-catenin antibodies. Endogenous β-catenin migrates slightly slower than the transfected ΔN-β-catenin.

AdWt-Fd Induces Cell Death Selectively in Colon Cancer Cells with Aberrant Activation of β-Catenin/Tcf Signaling. To increase gene transfer efficiency, we constructed AdCMV-Fd, AdWt-Fd, and AdO-Fd adenoviral vectors bearing the same Fadd expression cassettes shown in Fig. 2*A* (29). Cells were infected with the adenoviruses and cell viability was measured 48 h postinfection. As shown in Fig. 4*A*, SW48 cells, in which β-catenin/Tcf-mediated transcription was activated >30-fold (Fig. 1), were killed efficiently by AdWt-Fd, while not affected by AdO-Fd. The extent of AdWt-Fd-induced cell killing was comparable in SW48 and SW480 cells (Fig. 4*B*), in agreement with their similar elevation of β-catenin/Tcf activity (Fig. 1). HCT116 cells were partially susceptible to AdWt-Fd (Fig. 4*C*), which coincided with its moderately up-regulated β-catenin/Tcf signaling (Fig. 1). NCM460, a normal human colon epithelial cell line (27), and HRCE, a normal human renal cortical epithelial cell line, were resistant to the killing by AdWt-Fd (Fig. 4, *D* and *E*). Other cancer cell lines such as HeLa and A549, which have normal β-catenin/Tcf signaling activity (data not shown), were resistant to the killing by AdWt-Fd as well (Fig. 4, *F* and *G*). Parallel to cell death, Fadd expression from AdWt-Fd is also dependent on the activation of the β-catenin/Tcf pathway. As shown in Fig. 5*A*, comparable levels of Fadd were readily detected from AdCMV-Fd and AdWt-Fd infections in SW480 and SW48 cells. In contrast, although AdCMV-Fd gave high

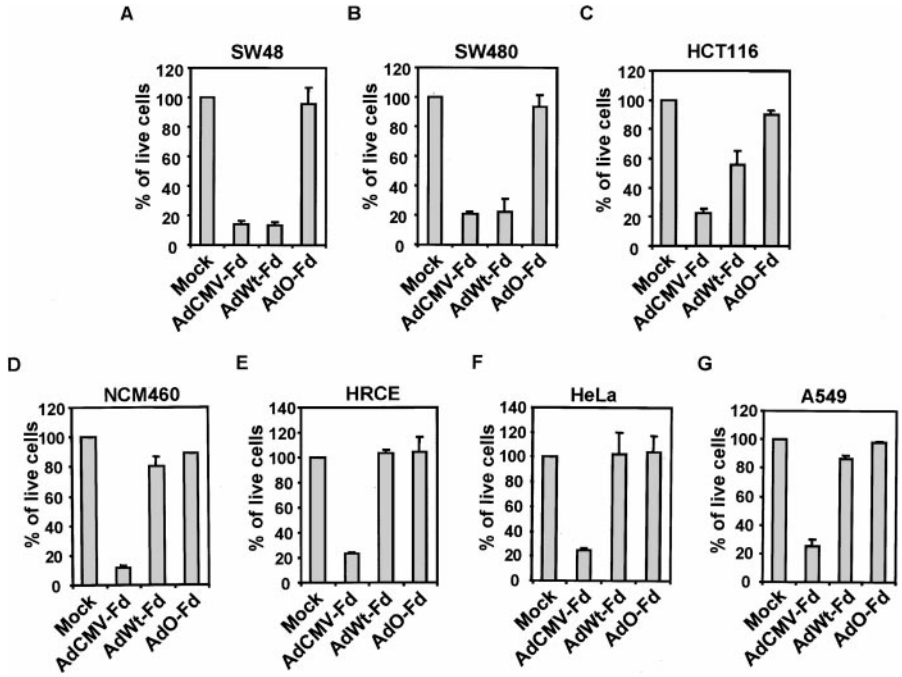

Fig. 4. AdWt-Fd selectively induces cell death in colon cancer cells. *A*, SW48; *B*, SW480; *C*, HCT116; *D*, NCM460; *E*, HRCE; *F*, HeLa; and *G*, A549 cells in 96-well plates were infected with the indicated GFP-tagged adenoviruses at a moi of 50. Ninety percent of cells were infected, as judged by the GFP fluorescence. Cell viability assay was performed 48 h postinfection. The results were expressed as percentages of live cells normalized to mock infection as 100%.

levels of Fadd expression, AdWt-Fd did not result in any detectable levels of Fadd in normal HRCE (Fig. 5*B*) and NCM460 cells (Fig. 5*C*). Furthermore, expression of the Fadd protein in different cells correlated precisely with the disappearance of the full-length Parp, an indication of apoptosis. Similar infection by different adenoviruses was assured via the GFP tag present on these viruses (*bottom panels*, Fig. 5). In conclusion, AdWt-Fd selectively targeted colon cancer cells with the activated β-catenin/Tcf pathway while leaving normal or other types of tumor cells without such activation unaffected. These results provided crucial evidence for further development of colon cancer therapy based on abnormal Wnt/β-catenin/Tcf signaling.

DISCUSSION

Events such as Wnt stimulation, loss of APC, and mutational activation of β-catenin all contribute to activation of β-catenin/Tcf-mediated transcription and have been implicated in tumorigenesis (1). Also, the pathway is greatly up-regulated in tumors relative to normal cells, making it a potential candidate target for cancer therapy. In this article, we demonstrated a specific targeting approach toward colon cancer cells in which β-catenin/Tcf signaling is up-regulated because of mutations in either APC or β-catenin.

A number of studies reported various approaches to target cancer cells selectively (36, 37). For example, Onyx-015, an adenovirus mutant lacking E1B 55K, targets selectively human tumor cells deficient in p53 functions (38, 39). Other strategies use tissue-specific promoters. The prostate-specific antigen or α-fetoprotein promoter has been used to construct replication-restricted adenoviruses that allow specific replication and killing of prostate-specific antigen-positive or α-fetoprotein-positive cancer cells. However, the underlining mechanisms for the overexpression of these markers, which may or may not account for the defects of these tumor cells, remain elusive. In another study reported by Parr *et al.* (40), the E2F-1 promoter has been shown to direct expression of β-gal in gliomas but not in normal proliferating hepatocytes *in vivo*. Parr *et al.* (40) proposed that the tumor selectivity is attributable to repression by RB-E2F complexes in normal tissue and loss of RB and activation of E2F in tumor tissue. Nevertheless, normal cells do require E2F activity at G_1-S transition for their cell cycle progression.

Our approach targets aberrant activation of the Wnt/β-catenin/Tcf pathway, an abnormality that affects the majority of colon cancer cells. When *fadd* is under the control of a defined promoter containing the Tcf/Lef-responsive elements, a strong correlation between the cell death

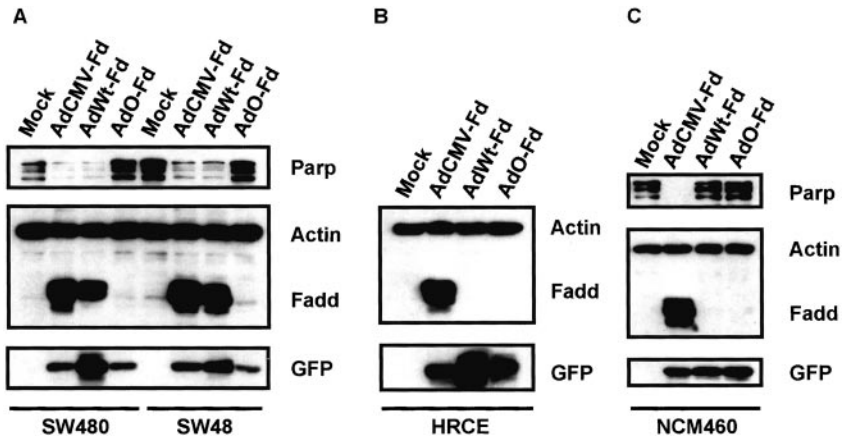

Fig. 5. Fadd expression from different adenoviruses in SW480, SW48, HRCE, and NCM460 cells. *A*, SW480 and SW48; *B*, HRCE; and *C*, NCM460 cells were infected with indicated GFP-tagged adenoviruses at a moi of 50. Forty-eight hours postinfection, cells were harvested and the normalized lysates were analyzed by Western blot using Parp, Actin, Fadd, and GFP antibodies. Parp was used as an indicator for apoptosis and GFP for infection efficiency.

induced by AdWt-Fd and the intrinsic activation of β-catenin/Tcf signaling is evident. Tcf/Lef reporter activity is at the basal level in normal cells compared to different degrees of aberrant activation in colon cancer cells harboring mutations in either APC or β-catenin. Our data from various cell lines show very promising therapeutic potential targeting cancer cells that have hyperactive β-catenin/Tcf4 activity. Indeed, AdWt-Fd kills efficiently colon cancer cells but exhibits no toxicity in normal cells. However, it still remains unknown whether in animal models this approach will be effective on colon cancers or safe on normal adult tissues such as colon crypts or hair follicles where Tcf/Lef activity is necessary for maintaining stem cells (41–43). To further develop this strategy, a replication-restrictive virus may be constructed in which essential viral genes are under the control of β-catenin/Tcf-responsive promoters. Therefore, expression of the viral genes as well as viral replication will be restricted to cells that have hyperactive β-catenin/Tcf signaling. We anticipate that such virus would allow us to evaluate efficacy and toxicity in animal models. Moreover, this strategy may be applicable to other tumors that have defects causing activation of β-catenin/Tcf-mediated transcription. In tumors such as skin cancers (melanomas and pilomatricomas), hepatocellular carcinomas, medulloblastomas, ovarian cancers, and prostate cancers, loss of function mutations in APC or activating mutations in β-catenin have been identified (20–26). In conclusion, specific defects that lead to abnormal transcriptional activation in human tumors can be used to target these tumors for cancer gene therapy.

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Note Added in Proof

Replicating adenoviruses controlled by the Tcf4-responsive promoters are reported by Brunori *et al*. in Journal of Virology, *75:* 2857–2865, 2001.

REFERENCES

1. Morin, P. J. β-Catenin signaling and cancer. BioEssays, *21:* 1021–1030, 1999.
2. Behrens, J., Jerchow, B. A., Wurtele, M., Grimm, J., Asbrand, C., Wirtz, R., Kuhl, M., Wedlich, D., and Birchmeier, W. Functional interaction of an axin homolog, conductin, with β-catenin, APC, and GSK3β. Science (Wash DC), *280:* 596–599, 1998.
3. 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.
4. Hart, M., Concordet, J. P., Lassot, I., Albert, I., del los Santos, R., Durand, H., Perret, C., Rubinfeld, B., Margottin, F., Benarous, R., and Polakis, P. The F-box protein β-TrCP associates with phosphorylated β-catenin and regulates its activity in the cell. Curr Biol., *9:* 207–210, 1999.
5. Munemitsu, S., Albert, I., Souza, B., Rubinfeld, B., and Polakis, P. Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. Proc. Natl. Acad. Sci. USA, *92:* 3046–3050, 1995.
6. 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.
7. Orford, K., Crockett, C., Jensen, J. P., Weissman, A. M., and Byers, S. W. Serine phosphorylation-regulated ubiquitination and degradation of β-catenin. J. Biol. Chem., *272:* 24735–24738, 1997.
8. Winston, J. T., Strack, P., Beer-Romero, P., Chu, C. Y., Elledge, S. J., and Harper, J. W. The SCFβ-TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IκBα and β-catenin and stimulates IκBα ubiquitination *in vitro*. Genes Dev., *13:* 270–283, 1999.
9. McCrea, P. D., Turck, C. W., and Gumbiner, B. A homolog of the armadillo protein in *Drosophila* (plakoglobin) associated with E-cadherin. Science (Wash DC), *254:* 1359–1361, 1991.
10. Polakis, P. The oncogenic activation of β-catenin. Curr. Opin. Genet. Dev., *9:* 15–21, 1999.
11. Eastman, Q., and Grosschedl, R. Regulation of LEF-1/TCF transcription factors by Wnt and other signals. Curr. Opin. Cell Biol., *11:* 233–240, 1999.
12. Cadigan, K. M., and Nusse, R. Wnt signaling: a common theme in animal development. Genes Dev., *11:* 3286–3305, 1997.
13. Nusse, R., and Varmus, H. E. Wnt genes. Cell, *69:* 1073–1087, 1992.
14. Kinzler, K. W., and Vogelstein, B. Lessons from hereditary colorectal cancer. Cell, *87:* 159–170, 1996.
15. Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, 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.
16. Sparks, A. B., Morin, P. J., Vogelstein, B., and Kinzler, K. W. Mutational analysis of the APC/β-catenin/Tcf pathway in colorectal cancer. Cancer Res., *58:* 1130–1134, 1998.
17. Ilyas, M., Tomlinson, I. P., Rowan, A., Pignatelli, M., and Bodmer, W. F. β-Catenin mutations in cell lines established from human colorectal cancers. Proc. Natl. Acad. Sci. USA, *94:* 10330–10334, 1997.
18. 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.
19. Tetsu, O., and McCormick, F. β-Catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature (Lond.), *398:* 422–426, 1999.
20. Rubinfeld, B., Robbins, P., El-Gamil, M., Albert, I., Porfiri, E., and Polakis, P. Stabilization of β-catenin by genetic defects in melanoma cell lines. Science (Wash DC), *275:* 1790–1792, 1997.
21. de La Coste, A., Romagnolo, B., Billuart, P., Renard, C. A., Buendia, M. A., Soubrane, O., Fabre, M., Chelly, J., Beldjord, C., Kahn, A., and Perret, C. Somatic mutations of the β-catenin gene are frequent in mouse and human hepatocellular carcinomas. Proc. Natl. Acad. Sci. USA, *95:* 8847–8851, 1998.
22. Zurawel, R. H., Chiappa, S. A., Allen, C., and Raffel, C. Sporadic medulloblastomas contain oncogenic β-catenin mutations. Cancer Res., *58:* 896–899, 1998.
23. Chan, E. F., Gat, U., McNiff, J. M., and Fuchs, E. A common human skin tumour is caused by activating mutations in β-catenin. Nat. Genet., *21:* 410–413, 1999.
24. Palacios, J., and Gamallo, C. Mutations in the β-catenin gene (*CTNNB1*) in endometrioid ovarian carcinomas. Cancer Res., *58:* 1344–1347, 1998.
25. Miyoshi, Y., Iwao, K., Nagasawa, Y., Aihara, T., Sasaki, Y., Imaoka, S., Murata, M., Shimano, T., and Nakamura, Y. Activation of the β-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. Cancer Res., *58:* 2524–2527, 1998.
26. Voeller, H. J., Truica, C. I., and Gelmann, E. P. β-Catenin mutations in human prostate cancer. Cancer Res., *58:* 2520–2523, 1998.
27. Moyer, M. P., Manzano, L. A., Merriman, R. L., Stauffer, J. S., and Tanzer, L. R. NCM460, a normal human colon mucosal epithelial cell line. In Vitro Cell Dev. Biol. Anim., *32:* 315–317, 1996.
28. Korinek, V., Barker, N., Morin, P. J., van Wichen, D., de Weger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. Constitutive transcriptional activation by a β-catenin-Tcf complex in APC−/− colon carcinoma. Science (Wash DC), *275:* 1784–1787, 1997.
29. He, T. C., Zhou, S., da Costa, L. T., Yu, J., Kinzler, K. W., and Vogelstein, B. A simplified system for generating recombinant adenoviruses. Proc. Natl. Acad. Sci. USA, *95:* 2509–2514, 1998.
30. Chinnaiyan, A. M., O'Rourke, K., Tewari, M., and Dixit, V. M. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell, *81:* 505–512, 1995.
31. Perez, D., and White, E. E1B 19K inhibits Fas-mediated apoptosis through FADD-dependent sequestration of FLICE. J. Cell Biol. *141:* 1255–1266, 1998.
32. Hedgepeth, C. M., Conrad, L. J., Zhang, J., Huang, H. C., Lee, V. M., and Klein, P. S. Activation of the Wnt signaling pathway: a molecular mechanism for lithium action. Dev. Biol., *185:* 82–91, 1997.
33. Stambolic, V., Ruel, L., and Woodgett, J. R. Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. Curr. Biol., *6:* 1664–1668, 1996.
34. Klein, P. S., and Melton, D. A. A molecular mechanism for the effect of lithium on development. Proc. Natl. Acad. Sci. USA, *93:* 8455–8459, 1996.
35. Chen, R. H., Ding, W. V., and McCormick, F. Wnt signaling to β-catenin involves two interactive components. Glycogen synthase kinase-3β inhibition and activation of protein kinase C. J. Biol. Chem., *275:* 17894–17899, 2000.
36. Hallenbeck, P. L., Chang, Y. N., Hay, C., Golightly, D., Stewart, D., Lin, J., Phipps, S., and Chiang, Y. L. A novel tumor-specific replication-restricted adenoviral vector for gene therapy of hepatocellular carcinoma. Hum. Gene Ther., *10:* 1721–1733, 1999.
37. Rodriguez, R., Schuur, E. R., Lim, H. Y., Henderson, G. A., Simons, J. W., and Henderson, D. R. Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. Cancer Res., *57:* 2559–2563, 1997.
38. Bischoff, J. R., Kirn, D. H., Williams, A., Heise, C., Horn, S., Muna, M., Ng, L., Nye, J. A., Sampson-Johannes, A., Fattaey, A., and McCormick, F. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. Science (Wash DC), *274:* 373–376, 1996.
39. McCormick, F. Cancer therapy based on p53. Cancer J. Sci. Am., *5:* 139–144, 1999.
40. Parr, M. J., Manome, Y., Tanaka, T., Wen, P., Kufe, D. W., Kaelin, W. G., Jr., and Fine, H. A. Tumor-selective transgene expression in vivo mediated by an E2F-responsive adenoviral vector. Nat. Med., *3:* 1145–1149, 1997.
41. Korinek, V., Barker, N., Moerer, P., van Donselaar, E., Huls, G., Peters, P. J., and Clevers, H. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat. Genet., *19:* 379–383, 1998.
42. van Genderen, C., Okamura, R. M., Farinas, I., Quo, R. G., Parslow, T. G., Bruhn, L., and Grosschedl, R. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. Genes Dev., *8:* 2691–2703, 1994.
43. Zhou, P., Byrne, C., Jacobs, J., and Fuchs, E. Lymphoid enhancer factor 1 directs hair follicle patterning and epithelial cell fate. Genes Dev., *9:* 700–713, 1995.