Molecular Cancer Therapeutics

APC Mutations as a Potential Biomarker for Sensitivity to Tankyrase Inhibitors in Colorectal Cancer

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Abstract

In most colorectal cancers, Wnt/β-catenin signaling is activated by loss-of-function mutations in the *adenomatous polyposis coli* (*APC*) gene and plays a critical role in tumorigenesis. Tankyrases poly(ADP-ribosyl)ate and destabilize Axins, a negative regulator of β-catenin, and upregulate β-catenin signaling. Tankyrase inhibitors downregulate β-catenin and are expected to be promising therapeutics for colorectal cancer. However, colorectal cancer cells are not always sensitive to tankyrase inhibitors, and predictive biomarkers for the drug sensitivity remain elusive. Here we demonstrate that the short-form *APC* mutations predict the sensitivity of colorectal cancer cells to tankyrase inhibitors. By using well-established colorectal cancer cell lines, we found that tankyrase inhibitors downregulated β-catenin in the drug-sensitive, but not resistant, colorectal cancer cells. The drug-sensitive cells showed higher Tcf/LEF transcriptional activity than the resistant cells and possessed "short" truncated APCs lacking all seven β-catenin-binding 20-amino acid repeats (20-AARs). In contrast, the drug-resistant cells possessed "long" APC retaining two or more 20-AARs. Knockdown of the long APCs with two 20-AARs increased β-catenin, Tcf/LEF transcriptional activity and its target gene *AXIN2* expression. Under these conditions, tankyrase inhibitors were able to downregulate β-catenin in the resistant cells. These results indicate that the long APCs are hypomorphic mutants, whereas they exert a dominant-negative effect on Axin-dependent β-catenin degradation caused by tankyrase inhibitors. Finally, we established 16 patient-derived colorectal cancer cells and confirmed that the tankyrase inhibitor–responsive cells harbor the short-form APC mutations. These observations exemplify the predictive importance of *APC* mutations, the most common genetic alteration in colorectal cancers, for molecular targeted therapeutics. *Mol Cancer Ther; 16(4); 752–62. ©2017 AACR.*

Introduction

Loss-of-function mutations in *adenomatous polyposis coli* (*APC*) gene, a negative regulator of Wnt/β-catenin signaling, frequently occur in colorectal cancer (1, 2). These *APC* mutations promote tumorigenesis together with several common mutations, such as those in *KRAS*, *SMAD4*, and *TP53* (3, 4). APC functions as a functional scaffold for the β-catenin destruction complex, which is composed of β-catenin, Axin, casein kinase 1α and glycogen synthase kinase 3β (GSK3β), and suppresses Wnt/β-catenin signaling (5–10). In the destruction complex, APC and Axin promote the ubiquitin-dependent proteasomal degradation of β-catenin via casein kinase 1α- and GSK3β-mediated phosphorylation of β-catenin (8, 10). In genetically engineered mouse models, *APC* mutation–derived activation of Wnt/β-catenin signaling was shown to be required not only for promotion of colorectal cancer but also for tumor maintenance (11, 12). These observations indicate that the Wnt/β-catenin signaling pathway is a rational therapeutic target for colorectal cancer. So far, however, this pathway lacks druggable molecular targets, which has hampered the development of therapeutic drugs targeting Wnt/β-catenin signaling.

Tankyrases (tankyrase-1/PARP-5a and tankyrase-2/PARP-5b) are members of the PARP family (13–15). This unique class of PARPs recognizes its binding partners via the multiple ankyrin repeat cluster domain and poly(ADP-ribosyl)ates (PARylates) them via the carboxyl terminal catalytic domain (13, 16, 17). Tankyrases enhance Wnt/β-catenin signaling by PARylation and subsequent destabilization of Axins (18, 19). Pharmacologic inhibition of tankyrases causes accumulation of Axins, which in turn facilitates degradation of β-catenin and suppresses the

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growth of several *APC*-mutated colorectal cancer cell lines (18, 20–23). These observations suggest that tankyrase inhibitors are promising drugs for colorectal cancer treatment. However, colorectal cancer cells are not equally sensitive to tankyrase inhibitors (22). Although appropriate stratification of patients has profound implications for new drug development, predictive biomarkers for sensitivity to tankyrase inhibitors remain elusive. In this study, we demonstrate that a class of *APC* mutations predicts the sensitivity of colorectal cancer cells to tankyrase inhibitors.

Materials and Methods

Cell culture and proliferation assay

Human colorectal cancer cell lines COLO-320DM, SW403, SW480, and DLD-1 were maintained in RPMI1640 medium (Gibco, Life Technologies) supplemented with 10% FBS. RKO cells were maintained in DMEM low glucose (Nacalai Tesque) with 10% FBS. These cell lines were obtained from ATCC. HCT-15, HCC2998, HT-29, KM12, and HCT-116 cells were maintained as described previously (24). All cell lines were authenticated by short tandem repeat profiling analysis (BEX) in 2016. Cell proliferation was evaluated by the MTT assay (5-day drug treatment) as previously described (25) or by colony formation assay (10-day drug treatment). Normalization was done to cells treated with DMSO as vehicle, which were defined as 100%. Final concentration of DMSO was constant and the same for all the treatments.

Plasmid construction

Total RNA was purified from COLO-320DM cells using the RNeasy Kit (Qiagen) and used as a template to amplify the truncated *APC* [amino acid (aa) 1–811] fragment by reverse transcription-PCR. The primer sets were: 5′-ATCGGATCCGCTGCCACCATGGAACAAAAGCTGATTTCTGAAGAAGATCTGGAATTCGCTGCAGCTTCATATGATCAGTTGTTA-3′ (forward) and 5′-ACACTCGAGATATGGTGAAAGGACAGTCATGTTGC-3′ (reverse). The forward and reverse primers contain *Bam*HI and *Xho*I sites, respectively. The PCR product was cloned into *Bam*HI and *Xho*I sites of the pLPC vector to generate the pLPC-APC811 plasmid.

Luciferase assay

Cells were transiently transfected with the β-catenin-responsive reporter vectors pTcf7wt-luc (carrying seven repeats of the Tcf-binding consensus sequence) or pTcf7mt-luc (carrying seven repeats of a mutated Tcf-binding consensus sequence, to which Tcf cannot bind; provided by Dr. Kunitada Shimotohno, National Center for Global Health and Medicine, via Riken BioResource Center, Ibaraki, Japan) and phRLuc (Promega) by Lipofectamine 2000 reagent (Thermo Fisher Scientific) or by electroporation using the Neon Transfection System (Thermo Fisher Scientific) with two pulses for 20 ms and 1,300 V each time. Luciferase assays were performed using the Dual-Glo Luciferase Assay System (Promega) and firefly and *Renilla* luminescence were measured with a multifunctional reader Genios (Tecan).

Immunoblotting

Whole cell lysates were prepared using lysis buffer (1% Nonidet P-40, 150 mmol/L NaCl, 50 mmol/L Tris-HCl, pH 8.0) with 2% (v/v) of protease inhibitor cocktail (Nacalai Tesque) and 1% (v/v) of phosphatase inhibitor cocktail (Nacalai Tesque). Immunoblotting was performed as previously described (17). The primary antibodies were anti-tankyrase-1/2 (H350; Santa Cruz Biotechnology), anti-active β-catenin (8E7; Millipore), anti-phospho-β-catenin (Ser33/37/Thr41; Cell Signaling Technology), anti-Axin1 (C76H11; Cell Signaling Technology), anti-Axin2 (76G6; Cell Signaling Technology), anti-GAPDH (6C5; Fitzgerald), anti-APC (ALi 12-28; Santa Cruz Biotechnology) for detecting the short APC (aa 1–811), and anti-APC (2504; Cell Signaling Technology) for APC larger than 811 aa.

siRNA transfection

ON-TARGETplus (human *CTNNB1* and nontargeting control) and Silencer Select [*APC* (s1433: siAPC#1; s1434: siAPC#2; s1435: siAPC#3) and negative control no. 1] siRNAs were purchased from GE Healthcare Dharmacon and Thermo Fisher Scientific, respectively. siRNAs were introduced into cells with reverse transfection method using Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific).

qRT-PCR

Total RNAs were purified with the RNeasy Mini Kit (Qiagen) and cDNA were synthesized with the SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Thermo Fisher Scientific). The expression levels of *AXIN2* or *Naked1* (*NKD1*) mRNA were quantified by real-time PCR analysis with the LightCycler 480 Real-Time PCR System with Universal ProbeLibrary Probe #36 (Roche). The primers were as follows: *AXIN2*, 5′-CACACCCTTCTCCAATCCAA-3′ (forward) and 5′-TGCCAGTTTCTTTGGCTCTT-3′ (reverse); and *NKD1*, 5′-TCTCGCCGGGATAGAAAAC-3′ (forward) and 5′-TCTCGCCGGGATAGAAAAC-3′ (reverse).

Immunofluorescence staining

Cells on glass coverslips were fixed with 2% formaldehyde/PBS and permeabilized with 0.5% Nonidet P-40/PBS. Immunofluorescence staining was performed as previously described (17) with antibody against nonphosphorylated (i.e., active) β-catenin (D13A1; Cell Signaling Technology). The nuclei were stained with VECTASHIELD Antifade Mounting Medium with 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories).

Patients

Surgical specimens of colorectal cancer from 16 patients were obtained between September 2013 and December 2015. Standard histopathologic analysis was performed to confirm the diagnosis of malignancy and the histological subtype. For all samples, total RNA and genomic DNA were isolated as described below. Electronic medical records were retrospectively reviewed to obtain clinical information in accordance with an institutional review board (IRB)-approved protocol. The patients submitted written informed consent for genetic and cell biological analyses, which were performed in accordance with protocols approved by the IRBs of the Japanese Foundation for Cancer Research (Tokyo, Japan).

Establishment of patient-derived cancer cell lines

A few pieces of tumors from colorectal cancer patients were obtained after surgical resection. Tumor pieces were immediately placed in ice-cold culture medium with antibiotic-antimycotic (Gibco). Tumor tissues were cut into small fragments, and washed with ice-cold PBS supplemented with antibiotic–antimycotic. Tumor pellets were enzymatically digested with collagenase/dispase (Roche) and DNase I in StemPro ESC culture medium (Invitrogen) for 30 to 60 minutes. After washing with

antibiotic–antimycotic and 0.2% BSA-containing PBS, the cell pellets were cultured in the StemPro ESC medium supplemented with 10 μmol/L of Y-27632 to establish the patient-derived JC (JFCR-Colorectal) cell lines. Before subsequent experiments, the cells were subcultured until the coexisting stromal cells were scarcely detected under the microscope.

Isolation of genomic DNA and total RNA and sequencing of *APC* gene

Genomic DNA was isolated from cell pellets or fresh frozen normal/tumor specimens using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's protocol. The isolated DNA was processed on the MiSeq platform (Illumina, Inc.) with a Haloplex custom panel (Agilent), which is designed to detect well-known cancer-associated somatic mutations. Somatic variants were called using the Somatic Variant Caller (Illumina) and annotated using the SureCall software tool (Agilent). Total RNA was isolated from cell pellets or fresh frozen specimens using the RNeasy Mini Kit (Qiagen). Mutated APC cDNAs were PCR-amplified from cDNA synthesized from total RNA with oligo-dTs using the KOD Plus Neo Kit (Toyobo Co., Ltd.) and bidirectionally sequenced by the Sanger method.

Statistical analysis

Tukey–Kramer tests were performed to examine every combination of multiple experimental groups. Multiple regression analysis was performed with sensitivities to tankyrase inhibitors and existence of genetic mutations (*APC*, *KRAS*, *PIK3CA*, and *TP53*), which were defined as dependent and independent variables, respectively.

Results

Tankyrase inhibitor–sensitive colorectal cancer cells retain highly activated Wnt/β-catenin signaling

To identify molecular parameters that are associated with cellular sensitivity to tankyrase inhibitors, we first evaluated the growth-inhibitory effects of tankyrase inhibitors G007-LK, IWR-1 and XAV939 (18, 20, 22) on nine established colorectal cancer cell lines. COLO-320DM, SW403, HCC2998, DLD-1, HCT-15, HT-29, and KM12 cells are *APC*-mutated, whereas HCT-116 and RKO cells retain wild-type *APC*. As expected, none of the inhibitors repressed the growth of HCT-116 or RKO cells (Fig. 1A) because HCT-116 cells harbor a constitutively active mutation in β-catenin (*CTNNB1*, deletion of Ser45, a phosphorylation site of casein kinase 1α) and RKO cells have no Wnt/β-catenin signal-related mutations. Among the rest of the cell lines with *APC*-truncated mutations, COLO-320DM and SW403 cells showed high sensitivity to the three tankyrase inhibitors (Supplementary Table S1), whereas the other cell lines were resistant.

We next examined transcriptional activity of Tcf/LEF, a downstream signaling component of the Wnt/β-catenin pathway, using a Tcf/LEF luciferase reporter construct. Figure 1B shows that COLO-320DM and SW403 cells exhibit high Tcf/LEF transcriptional activity compared with the tankyrase inhibitor–resistant cells. These transcriptional activities roughly correlated with β-catenin dependency of the cells (Fig. 1C and D; Supplementary Fig. S1). Importantly, COLO-320DM and SW403 cell growths were sensitive to β-catenin depletion, ensuring the mode-of-action of tankyrase inhibitors. These observations so far indicate that tankyrase inhibitor-sensitive colorectal cancer cells possess three characteristic properties: (i) *APC* truncated mutation, (ii) highly activated Wnt/β-catenin signaling, and (iii) β-catenin–dependent cell growth.

Sensitivity to tankyrase inhibitors is coupled with drug-induced suppression of β-catenin signaling

Tankyrase promotes Axin degradation and thereby induces β-catenin stabilization (18). Therefore, the pharmacodynamic response of the tankyrase–Axin–β-catenin axis could be a clue to determine the cellular sensitivity to tankyrase inhibitors. When we examined the basal expression levels of tankyrase, Axins, and active β-catenin in the colorectal cancer cell lines used, we did not observe any correlation between their expression levels and sensitivity to tankyrase inhibitors (Supplementary Fig. S2A–S2C). β-Catenin is localized both in adherence junctions and in the nucleus (26). In *APC*-mutated cell lines, subcellular localization or expression levels of β-catenin did not correlate with tankyrase inhibitor sensitivity (Supplementary Fig. S2D). We next monitored the effect of tankyrase inhibitors on the tankyrase–Axin–β-catenin axis. When nine colorectal cancer cell lines were treated with G007-LK or IWR-1, accumulation of Axins (Axin2, especially) and subsequent decrease of nonphosphorylated β-catenin (i.e., active β-catenin) was detected in the inhibitor-sensitive COLO-320DM and SW403 cells (Fig. 2A; Supplementary Fig. S1). In contrast, in tankyrase inhibitor–resistant cell lines, protein levels of active β-catenin did not change after inhibitor treatment. Consistent with these observations, in the inhibitor-sensitive cells, tankyrase inhibitors efficiently suppressed the highly activated Tcf/LEF activity (Fig. 2B) and mRNA expression of *AXIN2* and *NKD1* (27, 28), two target genes of Wnt/β-catenin signaling (Fig. 2C). However, in the inhibitor-resistant colorectal cancer cells with *APC* mutations (DLD-1, HCT-15, HCC2998, and KM12 cells), the levels of Tcf/LEF activity and downstream gene expression were relatively low and suppressive effects of tankyrase inhibitors on these cells were less clear. Of note, in HCC2998, HCT-15, and DLD-1 cells, tankyrase inhibitors caused Axin accumulation but failed to downregulate β-catenin. These results indicate that the pharmacodynamic effect of tankyrase inhibition is not transmitted to β-catenin downregulation in these resistant cell lines.

Mutation status of *APC* correlates with sensitivity to tankyrase inhibitors

Previous studies have shown that mutations of *APC* gene occur preferentially within the mutation cluster region (around aa 1282–1581) in colorectal cancers (2, 29–31). This results in *APC* gene products that lack several or all of the seven 20-amino acid repeats (20-AARs), which contribute to β-catenin binding and degradation. We next focused on the relationship between the positions of *APC* mutations and the cellular sensitivity to tankyrase inhibitors. As shown in Fig. 3, we noticed that the cell lines sensitive to tankyrase inhibitors harbored short, truncated *APC* mutants that lacked all seven 20-AARs (referred to as "short APC" hereafter), whereas the tankyrase inhibitor-resistant cell lines retained longer *APC* mutants containing two or more 20-AARs (referred to as "long APC" hereafter). We found that another colorectal cancer cell line, SW480, which retained a single 20-AAR, was resistant to tankyrase inhibitors (Supplementary Fig. S3A). Supplementary Table S2 shows statistically significant correlation between the short *APC* mutations and sensitivities to G007-LK ($P = 0.015$) and IWR-1 ($P < 0.001$). Moreover, *PIK3CA* mutations were correlated with sensitivities to IWR-1 ($P = 0.044$) but not

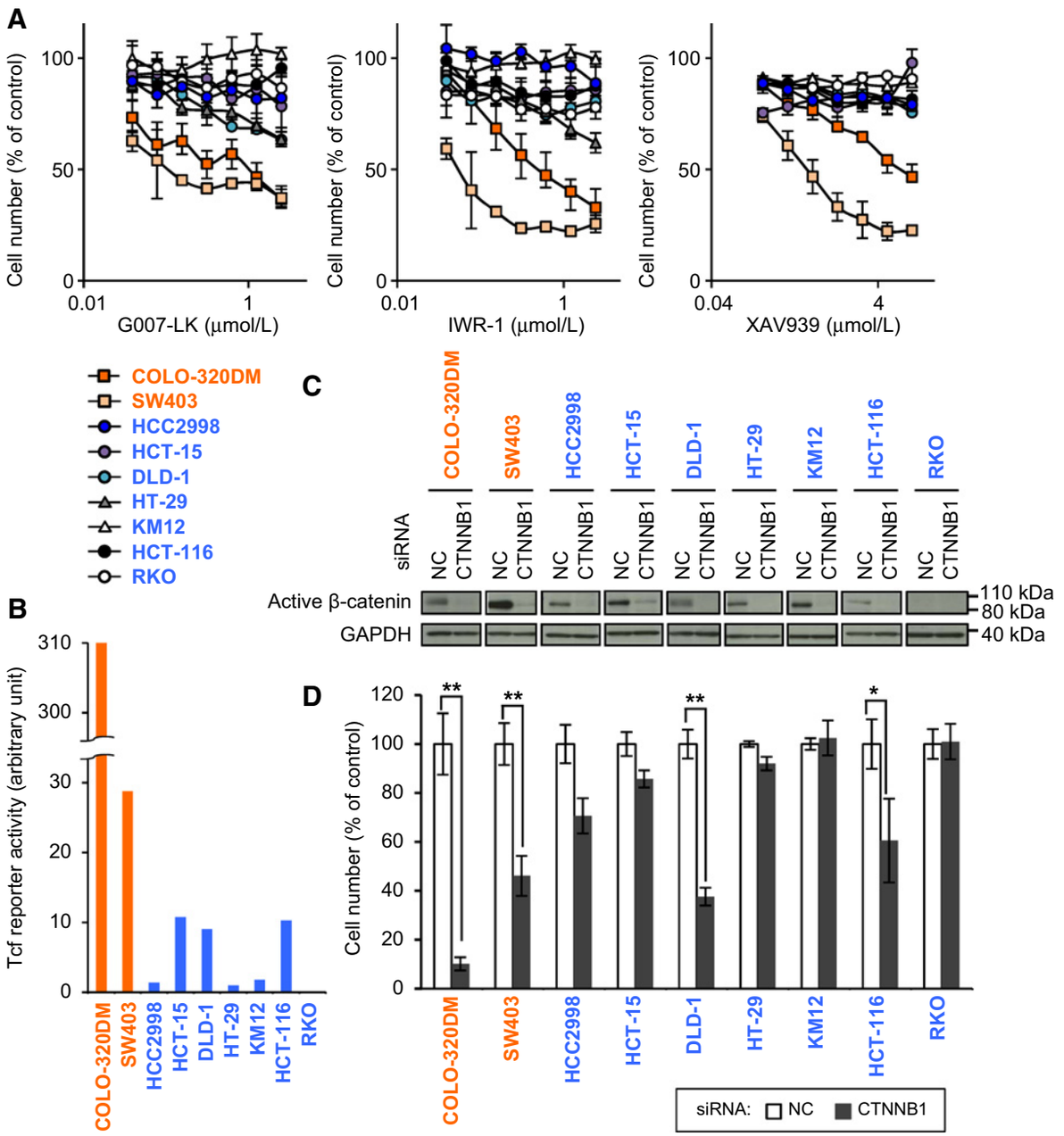

Figure 1.

Sensitivity of colorectal cancer cell lines to tankyrase inhibitors. **A,** MTT assay was performed with human colorectal cancer cell lines after 5-day treatment with tankyrase inhibitors G007-LK, IWR-1, and XAV939 at indicated doses. **B,** Luciferase reporter assay was performed to evaluate the Wnt/β-catenin signaling activity of each cell line. Relative light units (RLU) of pTcf7wt-luc-derived firefly luciferase activities (Luc) were determined by normalization with those of the control *Renilla* luciferase (RLuc), and the firefly/*Renilla* RLU was further normalized with that of pTcf7mt-luc-transfected samples. **C** and **D,** Antiproliferative effect of β-catenin knockdown. Cell lysates were prepared after 2 days of siRNA transfection and subjected to immunoblot analysis with indicated primary antibodies (**C**). MTT assay was performed after five days of transfection (**D**). NC, nonsilencing control siRNA; *CTNNB1*, β-catenin siRNA. Asterisk indicates statistical significance (**, $P < 0.01$; *, $P < 0.05$, Tukey–Kramer test).

G007-LK ($P = 0.141$). None of the other recurrent mutations in colorectal cancers showed correlation with the tankyrase inhibitor sensitivity. These observations suggest that *APC* mutation status could be a potential biomarker for prediction of tankyrase inhibitor sensitivity of colorectal cancer cells.

Long APC mutants block tankyrase inhibitor–induced β-catenin degradation

As shown earlier, tankyrase inhibitor-resistant colorectal cancer cell lines possess long APCs with two or more 20-AARs and fail to downregulate β-catenin upon tankyrase inhibition.

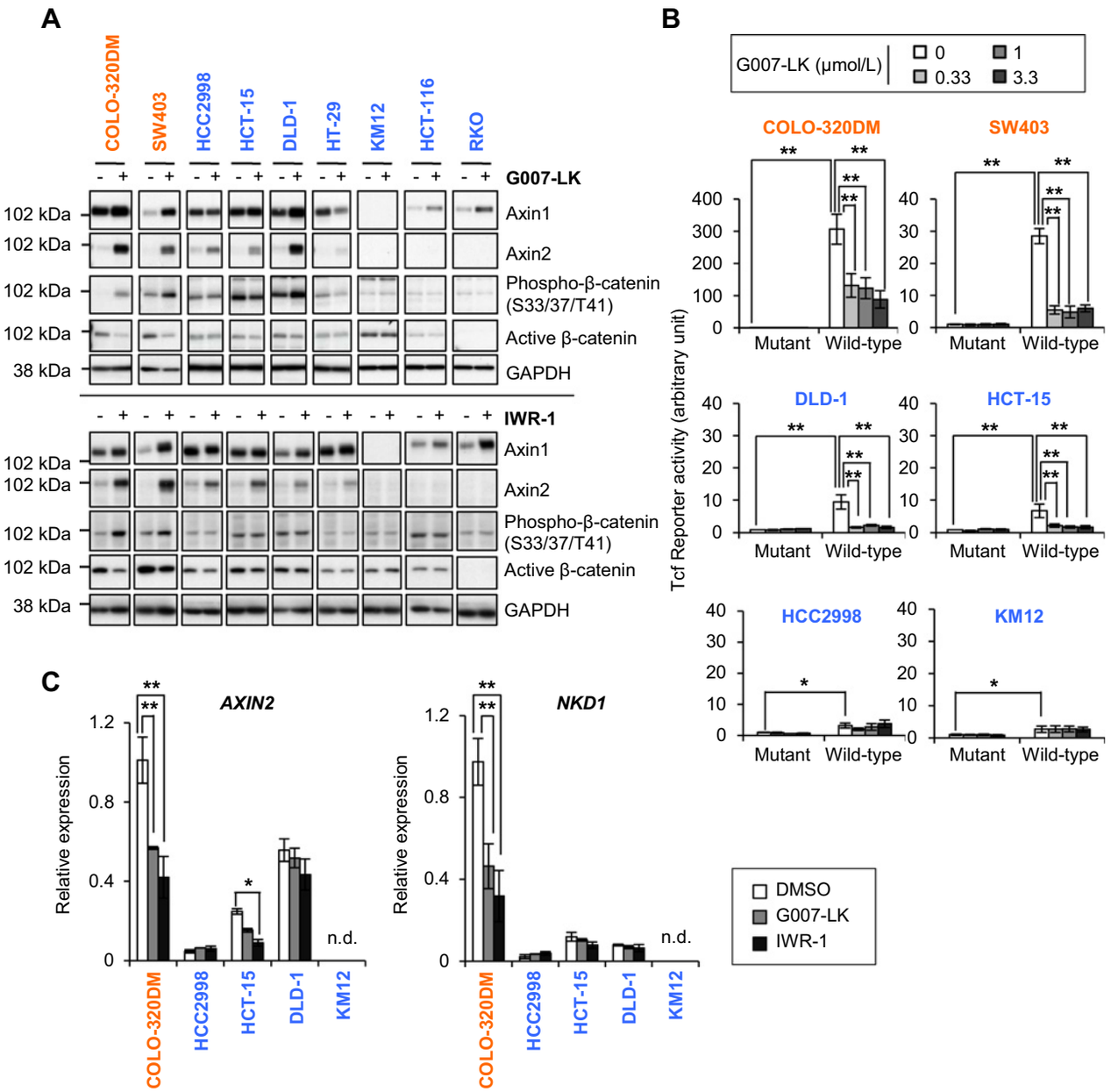

Figure 2.

Sensitivity to tankyrase inhibitors correlates with β-catenin downregulation. **A,** Immunoblot analysis. Cells were treated with G007-LK (1 μmol/L, top) or IWR-1 (10 μmol/L, bottom) for 16 hours. Cell lysates were prepared and subjected to immunoblot analysis. **B,** Luciferase reporter assays. Cells were treated with indicated concentrations of G007-LK for 18 hours after transfection of the reporter vectors. Wild-type, pTcf7wt-luc; Mutant, pTcf7mt-luc. **C,** Quantitative RT-PCR analysis of *AXIN2* and *NKD1* gene expression. Cells were treated with G007-LK (1 μmol/L) or IWR-1 (10 μmol/L) for 24 hours. Data were normalized with β-actin expression. n.d., not detected. Asterisk indicates statistical significance (**, $P < 0.01$; *, $P < 0.05$, Tukey–Kramer test).

To examine the functional involvement of the long APCs in the resistance of β-catenin to tankyrase inhibitor-mediated downregulation, we knocked down the expression of the long APCs in these cells. We particularly focused on the three cell lines expressing long APC with two 20-AARs, HCC2998, HCT-15 and DLD-1 cells, because the signaling from Axin2 accumulation to β-catenin downregulation was prevented in these cell lines. Depletion of the long APC resulted in elevation of active β-catenin levels (Fig. 4A; Supplementary Fig. S1) and downstream *AXIN2* mRNA expression (Fig. 4B), although the effects on active β-catenin and *AXIN2* expression in DLD-1 cells were not detected or detected only marginally, if any. These observations support the notion that the long APCs that partially retain several 20-AARs are hypomorphic mutants in terms of the ability to suppress intracellular β-catenin levels. Under long APC-depleted conditions, G007-LK–induced Axin2

Cell line	APC mutation	# of 20-AARs	*APC* gene product 20-AAR x 7
COLO-320DM	S811X	0	
SW403	S1197X2	0	
	S1278X		
HCC2998	R1450X	2	
	L665X		
DLD-1	G1417fs*2	2	
HCT-15	G1417fs*2	2	
HT-29	E853X	3	
	T1556fs*3		
KM12	N1819fs*7	11	
	WT		
HCT-116	WT	14	
RKO	WT	14	

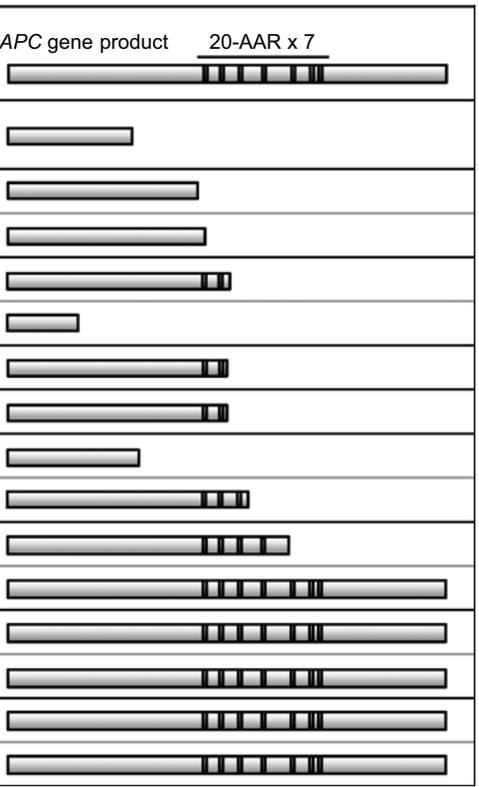

Figure 3.
Mutation status of *APC* in colorectal cancer cell lines. "*APC* mutation" column describes the amino acid position of the APC mutation. X stands for nonsense mutation, fs for frameshift and WT for wild-type. The numbers of 20-AARs in APC were counted as a total of both alleles. Schematic views of APC length and 20-AARs are shown in the "*APC* gene product" column.

accumulation and was able to downregulate β-catenin. Meanwhile, SW480, which retained a single 20-AAR, was able to downregulate β-catenin upon G007-LK treatment, and depletion of this mutant APC did not affect β-catenin stability (Supplementary Fig. S3B). Of note, in this cell line, the basal level of active β-catenin was very high and significant amounts remained even after the drug treatment, which would allow the drug-resistant cell growth (Supplementary Fig. S3A). Immunofluorescence staining confirmed that depletion of the long APC with two 20-AARs led to accumulation of active β-catenin in the cell nucleus, which was reversed by G007-LK (Fig. 4C). Similar results were obtained when other *APC* siRNAs and IWR-1 were used (Supplementary Fig. S4A and S4B). Knockdown of the long APCs did not affect the cellular sensitivities to tankyrase inhibitors (Supplementary Fig. S4C and S4D).

To investigate the functional difference of the short and long APCs, we evaluated the influence of short APC expression on tankyrase inhibitor–induced β-catenin degradation. Again, knockdown of the endogenous long APC in HCC2998 cells enabled G007-LK and IWR-1 to induce degradation of β-catenin (Fig. 5A; Supplementary Fig. S5A, lanes 1, 2, 5, 6). Exogenous expression of short APC (APC811, derived from COLO-320DM cells) in the absence of the endogenous long APC in HCC2998 cells did not affect tankyrase inhibitor–induced β-catenin degradation (Fig. 5A; Supplementary Fig. S5A, lanes 5–8). Ectopic expression of the short APC in HCC2998 cells did not affect the sensitivity to tankyrase inhibitors in the presence or absence of the long APC (Supplementary Fig. S5C and S5D). Because the long APC is a hypomorphic mutant, its depletion in HCC2998 cells increased Tcf/LEF transcriptional activity (Fig. 5B). Consistent with the results in Fig. 5A, ectopic expression of APC811 had little influence on this reporter activity. In contrast, overexpression or knockdown of APC811 in COLO-320DM cells did not affect the levels of active β-catenin or Tcf/LEF activity (Fig. 5C and D; Supplementary Fig. S5B). Tankyrase inhibitor–mediated degradation of β-catenin in long APC-depleted HCC2998 cells depended on Axin2 but not Axin1 (Fig. 5E). COLO-320DM cells also required Axin2 for tankyrase inhibitor–mediated degradation of β-catenin, on which APC811 ('short' APC) had no effect (Fig. 5F). Together, these observations indicate that the long APC, but not the short APC, disturbs tankyrase inhibitor–induced β-catenin downregulation (Supplementary Fig. S6).

Short *APC* mutations in patient-derived colorectal cancer cells predict response to tankyrase inhibitors

To validate the short *APC* mutations as a potential biomarker to predict tankyrase inhibitor sensitivity in more clinically relevant cells, we established patient-derived cells (PDC) from surgical specimens of colorectal cancer patients and analyzed the sequence of the *APC* gene (Supplementary Fig. S7A). These PDCs exhibited differential sensitivities to G007-LK and IWR-1 (Fig. 6A). On the basis of the cell growth inhibition rate, we divided these cells into three groups: sensitive, intermediate, and resistant (Supplementary Fig. S7B). Integrated analysis of *APC* mutation status and tankyrase inhibitor sensitivity revealed that PDCs in the resistant group tended to possess long *APC* mutants compared with the sensitive and intermediate groups together (Supplementary Fig. S7C). Importantly, PDCs with short *APC* mutants (20-AAR = 0) were classified as sensitive/intermediate, without exception (Fig. 6B). On the other hand, PDCs with long *APC* mutants (20-AAR $\geq$ 1) were either sensitive/intermediate or resistant to tankyrase inhibitors with

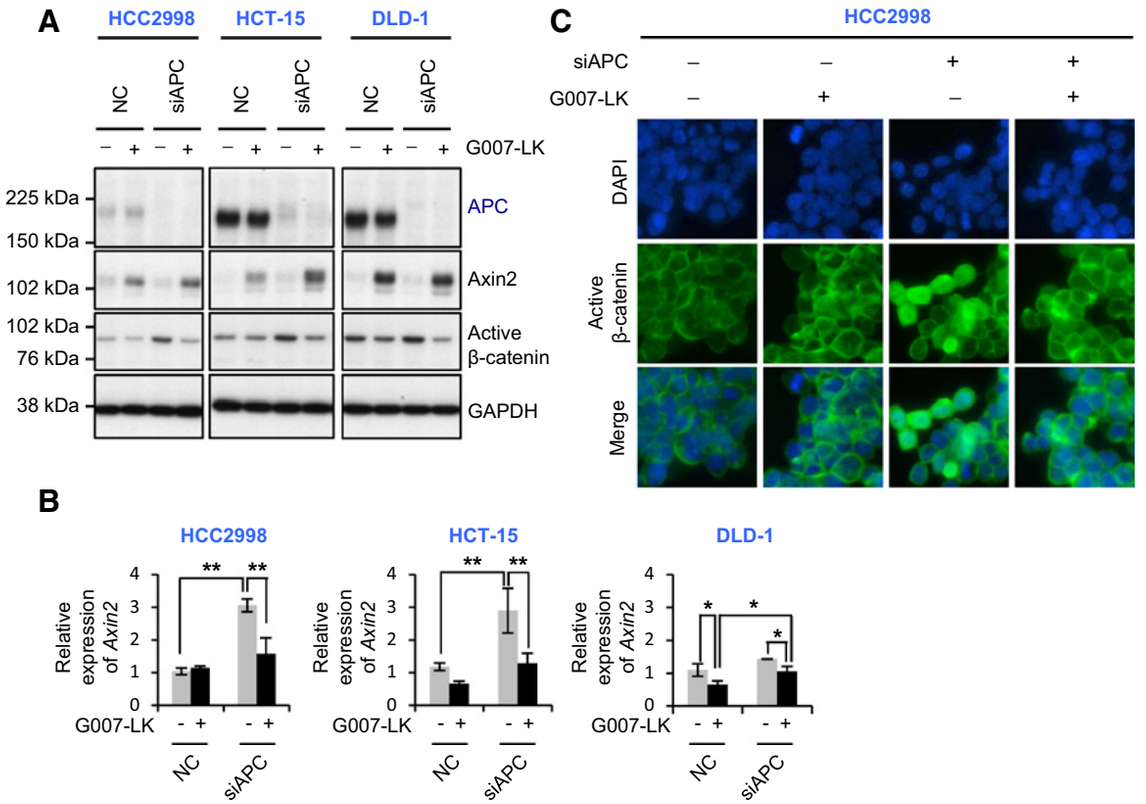

Figure 4.

The long APC mutant is hypomorphic and suppresses G007-LK-mediated β-catenin degradation. Twenty-four hours after transfection with *APC* siRNA#1, cells were incubated with G007-LK (1 μmol/L) for 16 hours. **A,** Immunoblot analysis. **B,** Expression level of *AXIN2* mRNA evaluated by quantitative RT-PCR analysis. *AXIN2* expression is shown as relative value normalized with β-actin expression. NC stands for nonsilencing control siRNA. Asterisk indicates statistical significance (**, $P < 0.01$; *, $P < 0.05$, Tukey–Kramer test). **C,** Immunofluorescence staining of nonphosphorylated (i.e., active) β-catenin.

comparable tendency. Collectively, these observations indicate that the short APC lacking all 20-AARs could potentially be a predictive biomarker for sensitivity of colorectal cancer cells to tankyrase inhibitors. Colony formation assay of six PDCs gave essentially similar results with MTT assay, although the cells tended to be more sensitive to tankyrase inhibition in colony formation assay because of longer drug exposure (Supplementary Fig. S8A). Knockdown of β-catenin inhibited the growth of these PDCs to comparable extents with tankyrase inhibitors although the tankyrase inhibitor–sensitive JC-35 was resistant to β-catenin depletion presumably due to high basal level expression of β-catenin and incomplete knockdown of the gene (Supplementary Fig. S8B and S8C).

Discussions

In this study, we demonstrated that the colorectal cancer cell lines that are sensitive to tankyrase inhibitors (COLO-320DM and SW403 cells) highly depend on β-catenin signaling: they exhibit (i) elevated Tcf/LEF transcriptional activities, (ii) higher expression of the downstream genes, and (iii) susceptibility to growth inhibition by β-catenin knockdown. As reported previously (18, 22), we confirmed that either the gain-of-function mutation of the *CTNNB1* gene (HCT-116 cells) or the absence of mutations in the Wnt/β-catenin pathway (RKO cells) could be rendered as exclusion criteria for therapeutic application of tankyrase inhibitors in colorectal cancers.

Lau and colleagues classified colorectal cancer cells into either tankyrase inhibitor-"sensitive" or "resistant" according to the β-catenin signaling (i.e., TCF reporter activity and target gene expression) but not cell proliferation (22). They have demonstrated that response of β-catenin signaling to tankyrase inhibitors does not necessarily correlate with the cell sensitivity to the drug-induced growth inhibition or positions of APC mutations. In HCT-15 cells, for example, (i) tankyrase inhibitors blocked β-catenin signaling but did not inhibit the cell growth, (ii) APC knockdown upregulated β-catenin signaling. These results are consistent with our present results (Figs. 1A, 2B and C, 4B). Meanwhile, the level of β-catenin signaling in HCT-15 cells was not so high (Fig. 1B). We assumed that such a low level of β-catenin signaling would not significantly affect the growth of HCT-15 cells, if any. In fact, β-catenin knockdown did not inhibit the growth of HCT-15 cells (Fig. 1D). In total, the observations by Lau and colleagues (22) do not oppose our present conclusion that positions of APC mutations correlate with sensitivities of colorectal cancer cell growth to tankyrase inhibitors.

In colorectal cancer cells, activation of multiple driver pathways, such as Wnt/β-catenin, receptor tyrosine kinase (RTK)/RAS

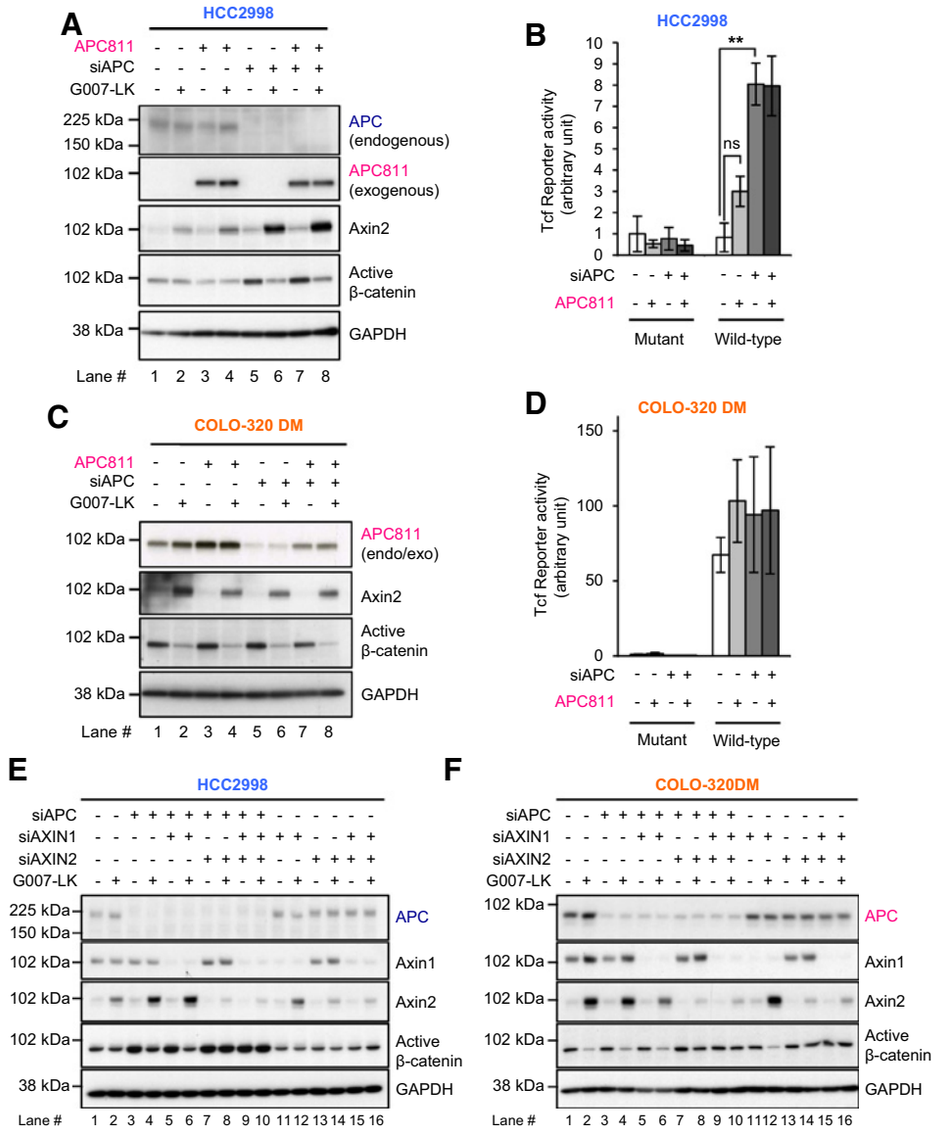

Figure 5.

Long but not short APC represses Wnt/β-catenin signal and its knockdown sensitizes β-catenin to tankyrase inhibitor-mediated downregulation. **A** and **C,** Immunoblot analysis of cell lysates. Cells were transfected with the indicated siRNAs directly after electroporation of pLPC-APC811, an expression vector for the short APC (APC811). Twenty-four hours after transfection, cells were treated with G007-LK (1 μmol/L) for 16 hours. **B** and **D,** Luciferase assay was performed 2 days posttransfection of Tcf reporter vectors, pLPC-APC811 and *APC* siRNA. *APC* siRNA#3 was used for the experiments in this figure. Asterisk indicates statistical significance (**, $P < 0.01$, Tukey–Kramer test). ns, not significant. **E** and **F,** Axin dependency of β-catenin degradation in G007-LK-treated cells. Cells were transfected with indicated siRNAs and treated with G007-LK (1 μmol/L) for 16 hours. Cell lysates were subjected to Western blot analysis.

and PI3K/AKT, and p53 inactivation are not mutually exclusive (4). In this study, we did not observe any significant correlation between the sensitivity to tankyrase inhibitors and mutations in *PIK3CA*, *KRAS*, *BRAF*, and *TP53*, although *PIK3CA* mutations were correlated with sensitivity to one of the two inhibitors, IWR-1. However, tankyrase inhibitor sensitivities of *APC*-mutated colorectal cancer cell lines examined here were inversely correlated with their sensitivities to regorafenib, a multitargeted kinase inhibitor (32). Thus, COLO-320DM and SW403 cells are resistant to regorafenib, whereas tankyrase inhibitor–resistant HT-29 cells are sensitive to regorafenib. These observations suggest two hypotheses: (i) responders to tankyrase inhibitors and those to inhibitors of RTK pathways may not overlap; and (ii) colorectal cancer patients who do not respond to either drug may obtain benefit from combination therapy of tankyrase inhibitors and RTK pathway inhibitors.

Tankyrase inhibitor-resistant colorectal cancer cells that had mutant APCs with two or three 20-AARs did not allow Axin-dependent β-catenin degradation caused by tankyrase inhibitors. These long APC mutants would have a dominant negative effect on the Axin-dependent β-catenin degradation because the siRNA-mediated knockdown resumed β-catenin degradation upon exposure to tankyrase inhibitors (Supplementary Fig. S6). Of note, a previous study showed that the long APC mutant (APC1450) forms a hetero-oligomer with the wild-type APC and exerts a dominant-negative effect on APC interaction with a plus-end microtubule-binding protein (EB1) and thus blocks the appropriate dynamics of microtubules (33). As APC requires its carboxyl terminal 284 residues (codons 2560-2843) to interact with EB1 (34), all of the APC-mutated cell lines used in this study should be lacking the EB1–APC interactions. Thus, diverse numbers of retained 20-AARs in APC mutants may not give differential effects on EB1-mediated function of APC, if any.

Importantly, these long *APC* mutants occur most frequently in colorectal cancers and are hypomorphic (29, 31). In fact, colorectal cancers adopt the so-called "just right signaling" of the

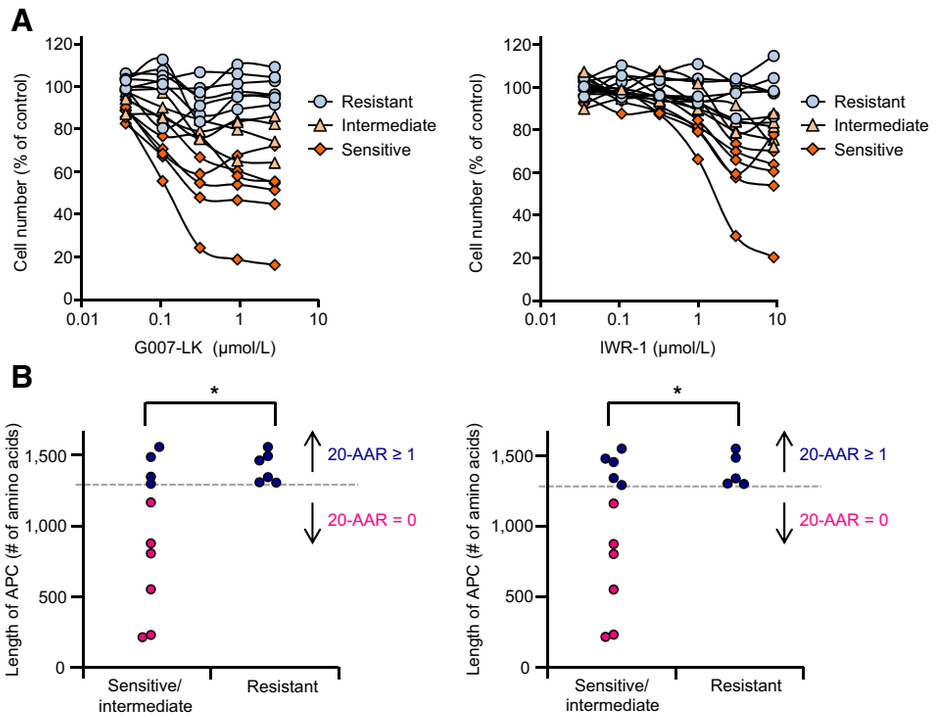

Figure 6.
Short *APC* mutations as a potential predictive biomarker for the sensitivity of PDCs to tankyrase inhibitors. **A,** Effects of tankyrase inhibitors (5-day treatment) at indicated doses on PDC growth were evaluated by MTT assay. **B,** Correlation between APC length of each PDC and sensitivity to tankyrase inhibitors were analyzed by dot plots (left: G007-LK, right: IWR-1). The cells harboring short *APC* mutations (20-AAR = 0) are shown as pink dots. *, $P < 0.05$ (*t* test).

Wnt/β-catenin pathway and prefer partially inactivated APC mutants (29, 31). This unique property of *APC* mutations will provide a second explanation for why the depletion of long APCs restores β-catenin degradation by tankyrase inhibitors: the long APC knockdown derepresses the target gene, *AXIN2*, and increases its mRNA pool (Fig. 4B and Supplementary Fig. S4B), which further enhances accumulation of Axin2 protein upon treatment with tankyrase inhibitors and facilitates β-catenin degradation. Axins serve as a master scaffold for the β-catenin degradation complex and are the rate-limiting factor of β-catenin degradation (35). In fact, overexpression of Axin in APC-mutated SW480 cells causes a drastic reduction of the β-catenin level (36). These observations support that tankyrase inhibitor-mediated accumulation of Axin induces β-catenin degradation even in the absence of APC, although the degradation efficiency might be reduced by APC dysfunction (22).

COLO-320DM and SW403 cells, in which APC had no 20-AARs, increased Axins and downregulated β-catenin upon treatment with tankyrase inhibitors. Neither overexpression nor knockdown of the short APC affected the Tcf/LEF transcriptional activity or the Axin-dependent degradation of β-catenin. Therefore, these *APC* mutants are not hypomorphic or dominant-negative in terms of β-catenin regulation and response to tankyrase inhibitors, respectively. We propose that short *APC* mutants induce the highest activation of the β-catenin signaling and render susceptibility to Axin-dependent degradation of β-catenin, both of which contribute to the sensitivity to tankyrase inhibitors. Consistent with our observations, Waaler and colleagues reported that a tankyrase inhibitor JW55 efficiently blocks polyposis formation in conditional *Apc* knockout mice (21). These mice produce a short APC (1-580) mutant, which lacks all 20-AARs and is classified as tankyrase inhibitor–sensitive, according to our present study.

Our finding that PDCs with short *APC* mutants were either sensitive or responsive to tankyrase inhibitors corroborates the idea that APC with complete deletion of seven 20-AARs could be a predictive biomarker for the sensitivity to tankyrase inhibitors. Although the number of established cell lines examined might be small, we examined totally 25 different cells (9 established cell lines and 16 PDCs). Among them, 8 cells were classified as the short APC mutants without 20-AARs. Strikingly, all of these short APC cells responded to two different tankyrase inhibitors, G007-LK and IWR-1 (six were sensitive, two were intermediate, and none were resistant). The antiproliferative effect of tankyrase inhibitors on PDCs seems to be relatively weaker than the established colorectal cancer cell lines. This could be a result of relatively slower growth of the PDCs, particularly under the serum-free culture conditions optimized for PDC culture.

Meanwhile, depletion of the long APCs retaining two or more 20-AARs did not affect the cellular sensitivities to tankyrase inhibitors. These observations are consistent with that HCC2998 and HCT-15 cells were resistant to growth inhibition by β-catenin knockdown (Fig. 1D). In case of DLD-1 cells, tankyrase inhibitor–mediated degradation of β-catenin gave less deleterious effect than direct knockdown of β-catenin (Fig. 1D). This would be due to that tankyrase inhibitors only partially downregulated β-catenin in the mutant APC-depleted DLD-1 cells and the cells still maintained significant amounts of β-catenin (Supplementary Fig. S4A). Accordingly, we propose that APC mutations are potential biomarkers but not functional determinants of tankyrase inhibitor sensitivities of colorectal cancer cells.

We could not define the long *APC* mutants retaining two or more 20-AARs as exclusion criteria for application of tankyrase inhibitors. APC regulates not only the stability of β-catenin in the destruction complex but also regulates localization of β-catenin with its nuclear localization and nuclear export

signals (37, 38). However, there was no general correlation between the nuclear localization of β-catenin and sensitivity to tankyrase inhibitors (H. Yoshida and N. Tanaka, unpublished observations). A recent report showed that APC2 contributes to the activity of the destruction complex upon tankyrase inhibition (39). Upon treatment with tankyrase inhibitors, tankyrase and Axin bind each other to form an oligomer complex, which is important for destabilizing β-catenin (40). These phenomena may provide a key to solve the tankyrase inhibitor sensitivity of PDCs with long APCs. Alternatively, tankyrase inhibitors may exert their therapeutic effect through the blockade of other functions of the proteins, including telomere maintenance, mitosis, downregulation of the tumor suppressor PTEN, and oncogenic YAP activation (41–44).

Although the Wnt/β-catenin pathway has been thought as a less-druggable target for colorectal cancers, tankyrase inhibitors are promising for blocking this signaling pathway. Our demonstration of *APC* mutations as a predictive biomarker for tankyrase inhibitor sensitivity has a profound implication, because mutations in this tumor suppressor gene are the most fundamental molecular alteration in the complicated etiology of colorectal cancers. Clarifying further mechanisms for the sensitivity to tankyrase inhibitors, especially in the long *APC*-mutated colorectal cancer cells, will enable the selection of appropriate patients for treating with tankyrase inhibitors and support the development of tankyrase inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. Tanaka, T. Mashima, B. Gong, M. Matsuura, R. Katayama, H. Seimiya
Writing, review, and/or revision of the manuscript: T. Mashima, R. Katayama, S. Nagayama, Y. Sugimoto, H. Seimiya
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Katayama, S. Nagayama, N. Fujita
Study supervision: Y. Sugimoto, H. Seimiya

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References

1. Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 1991;253:665–9.
2. Fodde R, Smits R, Clevers H. APC, signal transduction and genetic instability in colorectal cancer. Nat Rev Cancer 2002;1:55–67.
3. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525–32.
4. The Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012;487:330–7.
5. Rubinfeld B, Albert I, Porfiri E, Fiol C, Munemitsu S, Polakis P. Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. Science 1996;272:1023–6.
6. Hart MJ, de los Santos R, Albert IN, Rubinfeld B, Polakis P. Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK3 beta. Curr Biol 1998;8:573–81.
7. Behrens J, Jerchow BA, Wurtele M, Grimm J, Asbrand C, Wirtz R, et al. Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta. Science 1998;280:596–9.
8. Liu C, Li Y, Semenov M, Han C, Baeg GH, Tan Y, et al. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 2002;108:837–47.
9. Amit S, Hatzubai A, Birman Y, Andersen JS, Ben-Shushan E, Mann M, et al. Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. Genes Dev 2002;16:1066–76.
10. Li VS, Ng SS, Boersema PJ, Low TY, Karthaus WR, Gerlach JP, et al. Wnt signaling through inhibition of beta-catenin degradation in an intact Axin1 complex. Cell 2012;149:1245–56.
11. Hung KE, Maricevich MA, Richard LG, Chen WY, Richardson MP, Kunin A, et al. Development of a mouse model for sporadic and metastatic colon tumors and its use in assessing drug treatment. Proc Natl Acad Sci U S A 2010;107:1565–70.
12. Dow LE, O'Rourke KP, Simon J, Tschaharganeh DF, van Es JH, Clevers H, et al. Apc restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer. Cell 2015;161:1539–52.
13. Smith S, Giriat I, Schmitt A, de Lange T. Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. Science 1998;282:1484–7.
14. Seimiya H. The telomeric PARP, tankyrases, as targets for cancer therapy. Br J Cancer 2006;94:341–5.
15. Riffell JL, Lord CJ, Ashworth A. Tankyrase-targeted therapeutics: expanding opportunities in the PARP family. Nat Rev Drug Discov 2012;11:923–36.
16. Seimiya H, Muramatsu Y, Smith S, Tsuruo T. Functional subdomain in the ankyrin domain of tankyrase 1 required for poly(ADP-ribosyl)ation of TRF1 and telomere elongation. Mol Cell Biol 2004;24:1944–55.
17. Seimiya H, Smith S. The telomeric poly(ADP-ribose) polymerase, tankyrase 1, contains multiple binding sites for telomeric repeat binding factor 1 (TRF1) and a novel acceptor, 182-kDa tankyrase-binding protein (TAB182). J Biol Chem 2002;277:14116–26.
18. Huang SM, Mishina YM, Liu S, Cheung A, Stegmeier F, Michaud GA, et al. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. Nature 2009;461:614–20.
19. Zhang Y, Liu S, Mickanin C, Feng Y, Charlat O, Michaud GA, et al. RNF146 is a poly(ADP-ribose)-directed E3 ligase that regulates axin degradation and Wnt signalling. Nat Cell Biol 2011;13:623–9.
20. Chen B, Dodge ME, Tang W, Lu J, Ma Z, Fan CW, et al. Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat Chem Biol 2009;5:100–7.
21. Waaler J, Machon O, Tumova L, Dinh H, Korinek V, Wilson SR, et al. A novel tankyrase inhibitor decreases canonical Wnt signaling in colon carcinoma cells and reduces tumor growth in conditional APC mutant mice. Cancer Res 2012;72:2822–32.
22. Lau T, Chan E, Callow M, Waaler J, Boggs J, Blake RA, et al. A novel tankyrase small-molecule inhibitor suppresses APC mutation-driven colorectal tumor growth. Cancer Res 2013;73:3132–44.

23. Okada-Iwasaki R, Takahashi Y, Watanabe Y, Ishida H, Saito J, Nakai R, et al. The discovery and characterization of K-756, a novel Wnt/beta-catenin pathway inhibitor targeting tankyrase. Mol Cancer Ther 2016; 15:1525–34.
24. Mashima T, Oh-hara T, Sato S, Mochizuki M, Sugimoto Y, Yamazaki K, et al. p53-defective tumors with a functional apoptosome-mediated pathway: a new therapeutic target. J Natl Cancer Inst 2005;97:765–77.
25. Migita T, Okabe S, Ikeda K, Igarashi S, Sugawara S, Tomida A, et al. Inhibition of ATP citrate lyase induces triglyceride accumulation with altered fatty acid composition in cancer cells. Int J Cancer 2014; 135:37–47.
26. Kemler R. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. Trends Genet 1993;9:317–21.
27. Jho EH, Zhang T, Domon C, Joo CK, Freund JN, Costantini F. Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. Mol Cell Biol 2002;22:1172–83.
28. Yan D, Wiesmann M, Rohan M, Chan V, Jefferson AB, Guo L, et al. Elevated expression of axin2 and hnkd mRNA provides evidence that Wnt/beta-catenin signaling is activated in human colon tumors. Proc Natl Acad Sci U S A 2001;98:14973–8.
29. Albuquerque C, Breukel C, van der Luijt R, Fidalgo P, Lage P, Slors FJ, et al. The 'just-right' signaling model: APC somatic mutations are selected based on a specific level of activation of the beta-catenin signaling cascade. Hum Mol Genet 2002;11:1549–60.
30. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer 2008;8:387–98.
31. Christie M, Jorissen RN, Mouradov D, Sakthianandeswaren A, Li S, Day F, et al. Different APC genotypes in proximal and distal sporadic colorectal cancers suggest distinct WNT/beta-catenin signalling thresholds for tumourigenesis. Oncogene 2013;32:4675–82.
32. Schmieder R, Hoffmann J, Becker M, Bhargava A, Muller T, Kahmann N, et al. Regorafenib (BAY 73-4506): antitumor and antimetastatic activities in preclinical models of colorectal cancer. Int J Cancer 2013;135: 1487–96.
33. Green RA, Wollman R, Kaplan KB. APC and EB1 function together in mitosis to regulate spindle dynamics and chromosome alignment. Mol Biol Cell 2005;16:4609–22.
34. Su LK, Burrell M, Hill DE, Gyuris J, Brent R, Wiltshire R, et al. APC binds to the novel protein EB1. Cancer Res 1995;55:2972–7.
35. Lee E, Salic A, Kruger R, Heinrich R, Kirschner MW. The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway. PLoS Biol 2003;1:E10.
36. Nakamura T, Hamada F, Ishidate T, Anai K, Kawahara K, Toyoshima K, et al. Axin, an inhibitor of the Wnt signalling pathway, interacts with beta-catenin, GSK-3beta and APC and reduces the beta-catenin level. Genes Cells 1998;3:395–403.
37. Neufeld KL, Zhang F, Cullen BR, White RL. APC-mediated downregulation of beta-catenin activity involves nuclear sequestration and nuclear export. EMBO Rep 2000;1:519–23.
38. Rosin-Arbesfeld R, Townsley F, Bienz M. The APC tumour suppressor has a nuclear export function. Nature 2000;406:1009–12.
39. Croy HE, Fuller CN, Giannotti J, Robinson P, Foley AV, Yamulla RJ, et al. The Poly(ADP-ribose) polymerase enzyme tankyrase antagonizes activity of the beta-catenin destruction complex through ADP-ribosylation of axin and APC2. J Biol Chem 2016;291:12747–60.
40. Martino-Echarri E, Brocardo MG, Mills KM, Henderson BR. Tankyrase inhibitors stimulate the ability of tankyrases to bind axin and drive assembly of β-catenin degradation-competent axin puncta. PLoS One 2016;11:e0150484.
41. Seimiya H, Muramatsu Y, Ohishi T, Tsuruo T. Tankyrase 1 as a target for telomere-directed molecular cancer therapeutics. Cancer Cell 2005;7:25–37.
42. Dynek JN, Smith S. Resolution of sister telomere association is required for progression through mitosis. Science 2004;304:97–100.
43. Li N, Zhang Y, Han X, Liang K, Wang J, Feng L, et al. Poly-ADP ribosylation of PTEN by tankyrases promotes PTEN degradation and tumor growth. Genes Dev 2014;29:157–70.
44. Wang W, Li N, Li X, Tran MK, Han X, Chen J. Tankyrase inhibitors target YAP by stabilizing angiomotin family proteins. Cell Rep 2015;13:524–32.