Differential abundance of CK1α provides selectivity for pharmacological CK1α activators to target WNT-dependent tumors


Constitutive WNT activity drives the growth of various human tumors, including nearly all colorectal cancers (CRCs). Despite this prominence in cancer, no WNT inhibitor is currently approved for use in the clinic largely due to the small number of druggable signaling components in the WNT pathway and the substantial toxicity to normal gastrointestinal tissue. We have shown that pyrvinium, which activates casein kinase 1α (CK1α), is a potent inhibitor of WNT signaling. However, its poor bioavailability limited the ability to test this first-in-class WNT inhibitor in vivo. We characterized a novel small-molecule CK1α activator called SSTC3, which has better pharmacokinetic properties than pyrvinium, and found that it inhibited the growth of CRC xenografts in mice. SSTC3 also attenuated the growth of a patient-derived metastatic CRC xenograft, for which few therapies exist. SSTC3 exhibited minimal gastrointestinal toxicity compared to other classes of WNT inhibitors. Consistent with this observation, we showed that the abundance of the SSTC3 target, CK1α, was decreased in WNT-driven tumors relative to normal gastrointestinal tissue, and knocking down CK1α increased cellular sensitivity to SSTC3. Thus, we propose that distinct CK1α abundance provides an enhanced therapeutic index for pharmacological CK1α activators to target WNT-driven tumors.

INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent cancer in the United States (1), with ~50,000 CRC patients succumbing to their disease each year. The poor outcome of these patients underscores the urgent need for more effective CRC therapies. This need is especially great for patients harboring metastatic CRC, only 13% of whom survive beyond 5 years and for whom few targeted therapies exist (1). The mechanisms underlying the genesis and progression of CRC are now well established (2). Mutations in genes encoding components of the WNT signaling pathway (APC, which encodes adenomatous polyposis coli, and CTNNB1, which encodes β-catenin) were identified as early events in the vast majority of all CRCs, followed by mutations in additional oncogenic drivers such as KRAS and P53 (2, 3). More advanced stages of CRC remain addicted to WNT signaling (4), including metastasis (5). Despite the well-established mechanistic paradigm implicating WNT signaling in the development and progression of CRC, no WNT inhibitors are currently approved for clinical use.

The critical event in WNT signaling is the posttranslational regulation of the transcriptional coactivator β-catenin. In the absence of a WNT ligand, cytoplasmic β-catenin is maintained at low levels because of its constitutive degradation. This degradation occurs primarily via its association with a “destruction complex,” which consists of glycogen synthase kinase 3β (GSK3β), casein kinase 1α (CK1α), APC, and AXIN (6). The rate-limiting component in this complex is the scaffold protein AXIN, whose steady-state levels are tightly controlled by the adenosine diphosphate–ribose polymerase, tankyrase, targeting AXIN for ubiquitin-mediated proteolysis (7). All WNTs are palmitoylated in the endoplasmic reticulum by the membrane-bound O-acetyltransferase protein, Porcupine (8, 9). Post-translational modification of WNTs by palmitoylation is necessary for their exit from the endoplasmic reticulum and binding to Frizzled receptors (10–12). Upon Frizzled and co-receptor (LRP6) binding, β-catenin degradation is inhibited, and AXIN is ultimately degraded (13–16). In turn, β-catenin is translocated to the nucleus where it interacts with a variety of nuclear transcriptional regulators, such as BCL9 and PYGOPUS, to activate a T cell factor/lymphoid enhancer binding factor–mediated transcriptional program (17–20).

One emerging class of WNT inhibitors currently in clinical trials, Porcupine inhibitors, acts by blocking the palmitoylation of WNT ligands (21, 22). However, these inhibitors will likely not prove useful to target CRCs because the constitutive WNT activity driving CRC is ligand-independent. A second important class of WNT inhibitors is small-molecule Tankyrase inhibitors (TANKi) (7). Because tankyrase inhibition can attenuate the nonligand-driven WNT activity commonly found in CRC cells, such inhibitors represent a promising targeted therapeutic in CRC therapy (23). A significant hurdle to the clinical development of WNT inhibitors is overcoming the on-target toxicity that results from effects on the WNT-dependent intestinal stem cells that drive normal gastrointestinal (GI) homeostasis (24, 25). Such dose-limiting on-target toxicities have been observed using both Tankyrase
Thus, there appears to be only a limited therapeutic window for Porcupine inhibitors (23, 26, 27), with continuous dosing of both classes of small molecules disrupting normal GI structure and function. There, appears to be only a limited therapeutic window for Porcupine and TANKI, which might ultimately limit their clinical utility.

We previously described a mechanistically distinct WNT inhibitor, pyrvinium, which was already Food and Drug Administration (FDA)–approved as an anthelmintic agent (28, 29). We showed that pyrvinium potentially attenuated WNT activity by binding and activating CK1α. Pyrvinium also potently reduced the viability of CRC cell lines in culture, consistent with it attenuating WNT activity downstream of the common mutations that drive CRC growth. However, pyrvinium’s limited bioavailability precluded testing its efficacy against CRC growth in vivo and thus reduced its potential clinical utility as a WNT inhibitor for CRC patients (30, 31). Here, we sought to identify and characterize a second-generation CK1α activator with improved bioavailability, which would allow us to determine its efficacy against WNT-driven cancers in vivo.

**RESULTS**

**Activation of CK1α by SSTC3 inhibits WNT signaling**

To identify a CK1α activator with the pharmacokinetic properties necessary to advance into the clinic, we used an in silico scaffold screening approach (32). These scaffolds were prioritized on the basis of their predicted physical and pharmacokinetic properties and subsequently tested for their ability to attenuate a WNT-driven reporter gene assay. Subsequent cycles of medicinal chemistry and assaying WNT reporter gene activity led to the identification of SSTC3 (fig. S1A). SSTC3 attenuated the activity of a WNT reporter gene in a potent, dose-dependent manner [median effective concentration (EC50) = 30 nM; Fig. 1A] and bound directly to purified recombinant CK1α with a similar binding constant (Kd = 32 nM; Fig. 1B). SSTC3 treatment rapidly enhanced phosphorylation of CK1α substrates in vitro and in SW403 cells (Fig. 1, C and D). Further, SSTC3 coupled agarose beads associated with cellular CK1α (Fig. 1E and fig. S1B), and this association was reversible in the presence of uncoupled SSTC3 (fig. S1G) and pyrvinium (Fig. 1E). SSTC3 also attenuated WNT reporter activity (Fig. 1F) in a manner dependent on CK1α-dependent manner (fig. S1C). Injection of SSTC3 into Xenopus laevis embryos inhibited the ability of exogenous WNT to induce a secondary body axis (Fig. 1G), a classic WNT-driven phenotype (33), consistent with SSTC3 attenuating WNT signaling in vivo. Together, these results suggest that SSTC3 inhibits WNT signaling via activation of CK1α in a manner similar to that of pyrvinium.
**Activation of CK1α inhibits Apc mutation-driven tumor growth**

Loss of the tumor suppressor Apc leads to sustained activation of WNT signaling and intestinal adenoma formation in various mouse models (34–36). Organoids derived from these adenomas can be isolated and grown in a minimal culture that requires endogenous WNT activity (37). SSTC3 attenuated the growth of such Apc mutant organoids in a dose-dependent manner (Fig. 2A and fig. S2A). Notably, we observed that the EC50 of SSTC3 in wild-type organoids (2.9 μM) was significantly higher than that in Apc mutant organoids (150 nM for Apc+/− and 70 nM for Apc−/−) (Fig. 2A and fig. S2A). The expression of WNT target genes, Axin2 and the crypt stem cell marker Lgr5, in the intestines of Apc−/− mice were also reduced upon acute treatment with SSTC3 (EC50 = 3.1 μM; fig. S2B). We noted that the EC50 doses of SSTC3 for attenuating WNT reporter activity, CRC cell viability, and the expression of two WNT target genes (EC50 = 100 nM for AXIN2 and 106 nM for LGR5; Fig. 3C) were comparable to its binding affinity for CK1α (Fig. 1B). Further, SSTC3 exposure resulted in reduced expression of WNT target genes in all three sensitive CRC cell lines (Fig. 3D). The capacity of SSTC3 to decrease the viability of HCT116 cells was also significantly reduced when the mutant CTNNB1 allele driving its carcinogenic properties was deleted (EC50 = 78 nM versus 1.5 μM; Fig. 3E). Furthermore, an inactive structural analog of SSTC3, SST111 (fig. S1A and Fig. 3F), had minimal effects on CRC cell viability (Fig. 3G) or WNT target gene expression (fig. S3, A and B). Finally, we determined the ability of a series of structurally distinct SSTC3 derivatives to reduce WNT activity in a reporter assay and to attenuate HCT116 cell viability, deriving a ratio of each compound’s EC50 in both of these assays as an indicator of its on-target effects on viability (Fig. 3H). We found that, although the EC50 range for these SSTC3 derivatives varied about 100-fold, 90% of them exhibited an EC50 ratio within 1 SD of the idealized EC50 ratio of 1. Together, these results suggest that SSTC’s effect on CRC viability is primarily through an on-target mechanism, the attenuation of WNT activity.
Activation of CK1α inhibits growth of a patient-derived CRC xenograft

We injected SSTC3 into CD-1 mice and measured the plasma concentration by liquid chromatography–mass spectrometry (LC-MS) at five time points over 24 hours. On the basis of escalating doses in nude mice, a strong dose-dependent correlation of both maximal concentration (Cmax) and area under the curve (AUC) for SSTC3 was obtained in the serum. Our data show that ~250 nM concentration of SSTC3 can be maintained for 24 hours after treatment (Fig. 4A). This result verifies that the pharmacokinetic properties of SSTC3 are significantly improved over that of pyrvinium (31). Taking advantage of the improved pharmacokinetic properties of SSTC3, we tested for the first time the capacity of a CK1α activator to attenuate the growth of CRC in vivo. SSTC3 significantly inhibited the growth of HCT116 xenografts, compared to the vehicle-treated mice (Fig. 4B). Hematoxylin and eosin (H&E) staining of the residual tumor samples showed substantial loss of cancer cells in the SSTC3-treated group (Fig. 4B and fig. S4A). The reduction of both WNT target gene expression (Fig. 4C) and the proliferation marker Ki67 (Fig. 4D) was also observed in the tumors exposed to SSTC3. We obtained a panel of patient-derived CRC samples, two of which contained common APC mutations (CRC1 and CRC2), and established organoid cultures from them. SSTC3 treatment significantly reduced the growth of all three patient-derived CRC organoid cultures (Fig. 4E), relative to those treated with vehicle. One of these APC mutant CRCs, derived from a lung metastasis, was also used to establish patient-derived xenografts (PDXs) in mice. SSTC3 attenuated the growth of this metastatic CRC PDX (Fig. 4F and fig. S4B). SSTC3 also reduced the expression of WNT biomarkers in this CRC PDX (Fig. 4G), consistent with it acting in an on-target manner.

CK1α activation does not inhibit the proliferation of intestinal epithelium cells in vivo

One hurdle to the clinical development of WNT inhibitors is the on-target GI toxicity observed upon chronic dosing in animal models. Notably, mice exposed to either of two structurally distinct CK1α activators did not exhibit the weight loss typical of chronic effects on GI homeostasis [fig. S5, A and B, and (29)]. To explore this unexpected observation further, we directly compared the biological effects of SSTC3 to that of a TANKi (G007-LK) reported to disrupt normal GI physiology (23). We first compared their potency using a WNT reporter gene assay and noted that the TANKi was about 20 times more potent than SSTC3 in reducing ligand-induced WNT signaling (Fig. 5A). We next compared the activity of each of these WNT inhibitors on tumor growth and GI homeostasis within the same mice. For this experiment, we implanted an APC mutant CRC cell line (SW403), previously reported to be sensitive...
cultures (SSTC3 (15 mg/kg) or vehicle (right) Representative H&E staining. Scale bar, 100

Fig. 4. SSTC3 suppresses the growth of colorectal carcinoma in vivo. (A) Plasma concentration of SSTC3 in CD-1 mice after intraperitoneal injection. Data are means ± 5D (n = 3; dashed line, 250 nM). (B) Tumor volume (left) and H&E staining (right) of HCT116 xenografts in mice treated with vehicle or SSTC3 (25 mg/kg ip) for the indicated days (n = 10 in each group). Scale bars, 100 μm. (C and D) WNT-associated gene expression by qRT-PCR (C; n = 7 mice per group) and Ki67 staining (D) in residual tumors from mice described in (B) (n = 6 mice per group). Scale bar, 50 μm. (E) Representative image showing organoids derived from a patient’s CRC (CRC1) treated with vehicle or SSTC3 (200 nM and 2 μM, respectively) for 7 days is shown (left). Scale bar, 500 μm. The diameter of organoid cultures (n = 12 separate cultures) derived from three distinct resected patient CRCs was quantitated (right). (F) Tumor growth in PDXs (CRC1) from mice treated with SSTC3 (15 mg/kg ip daily) or vehicle (n = 10 and 9 mice, respectively). Right: Representative H&E staining. Scale bar, 100 μm. (G) qRT-PCR analysis in CRC1 tumors from mice treated with SSTC3 (15 mg/kg) or vehicle (n = 5 each). Data are means ± SEM (Student’s t test, *P < 0.05).

to TANKi (23), into the flanks of nude mice. Both WNT inhibitors significantly reduced the growth of the CRC xenograft (Fig. 5B), with SSTC3 exhibiting greater efficacy than the TANKi. Because the average body weight of TANKi-treated mice declined significantly and a number of the mice became moribund, we stopped this treatment after 12 days (Fig. 5C). However, the body weight of the SSTC3-treated mice remained similar to vehicle-treated mice. Sections of the mouse intestine were obtained from treated mice, H&E-stained, and examined by a pathologist. Although the crypt/villus axes of TANKi-treated mice were severely disrupted, only minor differences in the intestinal tissue were observed between SSTC3- and vehicle-treated mice (Fig. 5D and fig. S5, C and D). Consistent with this finding, TANKi treatment disrupted the proliferation of WNT-dependent intestinal crypt base columnar cells, whereas SSTC3 did not (Fig. 5E). We next examined the ability of the two WNT inhibitors to attenuate the expression of WNT biomarkers in the intestines of nontumor-bearing mice, when acutely exposed to either inhibitor at doses capable of reducing CRC growth. The expression of Axin2 and Lgr5 were substantially reduced in the intestinal tissue of nontumor-bearing mice treated with TANKi, whereas SSTC3 exposure had no observable effects on their expression (Fig. 5F). Thus, the limited effects of SSTC3 on GI homeostasis were observed in both tumor- and nontumor-bearing mice. Comparing the two WNT inhibitors in vivo is likely complicated by differences in their pharmacodynamics and pharmacokinetics. Therefore, to more directly compare their effects on GI homeostasis, we took advantage of established procedures to grow and maintain mouse intestinal tissue as enteroids, which are composed of villi and primitive crypt-like intestinal stem cell compartments (40). Similar to what was observed in vivo, SSTC3 had little effect on intestinal crypt/villus structures ex vivo (Fig. 5G), whereas the TANKi severely attenuated their growth.

WNT activity regulates CK1α levels, modulating SSTC3 sensitivity

CK1α is an established negative regulator of the WNT signaling pathway, whose deficiency leads to hyperactivated WNT signaling, and contributes to metastatic CRC in mouse models (41). Thus, we hypothesized that the differential SSTC3 response we observe in tumor tissue, relative to normal intestinal tissue, results from distinct amounts of its cellular target, CK1α. To begin to test this hypothesis, we compared the abundance of CK1α in isolated intestinal crypts and tumors from Apcmin mice by immunoblotting and observed a 40% decrease in CK1α abundance in tumor tissue relative to normal intestinal crypts (Fig. 6A and fig. S6A). Similar differences were found in organoids derived from these tissues (Fig. 6B and fig. S6B). Knockdown of APC in 293T cells also resulted in decreased abundance of CK1α (Fig. 6C and fig. S6C), consistent with the notion that activation of WNT signaling negatively regulates steady-state amounts of CK1α.

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CK1α. In line with a previous report (42), exogenous WNT and nico-
tinamide transform wild-type enteroid cultures, which exhibit increased
proliferation and decreased differentiation reminiscent of organoids
derived from tumors. CK1α abundance was significantly reduced in
these hyperactivated enteroid cultures (Fig. 6, D and E, and fig. S6D).
Unlike its effects on wild-type enteroids, SSTC3 potently suppressed the
growth of these WNT-hyperactivated enteroids (Fig. 6E and fig. S6, E
and F). To more directly address the role of CK1α expression on WNT
activity and SSTC3 efficacy, we used different amounts of small
RNA (siRNA) to partially knock down CK1α expression, revealed that survival was significantly
decreased in patients whose tumors expressed relatively lower amounts of CK1α
(Fig. 6G). These data are consistent both with CK1α expression being modulated
during CRC progression to hyperactivate WNT signaling and with lower expres-
sion of CK1α being predictive of a poorer patient outcome.

**DISCUSSION**

We found that a novel small-molecule activator of CK1α, SSTC3, attenuates the
growth of CRC cells via a mechanism dependent on CK1α and in a manner that
attenuates WNT signaling. Taking advant-
age of the significantly improved bio-
availability of SSTC3, we show for the
first time the ability of CK1α activators to attenuate the growth of WNT-driven
CRC in vivo. We further show that CK1α
levels are decreased by constitutive WNT
signaling and that this modulation of
CK1α levels likely determines the sensi-
tivity of a tissue to CK1α activators (Fig.
6H). This result is consistent with the role
CK1α plays as a negative regulator of WNT
signaling and with increased CK1α activ-
ity acting to down-regulate WNT activity
(6, 41). Thus, normal intestinal tissue has
high amounts of CK1α and is relatively
insensitive to SSTC3, whereas colorectal tumors have hyperactivated
WNT signaling that effectively lowers the amount of CK1α and are sensitive to CK1α activators (Fig. 6H).
We further show that, in a co-
hort of CRC patients, decreased tumor expression of CK1α is asso-
ciated with a worse prognosis, similar to what has been reported for
a number of other human cancers (43–46). These findings validate
CK1α as a novel therapeutic target in CRC and identify a cohort of
patients most likely to benefit from treatment with a CK1α activator
such as SSTC3.

CK1α plays important roles in a number of distinct signaling path-
ways. Thus, it remains plausible that a subset of SSTC3’s effects on CRC
viability occur via a non-WNT-dependent mechanism. For example,
CK1α has been implicated in the p53/MDM2 (47–49), FOXO1/autophagy
(50–52), and sonic hedgehog–GLI (53–55) signaling pathways, all of which

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The expression of CSNK1A1 (hereafter referred to as CK1α) is decreased in vari-
ous human tumors relative to the tissue of origin, where it is thought to be tumor-
suppressive. Taking advantage of a recent high-density transcriptome database of
colorectal tumor samples linked to patient outcome, we noted that the expression of
CK1α was significantly lower in colorectal adenomas and carcinomas relative to
normal colonic tissue (fig. S6G). Segrega-
tion of this cohort of CRC patients into two groups, based on CK1α expression,
was significantly decreased in patients whose tumors expressed relatively lower amounts of CK1α
(Fig. 6G). These data are consistent both with CK1α expression being modulated
during CRC progression to hyperactivate WNT signaling and with lower expres-
sion of CK1α being predictive of a poorer patient outcome.
have also been implicated in aspects of CRC progression (56–59). Pyrvinium, the first-in-class CK1α activator, was reported to exert antitumor activity through suppression of GLI activity (60) or autophagy flux (61). Unlike its role in WNT signaling (62, 63), CK1α is able to function in multiple, and opposing, ways within these other signaling pathways (47, 48, 54, 55), complicating the ability to predict SSTC3 effects in different biological contexts. Because of this complexity, we cannot rule out that the simultaneous modulation of these other signaling pathways may affect the efficacy of SSTC3 in targeting WNT-dependent tumors, especially at high doses.

A number of WNT inhibitors are currently being evaluated in, or for, clinical trials (64). Given the importance of WNT signaling for stem cell function and homeostasis within numerous tissues (65), these trials are being scrutinized for both efficacy and on-target toxicities (64). On-target GI toxicity has been particularly problematic for both porcupine and TANKi (23, 26), with members of both classes of these drugs showing narrow concentration ranges between efficacy and GI toxicity in animal models (27). A number of biological agents that target WNT signaling components are also being evaluated in the clinic, where on-target bone toxicities emerged as a dose-limiting factor (64). CK1α activators inhibit the growth of WNT-driven CRC without obvious on-target toxicities. These findings are consistent with the lack of GI toxicity observed in mice chronically dosed with pyrvinium or reported in pinworm patients treated with the FDA-approved anthelminthic doses of pyrvinium (66). We have suggested a model in which CK1α activators preferentially target the lower levels of CK1α found in colorectal tumors (67). Thus, CK1α activators represent the first class of WNT inhibitors that preferentially target the hyperactivated signaling typically found in WNT-driven tumors while minimizing the on-target toxicities resulting from attenuation of WNT signaling in various regenerative tissues.

**MATERIALS AND METHODS**

**Synthesis of SSTC3 analogs**

To synthesize 4-((N-methyl-N-(4-(trifluoromethyl)phenyl)sulfonyl)benzoic acid, we mixed a solution of 4-(chlorosulfonyl)benzoic acid (0.5 g, 2.27 mmol) in methanol (10 ml) with N-methyl-4-(trifluoromethyl)aniline (1191 mg, 6.80 mmol). The reaction mixture was stirred at room temperature for 14 hours, concentrated, and then partitioned between ethyl acetate and 0.5 M HCl solution. The organics were separated and dried over magnesium sulfate. The resulting dark solid was partitioned between diethyl ether and 0.5 M sodium hydroxide solution. The aqueous layer was separated and acidified to pH 1 with concentrated HCl. The resulting suspension was extracted with ethyl acetate. The organic layers were combined, dried over magnesium sulfate, and concentrated. The resulting solid was triturated with diethyl ether to provide the desired benzoic acid as a white solid. The yield was 385 mg (47%). 1H-nuclear magnetic resonance (NMR): δ 8.11 (J = 8.5 Hz, 2H), 7.74 (d, J = 8.5 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 8.5 Hz, 2H), and 3.21 (s, 3H).

To synthesize 4-((N-methyl-N-(4-(trifluoromethyl)phenyl)sulfonyl)-N-(4-(pyridin-2-yl)thiazol-2-yl)benzamide (SSTC3), we added diisopropylamine (0.12 ml, 0.69 mmol) and PyBOP (179 mg, 0.345 mmol) to a solution of 4-((N-methyl-N-(4-(trifluoromethyl)phenyl)sulfonyl)benzoic acid (100 mg, 0.28 mmol) in dimethylformamide.
The mixture was stirred for 10 min and was treated with 4-(pyridin-2-yl)thiazol-2-amine (41 mg, 0.23 mmol). The reaction mixture was stirred at room temperature for 48 hours, concentrated, and then partitioned between ethyl acetate and 5% lithium chloride solution. The organic layers were combined, dried over magnesium sulfate, and concentrated. The resulting dark oil was purified by column chromatography eluting with 50 to 100% ethyl acetate in hexanes. The resulting solid was triturated with diethyl ether to give the desired amide (SSTC3) as an off-white solid. The yield was 42 mg (35%).

\[ \text{H-NMR (400 MHz, DMSO-d6): } J = 8.5 \text{ Hz, 2H}, 7.41 \text{ (d, } J = 8.5 \text{ Hz, 2H}), 7.84 \text{ (s, 1H), 7.75 \text{ (d, } J = 8.5 \text{ Hz, 2H})}. \]

Three-dimensional organoid culture

Wild-type organoids (enteroids) were established from the isolated intestinal crypts of BALB/c mice as previously described (40). Briefly, jejunal from ~8-week-old mice was removed and gavage-washed with phosphate-buffered saline (PBS), followed by incubation with cold PBS containing 1.5 mM dithiothreitol and 30 mM EDTA for 20 min. The intestinal tissue was then incubated with warm PBS containing 15 mM EDTA for 6 min, followed by extensive shaking to release the intestinal crypts. After centrifugation, the resulting crypt pellet was washed with 30× volume of organoid basal medium [Dulbecco’s modified Eagle medium/F12 supplemented with 2 mM GlutaMAX, 10 mM Hepes, penicillin-streptomycin (100 U/ml), and 1×N2 and 1×B27 supplements]. The purified crypts were filtered through a 100-μm cell strainer and embedded in growth factor–reduced Matrigel (Corning). The resultant organoids were maintained in basal medium supplemented with niche factors [epidermal growth factor (50 ng/ml), R-spondin1 (250 ng/ml), Noggin (100 ng/ml), and 1×N2 and 1×B27 supplements]. The purified crypts were filtered through a 100-μm cell strainer and embedded in growth factor–reduced Matrigel (Corning). The resultant organoids were maintained in basal medium supplemented with niche factors [epidermal growth factor (50 ng/ml), R-spondin1 (250 ng/ml), Noggin (100 ng/ml), and 1×N2 and 1×B27 supplements]. The purified crypts were filtered through a 100-μm cell strainer and embedded in growth factor–reduced Matrigel (Corning). The resultant organoids were maintained in basal medium supplemented with niche factors [epidermal growth factor (50 ng/ml), R-spondin1 (250 ng/ml), Noggin (100 ng/ml), and 1×N2 and 1×B27 supplements].

**Surface plasmon resonance sensorgram analysis**

Surface plasmon resonance (SPR) experiments were performed on a Biacore T200 instrument (GE Healthcare) at 25°C. SSTC3 linker molecule was covalently immobilized to the surface of a CM5 sensor chip by standard amine coupling. A reference flow cell was prepared by activation and deactivation of the surface. Different concentrations of CK1α (Thermo Fisher Scientific) in 50 mM tris buffer (pH 7.5), 150 mM NaCl, 1 mM EDTA, and 5% DMSO were injected for 60 s at 30 μl/ml. Regeneration of the surface was achieved with 30-s injections of Gly-HCl (pH 2.0) and 50% DMSO. Data were fitted to a 1:1 binding model using Biacore T200 evaluation software (GE Healthcare).

**X. laevis injections**

*Xenopus* embryos were in vitro fertilized, dejellied, cultured, and injected as previously described (70). Capped Xwnt8 was generated using mMESSAGE mMACHINE (Ambion) according to the manufacturer’s instructions. All the work performed on *Xenopus* embryos was approved by the Institutional Animal Care and Use Committee (IACUC) at Vanderbilt University and was in accordance with their policies and guidelines.

**Mouse experiments**

All studies were carried out in accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health and with the policies of the University of Miami IACUC. Age-matched *Apcmin* mice (*C57BL/6-J/ApcMin/f*) were obtained from The Jackson Laboratory. Cancer cell line xenografts were established using CD-1 nu/nu mice (Charles River Laboratories) and exposed to the indicated small molecules by intraperitoneal injection. Quantitation of intestinal tumor in *Apcmin* mice was performed as described (71). Briefly, whole intestine from vehicle- or SSTC3-treated *Apcmin* mice was isolated, flushed with PBS, opened, and mounted on a filter paper. Intestinal tissues were then fixed with formalin for 10 min and subject to methylene blue staining to visualize the polyps. For immunoblotting analysis of CK1α in *Apc* mutant tumors, small intestines...
were used to show log2-transformed Fig. S6. Decreased abundance of CK1
a contract research organization (Covance-Madison), and the results
three mice via intraperitoneal injection. Blood samples were collected at
H&E staining was assessed via light microscopy. Tumor sections were

REFERENCES AND NOTES


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Treating WNT-driven tumors
The WNT signaling pathway powers the growth of various tumors, particularly colorectal cancer (CRC). However, WNT-targeted inhibitors are very toxic to normal gastrointestinal tissue, precluding their approval for clinical use. Li et al. show that WNT could be targeted by activating a kinase that inhibits the pathway. A small-molecule activator of the kinase CK1α called SSTC3 suppressed WNT activity in CRC cell lines, prevented tumor growth, and increased survival in mouse models of primary and metastatic CRC. Because the effects of SSTC3 were selective to cells with high WNT activity and low CK1α abundance, SSTC3 was minimally toxic to normal gastrointestinal epithelium. Thus, SSTC3 and its future derivatives may be a promising therapeutic for CRC patients.