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Selective Small Molecule Targeting β-Catenin Function Discovered by In Vivo Chemical Genetic Screen

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SUMMARY

The canonical Wnt signaling pathway, mediated by the transcription factor β-catenin, plays critical roles in embryonic development and represents an important therapeutic target. In a zebrafish-based in vivo screen for small molecules that specifically perturb embryonic dorsoventral patterning, we discovered a compound named windorphen that selectively blocks the Wnt signal required for ventral development. Windorphen exhibits remarkable specificity toward β-catenin-1 function, indicating that the two β-catenin isoforms found in zebrafish are not functionally redundant. We show that windorphen is a selective inhibitor of p300 histone acetyltransferase, a coactivator that associates with β-catenin. Finally, windorphen robustly and selectively kills cancer cells that harbor Wnt-activating mutations, supporting the therapeutic potential of this Wnt inhibitor class.

INTRODUCTION

Canonical Wnt/β-catenin signaling plays critical roles in embryogenesis and adult tissue homeostasis (Clevers, 2006). In the absence of Wnt ligands, cytoplasmic β-catenin is phosphorylated and targeted for proteolytic degradation by the destruction complex Axin/GSK-3/adenomatous polyposis coli (APC). In the presence of Wnt ligands, this complex is disassembled, and stabilized β-catenin translocates to the nucleus to promote expression of specific Wnt target genes. Because aberrant Wnt signal activation is thought to cause a number of human diseases, especially cancers (Malekar et al., 2010; Moon et al., 2004; Reya and Clevers, 2005; Thorne et al., 2010), small molecules that selectively inhibit Wnt/β-catenin signaling are highly sought after as potential therapeutic leads (Chen et al., 2009; Emami et al., 2004; Huang et al., 2009; Thorne et al., 2010).

In zebrafish, canonical Wnt signaling is mediated by two distinct β-catenin isoforms: β-catenin-1, which is highly similar to mammalian and *Xenopus* β-catenin; and β-catenin-2, which is more divergent from others (Figure S4) (Bellipanni et al., 2006). Although the two β-catenins are highly conserved, with 96.1% sequence identity at the N terminal and the armadillo repeat domains, the greatest divergence is found within the 87-amino acid C-terminal transactivation domain, with 66.7% sequence identity. Despite similarities in the sequences and the expression patterns, only maternally contributed β-catenin-2 is required for dorsal organizer formation, a region equivalent to the Spemann-Mangold organizer in *Xenopus* (Bellipanni et al., 2006), indicating that the two β-catenin isoforms are not functionally redundant. Moreover, after formation of the dorsal organizer, zygotic contributions of β-catenin-1 are thought to promote ventral and posterior cell fates (Bellipanni et al., 2006; Varga et al., 2007). In summary, molecular genetic studies indicate that, in zebrafish, Wnt/β-catenin-2 signaling early in embryogenesis is required for dorsal developmental programs; whereas later, Wnt/β-catenin-1 signaling has the opposite effect of promoting ventral development.

RESULTS

Discovery of Windorphen, a Small Molecule Wnt Inhibitor, in a Phenotype-Based Screen

Given the critical roles of Wnt/β-catenin signaling in embryonic dorsoventral patterning, we devised a chemical screen to discover selective small molecule Wnt pathway modulators based on their ability to perturb dorsoventral patterning in zebrafish embryos. Briefly, fertilized zebrafish embryos were arrayed into 96-well microtiter plates, exposed at 3–4 hr postfertilization (hpf) to compound library, and then visually assessed for perturbations in the dorsoventral pattern (Extended Experimental

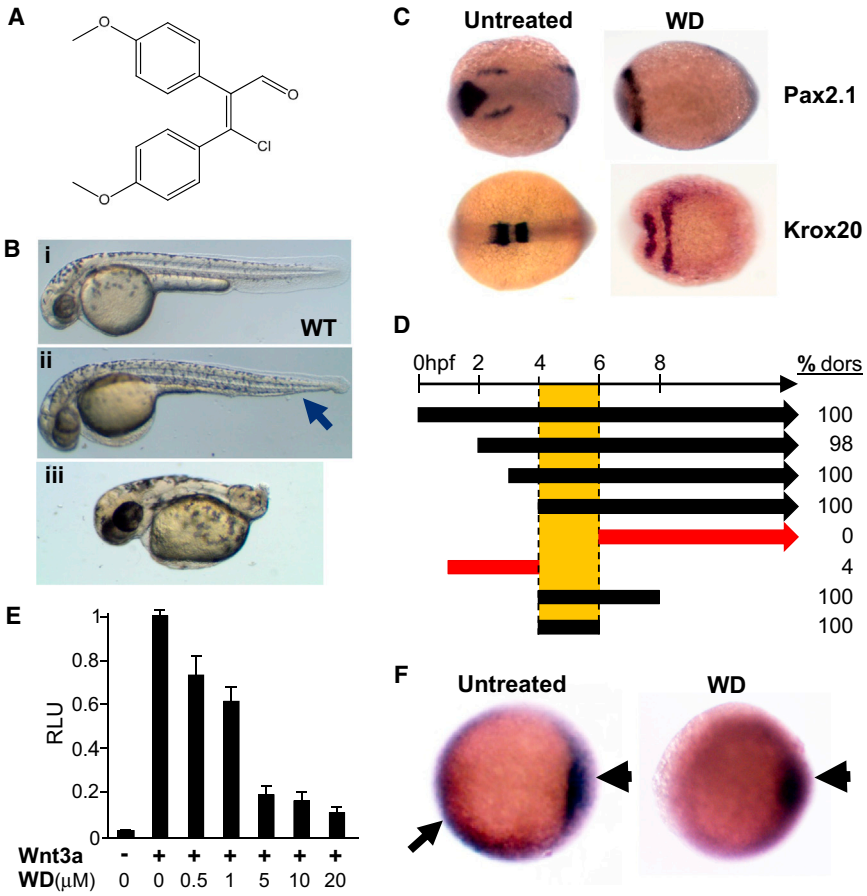


Figure 1. WD, which Dorsalizes Zebrafish Embryo, Inhibits Canonical Wnt Signaling

(A) Chemical structure of WD, (Z) 3-chloro-2,3-bis(4-methoxyphenyl)acrylaldehyde, is shown.

(B) WD dorsalizes zebrafish embryos. (i) Vehicle-treated WT zebrafish embryo at 48 hpf. (ii and iii) Dorsalized embryos treated with 10 μM WD. (i) Mildly dorsalized embryo is characterized by loss of ventral tail fin (arrow), whereas (ii) severely dorsalized embryo is characterized by severely shortened tail.

(C) In situ hybridization of six-somite-stage (12 hpf) embryos was performed to evaluate expression of the dorsal markers Pax2.1, which marks the midhindbrain boundary, and Krox20, which marks rhombomeres 3 and 5. In comparison to untreated embryos (left), Pax2.1 and Krox20 expression are dramatically expanded in WD-treated embryos (right). Dorsal view, anterior is to the left.

(D) Time window for dorsalization by WD (20 μM) is between 4 and 6 hpf. Numbers shown on right are percentage (%) of embryos dorsalized (dors) upon various WD treatment schemes. Left end of each bar or arrow represents the time when WD treatment was initiated, and the right end of each bar represents the time when WD was washed out (n > 100 embryos for each condition).

(E) WD inhibited Wnt3-induced signaling in β-catenin-responsive TOPFLASH reporter assay (n = 4; results are represented as mean RLU [relative luciferase units] ± SE).

(F) In situ hybridization for GFP expression in Wnt-dependent TOPFLASH-GFP transgenic zebrafish reveals that WD selectively abrogated Wnt signaling in the ventral and lateral regions (arrow) of the 50% epiboly stage (5.3 hpf) embryos, but not within the dorsal organizer (arrowhead). Ventral side is to the left.

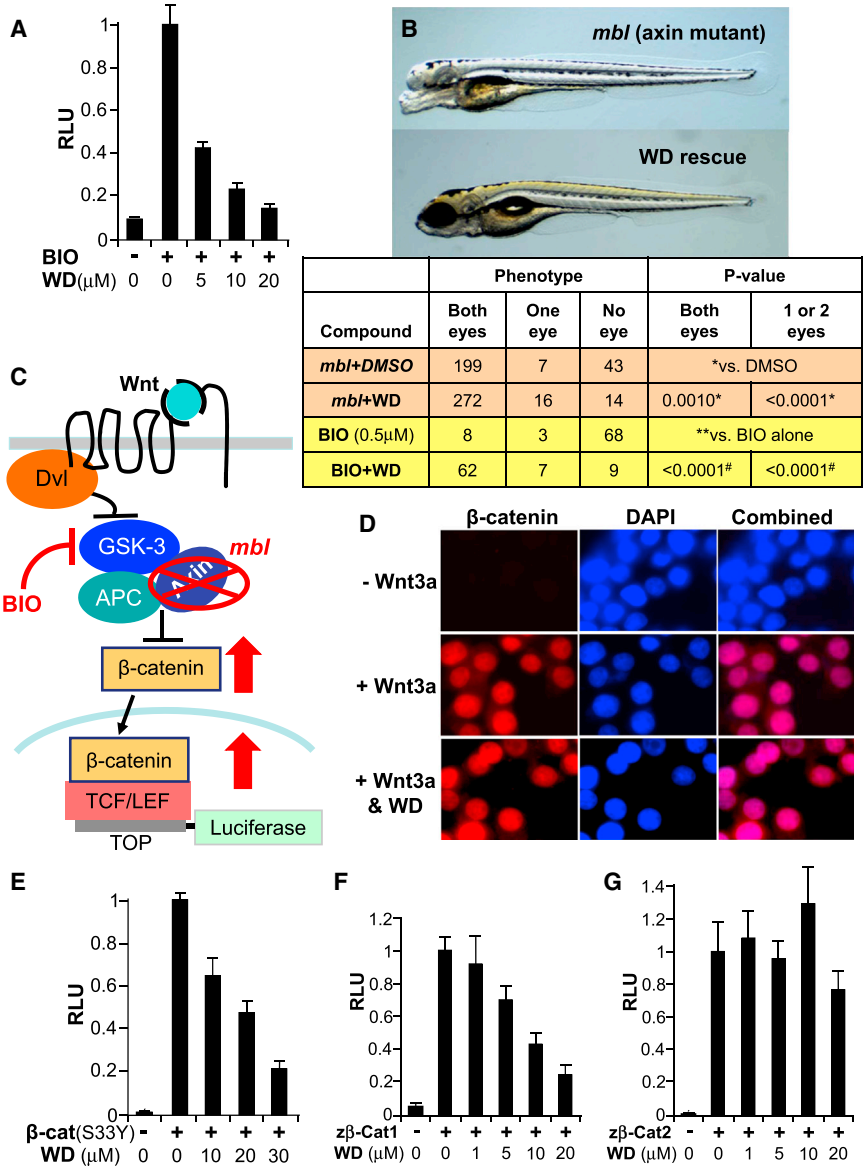
See also Figures S1, S2, and S3.

Procedures). This screen of 30,000 compounds discovered nine structurally independent hits. One of the hits, subsequently named windorphen (*W*nt *in*hibitor *dor*salizing; WD; Figure 1A), reproducibly dorsalized zebrafish embryos (Figure 1B). WD treatment induced marked expansion of the dorsal markers *pax2.1*, which demarcates the midhindbrain boundary, and *krox20*, a marker of rhombomeres 3 and 5 at the six-somite stage (Figure 1C). In contrast to our previously identified BMP inhibitor dorsomorphin, which dorsalized embryos when administered over a wide developmental time window (<1–8 hpf) (Yu et al., 2008), the temporal window for WD action was very narrow, between 4 and 6 hpf, corresponding to the early epiboly stage (Figure 1D). Moreover, in contrast to dorsomorphin, WD had no effect either on BMP-responsive luciferase reporter assay or on BMP4-induced phospho-SMAD1/SMAD5/SMAD8 activation, suggesting that WD-induced dorsalization did not involve BMP signal inhibition (Figures S1A and S1B).

In canonical Wnt/β-catenin reporter cell line STF293 (Thorne et al., 2010; Xu et al., 2004), WD inhibited Wnt3a-inducible TOPFLASH-luciferase activity in a dose-dependent manner with a calculated IC_{50} of about 1.5 μM (Figure 1E). By contrast, BAS, a structural analog of WD, neither dorsalized embryos nor inhibited Wnt reporter activity (Figures S1E and S1F). Additionally, WD did not affect either Hedgehog or TNF-α/NF-κB reporter activity (Figures S1C and S1D), indicating that it selectively targeted Wnt signaling. Thus, WD perturbs dorsoventral patterning through selective inhibition of canonical Wnt signaling. Interestingly, there was considerable batch-to-batch variability in WD's ability to both dorsalize embryos and inhibit Wnt signaling. Mass spectroscopic and nuclear magnetic resonance analyses of active and inactive WD batches revealed that the active batches were distinguishable by the presence of the Z isoform of WD that comprises a minor (~13%) constituent not present in the inactive batches (Figures S2 and S3). In all subsequent studies, only the batches capable of dorsalizing embryos were used, including potency determinations. Thus, the z isomer of WD perturbs dorsoventral patterning through selective inhibition of canonical Wnt signaling.

WD Selectively Targets Some, but Not All, Wnt Signals in Zebrafish Embryos

That WD is a Wnt inhibitor came as a surprise because such a compound was predicted to ventralize the embryo by blocking the formation of the dorsal organizer (Kelly et al., 2000; Pelegri and Maischein, 1998; Schier and Talbot, 2005). WD, however, never caused ventralization, and the temporal window of activity



Compound	Phenotype			P-value	
	Both eyes	One eye	No eye	Both eyes	1 or 2 eyes
mbl+DMSO	199	7	43	*vs. DMSO	
mbl+WD	272	16	14	0.0010*	<0.0001*
BIO (0.5μM)	8	3	68	**vs. BIO alone	
BIO+WD	62	7	9	<0.0001#	<0.0001#

Figure 2. WD Blocks Wnt Signaling Downstream of β-Catenin Destruction Complex

(A) WD inhibited Wnt signaling induced by BIO in TOPFLASH-luciferase assays in STF293 cells (n = 4; results are represented as mean RLU ± SE).

(B) WD (20 μM) rescued the loss of telencephalon and eyes in *mbl* mutant zebrafish with defective *Axin1* gene. Top view shows untreated *Axin1*/*mbl* mutant zebrafish embryo (3 days old). Bottom view presents WD-treated embryo with normal/restored eyes and telencephalon. Quantification of telencephalon/eye loss phenotype in *mbl* mutants and BIO-treated embryos, following treatment with WD or DMSO control, is shown. p value was determined using Student's two-tailed t test.

(C) Model illustrates TOPFLASH-luciferase assay, with components of the Wnt/β-catenin signaling and various means to perturb them. Briefly, disruption of the β-catenin degradation complex, either through pharmacological inhibition of GSK3β using the small molecule BIO or a genetic mutation in Axin (*mlb*), results in nuclear β-catenin accumulation and subsequent Wnt reporter activation.

(D) WD treatment does not block Wnt3a-induced β-catenin nuclear translocation in RKO cells. RKO cells were immunostained for β-catenin (red) and counterstained with DAPI (blue) following 24 hr incubation without Wnt3a, with Wnt3a, and with Wnt3a plus WD (20 μM).

(E) WD inhibited Wnt signaling induced by overexpression of the constitutively active human β-catenin (cat) mutant (S33Y) in TOPFLASH-luciferase assay (n = 4; results are represented as mean RLU ± SE).

(F) WD blocked Wnt signaling induced by overexpression of the zebrafish β-catenin-1 in TOPFLASH-luciferase assay in STF293 cells (n = 3).

(G) WD did not block Wnt signaling induced by overexpression of the zebrafish β-catenin-2 (n = 3) in STF293 cells.

See also Figure S4.

from 4 to 6 hpf (Figure 1D) suggested that it disrupted a patterning process subsequent to the formation of organizer. Indeed, using TOPFLASH-GFP transgenic zebrafish embryos, in which a Wnt/β-catenin-responsive promoter drives the GFP expression (Dorsky et al., 2002), we found that WD treatment selectively abrogated Wnt signaling in ventral and lateral regions of the 50% epiboly stage embryo (5.3 hpf), but not the Wnt signaling within the dorsal organizer (Figure 1F). These results suggest that WD selectively targets some, but not all, aspects of Wnt/β-catenin signaling involved in dorsoventral patterning.

WD Targets a Component Downstream of Both the β-Catenin Destruction Complex and β-Catenin Nuclear Translocation

To define where WD acts in the canonical Wnt signaling pathway, we utilized BIO, a selective GSK3 inhibitor that induces Wnt signaling by blocking β-catenin phosphorylation and subsequent degradation (Sato et al., 2004). In STF293 reporter cells, addition of BIO alone robustly induced β-catenin-dependent luciferase activity, and this BIO-induced reporter activity was blocked by WD in a dose-dependent manner (Figures 2A and 2C; Table S1). We sought corroborating in vivo evidence by using homozygous recessive *masterblind* (*mbl; Axin1*^{tm213}) mutant zebrafish, which carry the L399Q mutation in the *axin1* gene (Heisenberg et al., 2001; van de Water et al., 2001). In *mbl* mutants, a defective Axin1/β-catenin destruction complex leads to aberrant activation of Wnt signaling and the resultant loss of telencephalon and eyes (Figures 2B and 2C). Similar phenotype is also seen in embryos treated at 6 hpf with BIO. In both *mbl* mutants and BIO-treated embryos, WD exposure reproducibly rescued the telencephalon/eye phenotype (Figure 2B). Next, in human colon carcinoma RKO cells, we found that WD had no effect on Wnt3a-induced

translocation of β-catenin to the nucleus (Figure 2D). Taken together, these results indicate that WD selectively targets a signaling component in the Wnt pathway that lies downstream of both β-catenin destruction complex and β-catenin nuclear translocation. Indeed, WD abrogated TOPFLASH-luciferase induction in STF293 cells expressing the constitutively active human β-catenin mutant (S33Y), which is resistant to GSK3-mediated degradation (Figure 2E) (Morin et al., 1997).

WD Selectively Targets the Function of β-Catenin-1, but Not β-Catenin-2

Importantly, WD blocked reporter induction in STF293 cells overexpressing the zebrafish β-catenin-1 (Figure 2F), but not the zebrafish β-catenin-2 (Figure 2G), indicating that WD selectively targets the function of the zebrafish β-catenin-1 and the closely related mammalian homologs, but not the more divergent zebrafish β-catenin-2 (Figure S4). Together with the fact that WD treatment causes just dorsalization of the embryonic axis and disrupts Wnt signaling only in ventral and lateral regions of the epiboly stage embryo (Figure 1F), these results support the notion that the two zebrafish β-catenin isoforms are not functionally equivalent.

WD Functionally Targets the C-Terminal Transactivation Domain of β-Catenin-1, Disrupting Its Association with p300

Because β-catenin-2 is most divergent from the mammalian β-catenin at the C-terminal transactivation domain (Figure S4), we examined whether WD targets the mammalian β-catenin via this domain. We used an expression construct in which the fusion protein LEFΔN-βCTA (or LF-TA in Figure 3A; Table S1) comprised of the TCF/LEF1 DNA-binding domain fused to the C-terminal transactivation domain (amino acids 695–781) of mouse β-catenin (Vleminckx et al., 1999). As previously demonstrated, the LEFΔN-βCTA fusion protein was able to induce Wnt/β-catenin reporter expression. Importantly, WD blocked reporter induction by LEFΔN-βCTA in a dose-dependent manner (Figure 3A). However, WD did not block reporter induction by the LEFΔN-VP16 (or LF-VP in Figure 3B) fusion protein comprised of LEF1 DNA-binding domain and the transactivation domain of the herpes simplex virus VP16 protein (Figure 3B; Table S1), suggesting that inhibitory effects of WD were specific to the C-terminal transactivation domain of β-catenin-1 (Figure 3C; Table S1).

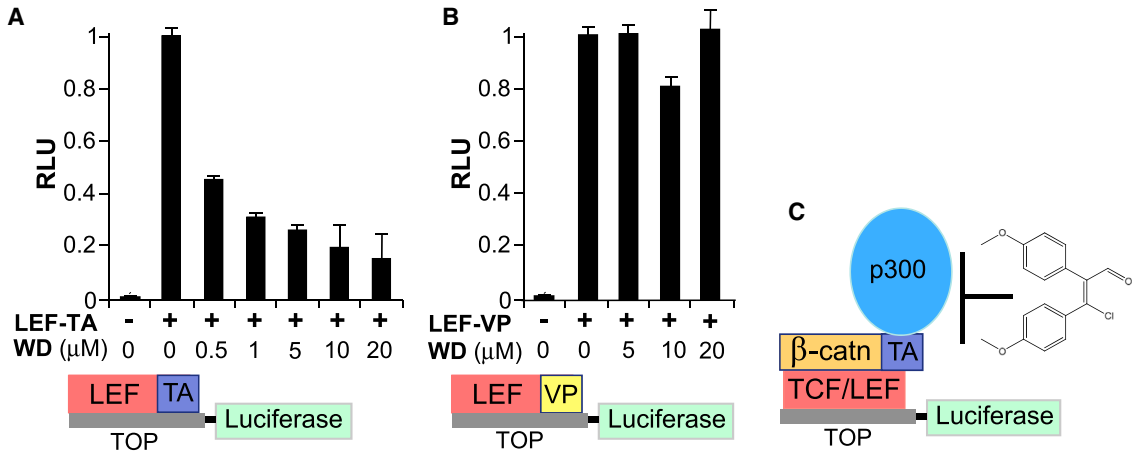
The closely related, yet nonredundant, histone acetyltransferases (HATs) CREB-binding protein (CBP) and p300 are known to associate with the C-terminal transactivation domain of β-catenin to mediate its transactivation activity (Hecht et al., 2000; Ma et al., 2005). To determine whether WD disrupted interaction of β-catenin with CBP or p300, we incubated cell lysates with the tested compounds and performed coimmunoprecipitation experiments. In human colon adenocarcinoma cell line SW480, endogenous CBP was readily coimmunoprecipitated with β-catenin in the presence of inactive analog BAS, or 30 μM WD (Figure 3D). In contrast, ICG-001, a Wnt inhibitor, specifically disrupted β-catenin and CBP interaction as previously reported (Figure 3D) (Emami et al., 2004). The β-catenin:p300 coimmunoprecipitation experiments were conducted in HEK293 cells following transfection with a HA-tagged p300 construct, as previously performed by Emami et al. (2004), because endogenous p300 was expressed at relatively low levels in SW480 cells, resulting in inconsistent results. In HEK293 cells overexpressing the HA-tagged p300, the physical association of β-catenin with p300 was disrupted by WD, but not BAS (Figure 3E). In addition, WD did not disrupt the interaction of β-catenin with its DNA-binding partner LEF1/TCF4 in HEK293 cells treated with Wnt3a (Figure S5). These results suggest that WD inhibits Wnt signaling by selectively targeting the association of β-catenin with p300, but not CBP or LEF1/TCF4.

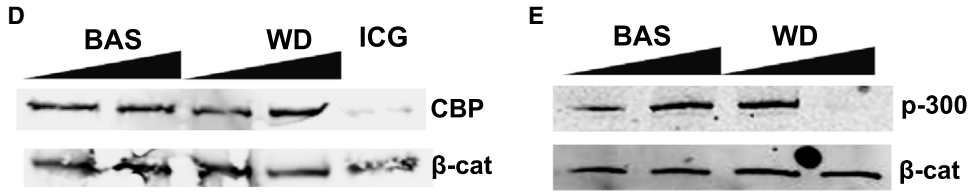
WD Directly and Selectively Targets the p300 HAT

Next, we examined whether WD directly targets p300, specifically its HAT activity. In in vitro acetyltransferase assays using purified human HATs p300, CBP, GCN5, KAT5, MYST2/KAT7, MYST4/KAT6B, and P300/CBP-associated factor (PCAF), WD, but not BAS, inhibited the p300 HAT activity with an IC_{50} (concentration causing 50% inhibition) of 4.2 μM. In contrast, WD's IC_{50} values toward other HATs, including CBP, were approximately 10-fold higher; much higher than the concentrations used to disrupt Wnt signaling in embryos and in cultured cells (Figure 3F). WD's selective effects on p300 versus CBP support the growing evidence that p300 and CBP, whereas traditionally considered to be functionally interchangeable, are functionally nonredundant (Emami et al., 2004; Ma et al., 2005; Ramos et al., 2010; Teo and Kahn, 2010). Indeed, we found that siRNA knockdown of either p300 or CBP led to a significant decrease in Wnt reporter activity, versus scrambled siRNA (Figure S6). Taken together, our study indicates that WD blocks Wnt signaling by selectively targeting p300, disrupting its association of the C-terminal transactivation domain of β-catenin-1.

WD Robustly Induces Apoptosis in Wnt-Dependent Tumor Cells

Canonical Wnt/β-catenin signaling has been strongly linked to the development of numerous malignancies, particularly gastrointestinal cancers of which 90% are thought to involve aberrant Wnt signal activation (Clevers, 2006; Moon et al., 2004; Reya and Clevers, 2005). To query the therapeutic potential of chemical inhibition of β-catenin, we evaluated WD's effects on the viability of cancer cells. In human colon adenocarcinoma SW480 cells, in which Wnt signaling is constitutively activated due to defective APC gene, WD (20 μM) caused widespread apoptosis after 72 hr of treatment (Figure 3G). In addition, in cell viability assays in the presence of increasing concentrations of WD for 72 hr, human carcinoma cell lines noted to have activation of Wnt signaling, such as human colon cancer cell lines SW480 and RKO and prostate cancer cell lines DU145 and PC3 (Lu et al., 2009; Morin et al., 1997; Suzuki et al., 2008), exhibited sensitivities to WD (IC_{50} values of 15.0, 19.2, 21.8, and 19.0 μM, respectively; Figure 3H). In contrast, WD did not display cytotoxic activity toward the human lung cancer cell line H460, which does not exhibit aberrant Wnt signal activation and is insensitive to β-catenin depletion or to the small molecule Wnt inhibitor IWR-3 (Figure 3H) (Chen et al., 2009). These





F	Histone Acetyl Transferase (HAT) Inhibition (iC50, μM)						
	p300	CBP	GCN5	KAT5	MYST2	MYST4	pCAF
WD	4.2	51.3	>100	38.2	62.2	59.5	>100
BAS	>100	>100	>100	>100	89.0	>100	>100

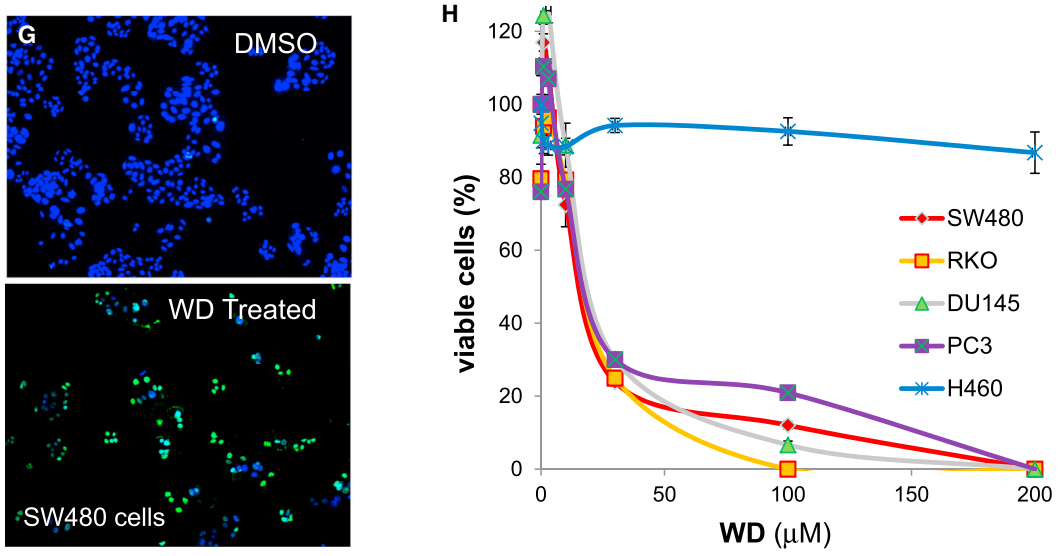


Figure 3. WD Targets the C-Terminal Transactivation Domain of β-Catenin-1, Disrupts Its Interaction with Coactivators CBP/p300, and Kills Human Carcinoma Cell Lines

(A) WD blocked Wnt signaling induced by overexpression of the LEFΔN-βCTA (LEF-TA) fusion protein comprised of the LEF1 DNA-binding domain and the β-catenin C-terminal transactivation domain in a dose-dependent manner.

(B) WD did not block Wnt signaling induced by overexpression of the LEFΔN-βCTA (LEF-VP) fusion protein comprised of the LEF1 DNA-binding domain and the transactivation domain of the herpes simplex virus VP16 protein.

(legend continued on next page)

observations raise the possibility that selective β-catenin inhibitors like WD may be attractive lead compounds for the development of Wnt pathway-targeted cancer therapeutics.

DISCUSSION

Here, we report the identification of a small-molecule Wnt/β-catenin signal inhibitor, named WD, in a large-scale in vivo chemical genetic screen. WD dorsalizes zebrafish embryos when administered during epiboly and rescues the loss of telencephalon caused by overactivation of Wnt signaling in GSK3-inhibited and *axin1* mutant embryos. Moreover, we provide evidence that WD specifically targets the function of the C-terminal transactivation domain of β-catenin-1, but not β-catenin-2. Among cofactors that interact with the C-terminal transactivation domain of β-catenin, WD directly and selectively targets p300, disrupting the association of the mammalian β-catenin with p300, but not CBP. Although p300 and CBP are highly homologous and often considered to be interchangeable, growing evidence suggests divergent roles of p300 and CBP (Ma et al., 2005; Ramos et al., 2010; Teo and Kahn, 2010). Indeed, small-molecule ICG-001 can selectively disrupt interaction between β-catenin and CBP but not β-catenin and p300 (Emami et al., 2004), consistent with our observation that, despite their close structural similarities, it is possible to develop small molecules that can discriminate between CBP and p300.

Our findings support earlier studies indicating that the two zebrafish β-catenin isoforms play nonredundant roles in embryonic dorsoventral patterning, with β-catenin-2 required for the formation of the dorsal organizer and β-catenin-1 involved primarily in the ventral development. However, how the developing embryo can differentiate between these two isoforms when their transcripts are present ubiquitously is unknown (Bellipanni et al., 2006). Our results suggest a potential mechanism in which the two β-catenin isoforms interact with distinct binding partners that initiate the divergent developmental programs. Moreover, our results indicate that the β-catenin-1:p300 interaction is specifically required for ventral development of the zebrafish embryo. Finally, we show that WD can selectively kill cancer cells in which Wnt signaling is known to be aberrantly activated. Because overexpression of p300, but not CBP, is associated with poor prognosis in human colon cancers, drugs that selectively target the β-catenin:p300 interaction like WD may have a therapeutic advantage as antineoplastic agents (Ishihama et al., 2007). In summary, our unbiased, in vivo chemical genetic approach demonstrates the feasibility of developing remarkably selective inhibitors of a nontraditional therapeutic target.

EXPERIMENTAL PROCEDURES

All zebrafish experiments were approved by Vanderbilt University Institutional Animal Care and Use Committee.

Chemical Screen

Chemical screen for small molecules that perturb dorsoventral axis was performed as previously described (Hao et al., 2010a; Hong, 2009; Yu et al., 2008) and are detailed in the Supplemental Information.

Compound Synthesis

Methods for synthesis of WD and BAS are detailed in the Supplemental Information and the synthesis schemes represented in Figure S7.

Luciferase Reporter Assays

For Wnt/β-catenin signaling assay, STF293 cells (HEK293 cells stably transfected with TOPFLASH [*T*CF/LEF1-optimized *p*romoter]-firefly luciferase reporter) (Thorne et al., 2010) were seeded in 96-well plates and incubated overnight with the various concentrations of the compound in the presence of Wnt3a-conditioned media (made according to directions in the American Type Culture Collection website). The cell-based assays for BMP signaling, Hedgehog signaling, and TNF-α signaling are detailed in the Supplemental Information.

Coimmunoprecipitation

Cells were lysed in M-PER lysis buffer (Thermo Scientific) supplemented with Complete Mini Protease Inhibitor Cocktail (Roche). Cell lysate was incubated with the tested compounds for 4 hr, followed by incubation with mouse Active-β-catenin antibody (Millipore; Clone 8E7, 1:50 dilution) at 4°C overnight. Antibody-antigen complex was conjugated to Protein A agarose beads (Thermo Scientific) for 2 hr at 4°C, followed by at least three cold TBS washes. The beads were spun down, and bound protein was eluted in LDS buffer (Invitrogen). Eluted protein was resolved in SDS-PAGE and transferred onto nitrocellulose membrane for western blotting.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, seven figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.07.047.

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(C) Model illustrates WD targeting the function of the C-terminal transactivation domain (TA) of β-catenin-1. In it, β-catenin-1 is brought to the promoter of the Wnt reporter gene (TOP-luciferase) via its association with TCF/LEF1 (LEF).
(D) Western blot showing coimmunoprecipitation of endogenous CBP and β-catenin in SW480 cells following incubation with increasing concentrations (5 and 30 μM) of WD, inactive analog BAS, and 10 μM ICG-001.
(E) Western blot showing coimmunoprecipitation of transfected HA-tagged p300 and β-catenin in HEK293 cells following incubation with increasing concentrations (5 and 30 μM) of BAS and WD.
(F) Inhibitory effects of WD and BAS on seven human HATs in in vitro assays. WD exhibits much lower IC_{50} (4.2 μM) against p300 than other HATs.
(G) TUNEL apoptosis assay in human colon adenocarcinoma SW480 cells following treatment with DMSO (top), or 20 μM WD for 72 hr (bottom), is presented. TUNEL signal is colored green, DAPI counterstain is blue, and yellow is merged.
(H) Percentage of viable human cancer cells (SW480, RKO, DU145, PC3, and H460), as determined by cell titer assays, following 72 hr treatment with increasing concentrations of WD (n = 4 for each data point).
For (A)–(C), n = 4 each. Results are represented as mean RLU ± SE. See also Figure S5.

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REFERENCES

Bellipanni, G., Varga, M., Maegawa, S., Imai, Y., Kelly, C., Myers, A.P., Chu, F., Talbot, W.S., and Weinberg, E.S. (2006). Essential and opposing roles of zebrafish beta-catenins in the formation of dorsal axial structures and neurectoderm. Development *133*, 1299–1309.

Chen, B., Dodge, M.E., Tang, W., Lu, J., Ma, Z., Fan, C.W., Wei, S., Hao, W., Kilgore, J., Williams, N.S., et al. (2009). Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat. Chem. Biol. *5*, 100–107.

Clevers, H. (2006). Wnt/beta-catenin signaling in development and disease. Cell *127*, 469–480.

Dorsky, R.I., Sheldahl, L.C., and Moon, R.T. (2002). A transgenic Lef1/beta-catenin-dependent reporter is expressed in spatially restricted domains throughout zebrafish development. Dev. Biol. *241*, 229–237.

Emami, K.H., Nguyen, C., Ma, H., Kim, D.H., Jeong, K.W., Eguchi, M., Moon, R.T., Teo, J.L., Kim, H.Y., Moon, S.H., et al. (2004). A small molecule inhibitor of beta-catenin/CREB-binding protein transcription [corrected]. Proc. Natl. Acad. Sci. USA *101*, 12682–12687.

Hao, J., Williams, C.H., Webb, M.E., and Hong, C.C. (2010a). Large scale zebrafish-based in vivo small molecule screen. J. Vis. Exp. (46), 2243.

Hecht, A., Vleminckx, K., Stemmler, M.P., van Roy, F., and Kemler, R. (2000). The p300/CBP acetyltransferases function as transcriptional coactivators of beta-catenin in vertebrates. EMBO J. *19*, 1839–1850.

Heisenberg, C.P., Houart, C., Take-Uchi, M., Rauch, G.J., Young, N., Coutinho, P., Masai, I., Caneparo, L., Concha, M.L., Geisler, R., et al. (2001). A mutation in the Gsk3-binding domain of zebrafish Masterblind/Axin1 leads to a fate transformation of telencephalon and eyes to diencephalon. Genes Dev. *15*, 1427–1434.

Hong, C.C. (2009). Large-scale small-molecule screen using zebrafish embryos. Methods Mol. Biol. *486*, 43–55.

Huang, S.M., Mishina, Y.M., Liu, S., Cheung, A., Stegmeier, F., Michaud, G.A., Charlat, O., Wiellette, E., Zhang, Y., Wiessner, S., et al. (2009). Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. Nature *461*, 614–620.

Ishihama, K., Yamakawa, M., Semba, S., Takeda, H., Kawata, S., Kimura, S., and Kimura, W. (2007). Expression of HDAC1 and CBP/p300 in human colorectal carcinomas. J. Clin. Pathol. *60*, 1205–1210.

Kelly, C., Chin, A.J., Leatherman, J.L., Kozlowski, D.J., and Weinberg, E.S. (2000). Maternally controlled (beta)-catenin-mediated signaling is required for organizer formation in the zebrafish. Development *127*, 3899–3911.

Lu, W., Tinsley, H.N., Keeton, A., Qu, Z., Piazza, G.A., and Li, Y. (2009). Suppression of Wnt/beta-catenin signaling inhibits prostate cancer cell proliferation. Eur. J. Pharmacol. *602*, 8–14.

Ma, H., Nguyen, C., Lee, K.S., and Kahn, M. (2005). Differential roles for the coactivators CBP and p300 on TCF/beta-catenin-mediated survivin gene expression. Oncogene *24*, 3619–3631.

Malekar, P., Hagenmueller, M., Anyanwu, A., Buss, S., Streit, M.R., Weiss, C.S., Wolf, D., Riffel, J., Bauer, A., Katus, H.A., and Hardt, S.E. (2010). Wnt signaling is critical for maladaptive cardiac hypertrophy and accelerates myocardial remodeling. Hypertension *55*, 939–945.

Moon, R.T., Kohn, A.D., De Ferrari, G.V., and Kaykas, A. (2004). WNT and beta-catenin signalling: diseases and therapies. Nat. Rev. Genet. *5*, 691–701.

Morin, P.J., Sparks, A.B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K.W. (1997). Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science *275*, 1787–1790.

Pelegri, F., and Maischein, H.M. (1998). Function of zebrafish beta-catenin and TCF-3 in dorsoventral patterning. Mech. Dev. *77*, 63–74.

Ramos, Y.F., Hestand, M.S., Verlaan, M., Krabbendam, E., Ariyurek, Y., van Galen, M., van Dam, H., van Ommen, G.J., den Dunnen, J.T., Zantema, A., and 't Hoen, P.A. (2010). Genome-wide assessment of differential roles for p300 and CBP in transcription regulation. Nucleic Acids Res. *38*, 5396–5408.

Reya, T., and Clevers, H. (2005). Wnt signalling in stem cells and cancer. Nature *434*, 843–850.

Sato, N., Meijer, L., Skaltsounis, L., Greengard, P., and Brivanlou, A.H. (2004). Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat. Med. *10*, 55–63.

Schier, A.F., and Talbot, W.S. (2005). Molecular genetics of axis formation in zebrafish. Annu. Rev. Genet. *39*, 561–613.

Suzuki, H., Toyota, M., Carraway, H., Gabrielson, E., Ohmura, T., Fujikane, T., Nishikawa, N., Sogabe, Y., Nojima, M., Sonoda, T., et al. (2008). Frequent epigenetic inactivation of Wnt antagonist genes in breast cancer. Br. J. Cancer *98*, 1147–1156.

Teo, J.L., and Kahn, M. (2010). The Wnt signaling pathway in cellular proliferation and differentiation: a tale of two coactivators. Adv. Drug Deliv. Rev. *62*, 1149–1155.

Thorne, C.A., Hanson, A.J., Schneider, J., Tahinci, E., Orton, D., Cselenyi, C.S., Jernigan, K.K., Meyers, K.C., Hang, B.I., Waterson, A.G., et al. (2010). Small-molecule inhibition of Wnt signaling through activation of casein kinase 1α. Nat. Chem. Biol. *6*, 829–836.

van de Water, S., van de Wetering, M., Joore, J., Esseling, J., Bink, R., Clevers, H., and Zivkovic, D. (2001). Ectopic Wnt signal determines the eyeless phenotype of zebrafish masterblind mutant. Development *128*, 3877–3888.

Varga, M., Maegawa, S., Bellipanni, G., and Weinberg, E.S. (2007). Chordin expression, mediated by Nodal and FGF signaling, is restricted by redundant function of two beta-catenins in the zebrafish embryo. Mech. Dev. *124*, 775–791.

Vleminckx, K., Kemler, R., and Hecht, A. (1999). The C-terminal transactivation domain of beta-catenin is necessary and sufficient for signaling by the LEF-1/beta-catenin complex in *Xenopus laevis*. Mech. Dev. *81*, 65–74.

Xu, Q., Wang, Y., Dabdoub, A., Smallwood, P.M., Williams, J., Woods, C., Kelley, M.W., Jiang, L., Tasman, W., Zhang, K., and Nathans, J. (2004). Vascular development in the retina and inner ear: control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair. Cell *116*, 883–895.

Yu, P.B., Hong, C.C., Sachidanandan, C., Babitt, J.L., Deng, D.Y., Hoyng, S.A., Lin, H.Y., Bloch, K.D., and Peterson, R.T. (2008). Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. Nat. Chem. Biol. *4*, 33–41.