Wnt Inhibitor Screen Reveals Iron Dependence of β-Catenin Signaling in Cancers

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Abstract

Excessive signaling from the Wnt pathway is associated with numerous human cancers. Using a high throughput screen designed to detect inhibitors of Wnt/β-catenin signaling, we identified a series of acyl hydrazones that act downstream of the β-catenin destruction complex to inhibit both Wnt-induced and cancer-associated constitutive Wnt signaling via destabilization of β-catenin. We found that these acyl hydrazones bind iron in vitro and in intact cells and that chelating activity is required to abrogate Wnt signaling and block the growth of colorectal cancer cell lines with constitutive Wnt signaling. In addition, we found that multiple iron chelators, desferrioxamine, deferasirox, and ciclopirox olamine similarly blocked Wnt signaling and cell growth. Moreover, in patients with AML administered ciclopirox olamine, we observed decreased expression of the Wnt target gene AXIN2 in leukemic cells. The novel class of acyl hydrazones would thus be prime candidates for further development as chemotherapeutic agents. Taken together, our results reveal a critical requirement for iron in Wnt signaling and they show that iron chelation serves as an effective mechanism to inhibit Wnt signaling in humans. Cancer Res; 71(24); 1–12. ©2011 AACR.

Introduction

Inappropriate activation of the canonical Wnt/β-catenin signaling pathway contributes to the development of a numerous human cancers (1–3). In colorectal cancers, loss-of-function mutations in the tumor suppressor, adenomatous polyposis coli (APC) are common. APC is an integral part of the destruction complex that controls cytoplasmic β-catenin levels by promoting ubiquitin-mediated degradation of β-catenin. Cancer-associated mutations in APC, thus lead to constitutively high levels of β-catenin and the concomitant expression of Wnt/β-catenin target genes that are important in cell growth, survival, and metastasis (1–3). In other cancers, constitutive Wnt signaling is achieved by a variety of means, such as increases in Wnt ligand or decreases in secreted inhibitors (2). Deregulation of the Wnt/β-catenin pathway has been reported in acute myeloid leukemias (AML) where expression of β-catenin is correlated with poor prognosis (4, 5). In addition, Wnt/β-catenin signaling has been shown to be required for the development of the highly proliferative leukemia stem cells (LSC), that are thought to maintain leukemias (6). The widespread deregulation of the Wnt pathway in diverse cancers makes it an attractive therapeutic target (7, 8) and while several chemical inhibitors of the Wnt pathway have been identified (7–11), there remains the need for effective small-molecule inhibitors appropriate for therapeutic development.

Cancer cells have an increased demand for iron to maintain robust cell proliferation, yet use of iron chelators for cancer treatment has only recently been rigorously considered (12–15). Here, we used a mammalian cell–based screen and identified a class of acyl hydrazones that block constitutive Wnt signaling and cell growth through their activity as iron chelating agents and thereby suggest that iron chelation–based therapies may be an effective means to target Wnt signaling in cancers.

Materials and Methods

Compounds

Compound 21H7 was purchased from Ryan Scientific, indirubin-3′-monoxide (I3M), desferrioxamine, and dimethylxoyl glycine (DMOG) from Sigma-Aldrich; deferasirox and ciclopirox olamine from ChemPacific Corporation; and M-110 (16), kindly provided by Jan Jongstra (Toronto Western Research Institute), as chemical inhibitors of the Wnt pathway.

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Institute, Toronto, ON, Canada), was synthesized by Sundia MediTech Company. OICR142 and OICR623 were synthesized by the OICR Medicinal Chemistry Group.

β-catenin-firely luciferase stabilization assay and high throughput screen

HEK293 cells, stably expressing a Flag-β-catenin-firely luciferase fusion protein were plated into 96-well dishes at 1,250 cell per well. The next day, cells were incubated 1 hour in Minimum Essential Media with 0.2% fetal calf serum, 1 hour with 1.2 μmol/L compounds, and then Wnt3A or control conditioned media, prepared as described (17, 18) was added for 16 hours. Luciferase activity was measured using Bright-Glo (Promega) 10 min after addition, using the CLIPR plate reader (Molecular Devices). Screens were conducted at the SMART Robotics Facility at the Samuel Lunenfeld Research Institute. Hits were identified by B-score analysis. For validation assays, Flag-β-catenin-firely luciferase was immunoprecipitated from cell lysates with anti-Flag antibody and collected using Protein G beads.

Transcriptional reporter, electrophoretic mobility shift assay, and cell growth assays

Cell lines, from American Type Culture Collection, were expanded, frozen, and thawed aliquots cultured for less than 6 months. Cells were routinely tested for Mycoplasma, and Wnt pathway status was monitored using TOPFLASH/FOPFLASH. Cells were transfected with CMV-βgal and either 5×HRE-luciferase (provided by Dr. Michael Ohh, University of Toronto, Toronto, ON, Canada), TOPFLASH or FOPFLASH using calcium phosphate (HEK293T) or Lipofectamine 2000 (SW480, DLD-1, SW620, and HCT116). HEK293T cells were incubated with compounds for 1 hour prior to overnight incubation with control or Wnt3A-conditioned medium. For all other cells, compounds were added 5 hours posttransfection, and cells were incubated for 24 hours in full serum containing media. Luciferase activity, normalized to β-galactosidase activity was measured as previously (17, 18). All results were statistically significant (P < 0.005) using the Student 2-tailed t test.

Clinical trial of oral ciclopirox olamine in patients with refractory or relapsed hematologic malignancies

Patients were treated with ciclopirox olamine at increasing concentrations from 5 to 80 mg/m² daily for 5 days, in a phase I clinical trial, following informed consent and REB approval by the Princess Margaret Hospital, Toronto, Canada (NCT00990587). Peripheral blood samples were obtained before, during, and after drug treatment. Mononuclear cells were separated by Ficoll-Hypaque (Sigma Chemical) density-gradient centrifugation and AML blasts isolated by magnetic bead separation.

Results

Identification of small-molecule inhibitors of Wnt signaling

The deleterious effects of constitutive Wnt signaling result from abnormally high levels of β-catenin protein (1). Thus, to identify small-molecule inhibitors that reverse β-catenin accumulation, we designed a homogeneous high throughput screening (HTS) assay using HEK293 cells stably expressing a fusion protein comprising firefly luciferase (FFluc) linked to β-catenin in which fusion protein levels were measured using a luciferase assay (Fig. 1A). Cells were treated with compounds...

Measurement of intracellular calcein-chelatable iron

Cells were loaded with 100 μmol/L ferric ammonium citrate (FAC) for 24 hours (22), washed twice with PBS containing 20 mmol/L HEPES, pH 7.3, loaded with 0.25 μmol/L calcein green acetoxyethyl ester (calcein-AM; Molecular Probes) in serum-free medium containing 20 mmol/L HEPES, pH 7.3 for 15 minutes at 37°C, and then were washed and plated at 50,000 cells/well in a 96-well plate (23). Intracellular fluorescence intensity of calcein (λex = 485 and λem = 520), a measure of the amount of labile iron (24), was determined as a function of time 1 minute before and 10 minutes after compound addition at 37°C using FLUOstar OPTIMA (BMG Labtech) microplate reader.

Microarray analysis and real-time PCR

SW480 cells were incubated with 10 μmol/L OICR623, 100 μmol/L desferioxamine or 50 μmol/L deferasirox or dimethyl sulfoxide (DMSO) as control for 6 hours. RNA was isolated using the PureLink Mini Kit (Invitrogen), and cDNA samples were hybridized to the GeneChip Human Gene 1.0 ST array (Affymetrix) and then scanned with the Affymetrix GeneChip Scanner 3000 at the Center for Applied Genomics. Raw data were prenormalized using RMA (robust multiarray average) algorithm, adjusted for batch effects and differentially expressed genes were identified using LIMMA (linear models for microarray data; ref. 25). Data are available at GEO (GSE32369). Real-time PCR was carried out using SYBR Green (Applied Biosystems) using validated primers (Supplementary Table S1). Gene expression was normalized to hypoxanthine phosphoribosyltransferase and relative quantitation was calculated using the ΔΔCt method. Results shown are statistically significant (P < 0.005) using the Student 2-tailed t test.
for 1 hour and then cultured with or without Wnt3A for 16 hours before determining β-catenin-FFluc levels (Fig. 1B).

Screening of 10,400 compounds from the Maybridge Collection (Fig. 1C) yielded 10 positive hits that were subsequently screened in a secondary assay for inhibition of the Wnt TOPFLASH reporter. One compound, 21H7, was identified as an inhibitor of the Wnt pathway that abrogated Wnt3A-dependent stabilization of β-catenin-FFluc and activation of TOPFLASH, but had no effect on FOPFLASH, a Wnt-insensitive mutant variant (Fig. 1D–F).
M-110, a structural variant of 21H7, also inhibited stabilization of β-catenin-FFLuc and Wnt/β-catenin–dependent activation of TOPFLASH with no effect on FOPFLASH (Fig. 1G–1) as did compound OICR623, which contains the more polar pyrimidine group and a 2-fluoro-4-chloro phenolic moiety (Fig. 1G and J). In contrast, OICR142, which lacks the orthohydroxy group rendered the compound inactive (Fig. 1G and J). Thus, our screen identified a class of acyl hydrazones that inhibit Wnt-induced transcriptional responses.

**Inhibition of Wnt/β-catenin signaling and cell proliferation in colorectal cancer cells**

Most colorectal cancer cell lines display constitutive Wnt signaling because of mutations in APC. M-110 treatment of APC-mutant DLD-1 cells (26), preferentially attenuated constitutive TOPFLASH activity with IC50 values in the range of 0.5 to 1.7 μmol/L (Fig. 2E). Real-time PCR analysis also revealed that M-110 inhibited expression of the endogenous Wnt targets, AXIN2 and SP5 (Fig. 2B). In other APC mutant colorectal cancer cell lines, including SW480, SW620, and Colo205, M-110 similarly reduced TOPFLASH activity (Supplementary Fig. S1A) and expression of Wnt target genes (Fig. 2C). OICR623 and 21H7 also inhibited Wnt activity, whereas compound OICR142, which lacks the orthohydroxy group did not (Fig. 2D; see also Fig. 5). The Wnt/β-catenin pathway regulates cell proliferation in colorectal cancer cells. Consistent with this, M-110, OICR623, and 21H7 inhibited the growth of DLD-1 and SW480 cells, with IC50 values in the range of 0.5 to 1.7 μmol/L (Fig. 2E and Supplementary Fig. S1B). Thus, this family of small molecules inhibits constitutive Wnt signaling and blocks the growth of colorectal cancer cell lines.

**Acyl hydrazones block Wnt signaling by promoting β-catenin degradation downstream of the destruction complex**

There are 2 cellular pools of β-catenin, plasma membrane E-cadherin-associated and Wnt-modulated cytoplasmic pools (27). In mouse L cells, where most of the β-catenin is cytoplasmic, M-110, OICR623, and 21H7 blocked Wnt-induced stabilization of total β-catenin and the active, nonphosphorylated (S37/T41) β-catenin, which mediates Wnt signaling (Fig. 3A). In SW480 cells, M-110 also decreased the levels of free cytoplasmic β-catenin as determined by E-cadherin pull down assays, which collects β-catenin that is not prebound to E-cadherin, and reduced the pool of the active, nonphosphorylated β-catenin (Fig. 3B). Total β-catenin levels, which include the E-cadherin-bound pool were not significantly affected (Fig. 3B), nor were β-catenin mRNA levels (Supplementary Fig. S1C). Degradation of β-catenin occurs in a destruction complex comprising Axin and APC, within which CK1 and GSK3β-mediated phosphorylation of β-catenin marks it for ubiquitin-mediated degradation (1). DLD-1 and SW480 cells have a mutant APC, suggesting that the compounds act downstream of the destruction complex. Consistent with this, M-110 also blocked Wnt signaling when other destruction complex components were disrupted, including abrogation of AXIN1/2 expression using siRNAs or inhibition of GSK3β activity using LiCl or I3M (Fig. 3C and D). The ability of M-110 to decrease the levels of active β-catenin was mitigated in the presence of MG132, a proteasome inhibitor, but not by chloroquine, a lysosomal inhibitor (Fig. 3E), indicating ubiquitin-mediated degradation of β-catenin. M-110, OICR623, and 21H7 also inhibited activation of the TOPFLASH reporter in HCT116 cells, which harbor a constitutively-active version of β-catenin and in HEK293T cells overexpressing versions of β-catenin lacking the phosphorylation sites (Fig. 3F and G; Supplementary Fig. S1D). Altogether, these data show that the compounds act downstream of the destruction complex to destabilize active β-catenin.

**Gene expression profiling reveals compounds induce an iron chelation signature**

To gain insights into molecular mechanisms, we used microarrays to examine changes in gene expression in SW480 cells treated for 6 hours with OICR623. Analysis of results from replicate runs revealed that OICR623 significantly (P < 0.05) upregulated the expression of 31 genes by 1.5-fold or greater (Fig. 4A and B) including several genes induced by iron chelators (28), such as DDIT4, VEGFA, and NDRG1. This gene profile was next compared with that obtained using 2 well-described iron chelators, desferoxamine and deferasirox in cells treated in parallel. At 6 hours of treatment, 64 and 72 genes were upregulated by greater than 1.5-fold (P < 0.05) by desferoxamine and deferasirox, respectively, of which 64 overlapped (Fig. 4A). Remarkably, all 31 genes upregulated by OICR623 were found within the desferoxamine/deferasirox signature. Similar results were observed in the case of significantly (1.5-fold, P < 0.05) downregulated genes, although this overlap set comprised only 4 genes (Fig. 4B). Of note, at this early time point, inhibition of Wnt target gene expression was not yet manifested. These results thus show that the OICR623-induced gene signature is entirely encompassed by that of the iron chelators, desferoxamine and deferasirox.

Iron depletion inhibits the activity of iron-dependent enzymes, including prolyl hydroxylases (PHD), which promote stabilization of the hypoxia inducible transcription factor, HIF1α (29, 30). Treatment of SW480 and DLD-1 cells with OICR623, M-110, and 21H7, stabilized HIF1α and activated a HIF1α transcriptional reporter, 5xHRE-luciferase (Fig. 4C and D). Furthermore, genes induced by OICR623, desferoxamine, and deferasirox in the microarray analysis included the known HIF1α targets (29), VEGFA, ADM, EGLN3, and NDRG1 (Fig. 4A). Verification of microarray results by real-time PCR showed that all 3 acyl hydrazones, OICR623, M-110, and 21H7, as well as the iron chelators, desferoxamine and deferasirox, rapidly induced expression of the iron-dependent and/or HIF1α target genes, NDRG1, DDIT4, VEGFA, and GLUT1 in SW480 cells, used for the microarray study and in DLD-1 and SW620 cells (Fig. 4E and Supplementary Fig. S2A and S2B).

Under conditions of iron depletion, IRPs recognize and bind to IREs in mRNAs such as Ferritin (Fh), to stall translation or to TIR mRNA to enhance mRNA stability and translation (14). As expected for iron chelators, treatment of SW480 cells with M-110 or deferasirox resulted in enhanced IRE–IRP binding activity, in an RNA-binding gel shift assay (Fig. 4F).
A concomitant reduction in Ferritin and increase in TfRI was observed by immunoblotting for M-110 (Fig. 4F) and OICR623, 21H7, and deferasirox (Supplementary Fig. S3A). Thus, given the gene expression profiles and characteristic cell-based responses of iron depletion, our data strongly suggest that OICR623 and related compounds act as iron chelators.
Figure 3. Compounds block Wnt signaling downstream of the destruction complex. A, compounds inhibit Wnt3A-induced stabilization of β-catenin in mouse L cells. Total lysates from cells incubated overnight with DMSO (−) or compounds and then stimulated with Wnt3A for 3 hours were subjected to immunoblotting (IB) to detect total β-catenin, active β-catenin, or actin. B, M-110 decreases the level of active β-catenin in colorectal cancer cells. SW480 cells were incubated overnight with 10 μmol/L M-110 and the levels of cytoplasmic (free) β-catenin was determined by GST-E-cadherin (E-Cad) pull down followed by anti-β-catenin IB. Levels of total and active β-catenin in total cell lysates are shown (input). Full-length blots of these cropped images are presented in Supplementary Fig. S4A and B. C and D, inhibition of TOPFLASH downstream of destruction complex components by M-110. HEK293T cells were transfected with TOPFLASH (C and D), siControl (siCtl), or siAXIN1/2 (C) and treated with M-110 with or without 25 mmol/L LiCl or 3M (D). E, the proteasome inhibitor MG132, but not chloroquine, blocks the M-110–mediated decrease in the level of active β-catenin in SW480 cells as determined by E-cadherin pull down assays and immunoblotting as in B. F and G, compounds inhibit TOPFLASH activity in β-catenin mutant, HCT116 cells, and in HEK293T cells overexpressing murine wild-type (WT) or Ser33/Ser37/Thr41/Ser45 to Ala mutant (mut) β-catenin-6xmyc.
Compounds bind iron in vitro and in cultured cells and this activity mediates inhibition of Wnt signaling and cell growth

The acyl hydrazone group is a characteristic metal chelating motif, thus, to test whether OICR623 can bind iron in vitro, we mixed Fe(NO₃)₃·C₉H₂O with OICR623 and analyzed the mixture by liquid chromatography/mass spectroscopy. An iron complex of OICR623 in a 1:2 ratio (iron:OICR623) with a molecular ion mass [M+1] of 698 was observed. On the basis of the reported crystal and molecular structure of Compound 311 and its iron (III) complex (31), we speculate that OICR623 is a tridentate chelator (Fig. 5A).

We next assessed the ability of the compounds to bind intracellular iron in colorectal cell lines by calcein assay. Upon cell entry, calcein-AM is cleaved into calcein, whose fluorescence is quenched upon chelation of labile iron (23). Calcein-AM–loaded SW480 cells were briefly incubated with M-110, OICR623, and 21H7. Similar to the iron chelator, deferasirox, both M-110 and OICR623 increased calcein fluorescence (Fig. 5B), whereas 21H7 yielded a more modest increase that was similar to that observed for desferrioxamine, an iron chelator with poor cell-permeability properties (32). We next tested the effect of excess iron on compound activity in cells. Analysis of TOPFLASH activity in SW480 cells treated with compounds...
Figure 5. Iron chelators block Wnt signaling and colorectal cancer cell growth. A, a modeled structure of the iron–OICR623 complex. B, compounds bind intracellular iron. SW480 cells were loaded with calcein-AM, and the fluorescence intensity change, after and before the addition of 5 μmol/L M-110, OICR623, 21H7, ciclopirox olamine (CPX), 100 μmol/L desferrioxamine (DFO), or 50 μmol/L deferasirox (DFX) is plotted. C, excess iron blocks compound-mediated inhibition of constitutive Wnt signaling. SW480 cells were transfected with TOPFLASH and treated with 5 μmol/L M-110, OICR623, or 21H7 in the absence or presence of 10 μmol/L of the indicated salts. Promoter activity is shown as the mean of 3 replicates ± SD. D, iron chelators decrease the expression of Wnt target genes. SW480 and DLD-1 cells were treated with 5 μmol/L M-110, OICR623, 21H7, 100 μmol/L desferrioxamine, or 100 μmol/L deferasirox overnight, and relative gene expression is plotted as the average of 3 PCR replicates ± the range. E, IC50 values for growth inhibition.
Iron Chelators Inhibit Wnt/β-Catenin Signaling

(5 μmol/L) coinubated with a 2-fold molar excess (10 μmol/L) of various divalent cations revealed that addition of FeSO₄ or FAC completely neutralized compound activity, whereas Mg²⁺, Mn²⁺, or Zn²⁺ salts had no effect (Fig. 5C). Moreover, in cell growth assays, addition of FeSO₄ at an equimolar ratio of iron to compound completely abrogated the inhibitory activity of the compounds in SW480 and DLD-1 cells (Supplementary Fig. S3B). Treatment of DLD-1 or SW480 cells with the structurally unrelated iron chelators, desferoxamine or deferasirox, similarly inhibited expression of Wnt target genes (Fig. 5D) and inhibited cell growth with ICₕ₀ values of 2.9 to 3.0 μmol/L (Fig. 5E), roughly 5- to 10-fold higher than the acyl hydrazones, but similar to previously reported ICₕ₀ values for these compounds (Fig. 5D and E; ref. 33). Stabilization of HIF1α and activation of the HIF1α reporter, 5xHRE-luciferase by DMOG, an inhibitor of 2-oxoglutarate–dependent enzymes including PHDs, to levels (0.1 mmol/L) comparable as that achieved by acyl hydrazones or deferasirox, had no effect on TOPFLASH activity, though at 10-fold higher DMOG doses, some inhibition was observed suggesting that HIF1α is unlikely to be the primary mechanism whereby iron chelators inhibit Wnt signaling (Supplementary Fig. S3C).

Altogether, our results showed that acyl hydrazones are potent iron chelators in cells and that depletion of intracellular iron abrogates cell proliferation and Wnt signaling by promoting β-catenin degradation downstream of the destruction complex.

Oral administration of the iron chelator, ciclopirox olamine, reduces Wnt target gene expression in the leukemic cells of patients with AML.

Ciclopirox olamine functions as an anticancer agent in both leukemic cell lines and primary AML patient samples via its intracellular iron chelation activity (34). We confirmed in SW480 cells that ciclopirox olamine also binds intracellular iron (Fig. 5B), induces the HIF1α responsive reporter, and inhibits TOPFLASH in an iron-dependent manner (Fig. 6A and B).

Mutations in Wnt pathway components have not been reported in AML, but expression of β-catenin is correlated with poor prognosis (5). Thus, to determine if iron chelators modulate Wnt signaling in vivo, we assessed the effect of systemic administration of ciclopirox olamine to patients with refractory hematologic malignancies. Expression of AXIN2 in patients with AML taking orally administered ciclopirox olamine as part of an on-going phase I dose escalation trial was determined in isolated leukemic blasts. Analysis of samples from 9 patients with relapsed AML receiving ciclopirox olamine doses between 5 and 80 mg/m² daily for 5 days revealed that 7 of 9 patients displayed a decrease in AXIN2 levels. For 4 patients (A–D), marked decreases were detected within 1 day of ciclopirox olamine administration (day 2) that became more pronounced from days 3 to 5 (Fig. 6C and D). In 3 patients (E–G), decreases in AXIN2 levels were transient, whereas in 2 patients (H and I), there was no reduction (Fig. 6C and D). Excluding the 2 patients (H and I) who showed no changes, the average reduction in AXIN2 levels from days 2 to 5 ranged from 60% to 74% (median) or 40% to 71% (mean; Fig. 6D). Of note, hematopoietic cells isolated from 2 ciclopirox olamine–administered patients (J–K) with myeloma or MDS, but lacking circulating malignant cells showed no reduction in AXIN2 (Fig. 6E), suggesting that leukemic cells may be more susceptible to iron chelation than non-malignant hematopoietic cells. Although only a small, short-term study, these results show that administration of iron chelators can decrease the expression of Wnt target genes in patients with hematologic malignancies and further indicate that iron chelators might be of therapeutic value for Wnt pathway driven tumors.

Discussion

Stabilization of β-catenin is an invariant feature of cancers with excessive Wnt signaling (1, 2). Our screen, designed to identify small molecules that destabilize β-catenin, identified a compound series that inhibits β-catenin stabilization and blocks Wnt-induced transcription. The compounds were shown to function downstream of the β-catenin destruction complex and were effective in blocking Wnt signaling and growth in APC and β-catenin mutant colorectal cancer cells with constitutive Wnt signaling. This is consistent with studies showing that downregulation of β-catenin inhibits proliferation of colon cancer cell lines grown in vitro or as xenografts (35, 36). Given the frequent occurrence of excessive Wnt signaling through a range of mechanisms, these inhibitors which act downstream of the destruction complex to control β-catenin levels could have widespread therapeutic utility in a diverse range of Wnt-driven cancers.

Investigation of the mechanism whereby the acyl hydrazones block the Wnt pathway showed that the compounds bind iron and that this iron chelation activity is essential for compound activity. Iron loading of colorectal cancer cells has been reported to promote Wnt signaling and cell proliferation (37) consistent with our observations that iron depletion can attenuate the pathway. Our conclusions were further supported by the observation that a series of structurally unrelated iron chelators, including desferoxamine, deferasirox, and ciclopirox olamine also inhibit Wnt signaling and that patients with AML taking ciclopirox olamine, show a marked reduction in the expression of the Wnt target gene, AXIN2. Our work along with a recent study (38) show that iron depletion attenuates Wnt signaling, but which of the plethora of iron-dependent proteins, are most relevant for inhibiting Wnt signaling remains to be determined.

Iron is essential for cell growth and metabolism and cancer cells in particular, have an increased demand for iron to maintain cell proliferation having acquired diverse alterations to ensure increased iron accumulation (12–15). In breast cancer, decreases of the intracellular iron exporter, ferroportin, is associated with reduced metastasis-free survival, whereas ferroportin overexpression decreases breast cancer cell growth in an orthotopic mouse model (39). Expression levels of ferroportin are correlated with levels of intracellular iron, indicating that altering iron levels can modulate tumor growth in vivo. It is thus tempting to speculate that an increase in iron accumulation promotes widespread increases in the proproliferative Wnt signaling pathway to promote tumorigenesis.
Figure 6. Ciclopirox olamine inhibits Wnt signaling in cultured cells and in vivo. A, ciclopirox olamine inhibits Wnt signaling and activates a HIF1α-dependent reporter. SW480 cells transfected with TOPFLASH or 5xHRE-luciferase reporters were treated overnight with ciclopirox olamine or M-110. B, excess iron blocks ciclopirox olamine–mediated inhibition of constitutive Wnt signaling in SW480 cells. Cells were transfected with TOPFLASH and treated with 5 μmol/L ciclopirox olamine in the absence or presence of 10 μmol/L of the indicated salts. C–E, ciclopirox olamine administration decreases AXIN2 expression in leukemic blasts isolated from patients with AML. The expression of AXIN2 on each day of treatment for individual patients was measured by real-time PCR and is calculated relative to levels prior to ciclopirox olamine (CPX) administration. Data for individual patients are plotted as a bar graph (C and E) or scatter plot (D). The horizontal lines indicate the median (dashed line) or mean (solid line) for patients A to G (filled symbols). Results for patients H and I are indicated by open symbols. Probabilities were determined using Student paired, 1-tailed t test: ***, P = 0.008; **, P = 0.05; *, P = 0.02. E, AXIN2 expression in 2 patients lacking circulating malignant cells.
Testing the utility of iron chelators, such as the acyl hydrazones identified herein, for cancer therapeutics is an important area for future investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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