A small-molecule inhibitor of Tcf/β-catenin signaling down-regulates PPARγ and PPARδ activities

Shlomo Handeli and Julian A. Simon

Clinical Research and Human Biology Divisions, Fred Hutchinson Cancer Research Center, Seattle, Washington

Abstract

Activation of the Wnt/β-catenin signaling pathway occurs in several types of cancers and thus it is an attractive target for anticancer drug development. To identify compounds that inhibit this pathway, we screened a chemical library using a cell-based β-catenin/Tcf–responsive reporter. We identified FH535, a compound that suppresses both Wnt/β-catenin and peroxisome proliferator–activated receptor (PPAR) signaling. FH535 antagonizes both PPARγ and PPARδ ligand–dependent activation and shows structural similarity to GW9662, a known PPARγ antagonist. The effect of FH535 on β-catenin/Tcf activity is reduced in cells carrying a deletion of the PPARγ gene, as well as by the PPARγ agonist lyso phosphatidic acid. Mechanistically, FH535 inhibits recruitment of the coactivators β-catenin and GRIP1 but not the corepressors NCoR and SMRT. Its repression of β-catenin recruitment, in comparison with GW9662, is linked to FH535’s unique capability to inhibit the Wnt/β-catenin signaling pathway. The antiproliferation effect of the compound observed on some transformed colon lung and liver cell lines is suggestive of its potential therapeutic value in the treatment of cancer. [Mol Cancer Ther 2008;7(3):521–9]

Introduction

The Wnt signaling pathway is important in normal development and in cancer (reviewed in refs. 1, 2). This signaling pathway is regulated by Wnt ligands, the APC–Axin complex and β-catenin. In development, signaling that stabilizes β-catenin is mainly mediated by the Wnt ligands. However, in human cancers such as hepatocellular and colorectal tumors, β-catenin stabilization is often the result of mutation in the tumor suppressor genes Axin and APC, or in the proto-oncogene β-catenin. β-Catenin stabilization leads to its accumulation and subsequent translocation to the nucleus, where it forms complexes with transcription factors of the Tcf/Lef family. The transcriptionally active β-catenin/Tcf complex exerts its cell proliferation and tumorigenic effects by promoting the transcription of growth controlling genes like c-Myc and cyclin D1. Wnt/β-catenin signaling pathway plays a central role in regulating the balance between stem cell growth and differentiation. Thus, the degree of Wnt signaling activation is an important modulator of the stem cells cancerous potential. In colorectal cancer, the necessary initiating APC or β-catenin mutations are not sufficient for maximum Wnt activation. Other intrinsic somatic mutations such as the oncogene Ras, as well as extrinsic factors like prostaglandins, are likely to play a rate-limiting role in Wnt signaling activation and contribute to the cancer development from the initial stem cell transformation to the metastasis stage (3). Increasing evidence shows that suppression of Wnt signaling can be achieved by targeting pathways that cross-talk with the Wnt signaling pathway. For example, both cyclooxygenase-2 and integrin-linked kinase small-molecule inhibitors attenuate β-catenin/Tcf–dependent transcription in colorectal cancer cells harboring mutated Wnt signaling (reviewed in ref. 4).

Peroxisome proliferator–activated receptors (PPAR) are members of the nuclear hormone receptor superfamily. The ligand-activated transcription by these receptors requires heterodimerization with retinoid X receptor, interaction with different coactivators as well as binding to PPAR–response elements (PPRE). There are three PPAR isotypes (α, γ, and δ), differing in their tissue distribution, physiologic functions, and ligand specificity. Fatty acids and their derivatives are natural PPAR agonists. Synthetic agonists have been reported for all PPAR isoforms, whereas antagonists have been identified only for PPARγ.

The physical interaction between β-catenin and PPARγ suggests a possible mechanism of cross-talk between the Wnt and the PPAR signaling pathways. On the transcriptional level, β-catenin enhances PPARγ activity whereas PPARδ is a target for β-catenin/Tcf regulation (5, 6). A clear interaction between these pathways is observed during adipogenesis. Adipogenic differentiation is regulated by reciprocal inhibitory signals between PPARγ and Wnt ligands; Wnt1 and Wnt10 promote the growth of preadipocytes whereas PPARγ agonists repress the Wnt/β-catenin signaling and advance their differentiation (7).

The contribution of PPARγ and PPARδ to Wnt/β-catenin–induced carcinogenesis remains unclear, as there are genetic and pharmacologic data suggesting that PPARs either promote or inhibit colon cancer (reviewed in refs. 8, 9). For example, treatment of Apcmin mice, which are predisposed to intestinal polyposis with the PPARγ agonist troglitazone, enhanced colon polyp development (10, 11), whereas treatment with pioglitazone, another PPARγ
agonist, suppressed polyp formation (12). Similarly, conflicting results were observed using the PPARδ agonists GW501516 and GW0742 (13, 14).

Another class of compounds that modulate both Wnt/β-catenin and PPAR activity is the nonsteroidal anti-inflammatory drugs (NSAID). The pharmacologic action of NSAIDs has been attributed to their inhibition of cyclooxygenase activity. However, a large number of studies have suggested that the anticarcinogenic efficacy of NSAIDs is independent of their cyclooxygenase inhibition. Several NSAIDs, including indomethacin, weakly interact with PPARγ and stimulate its activity (15, 16). Indomethacin and other NSAIDs were also reported to suppress PPARγ activity (6, 17). The role of PPAR regulation to the overall ability of NSAIDs to repress the Wnt/β-catenin pathway is not fully understood.

Here, we report the identification of a low molecular weight compound (FH535) that suppresses β-catenin/Tcf–mediated transcription. FH535 behaves as a dual PPARγ agonist and PPARδ antagonist that is able to inhibit GRIP1 and β-catenin recruitment. Comparisons between FH535 and the PPARγ antagonist GW9662, which allows β-catenin recruitment, suggest that inhibition of the Wnt/β-catenin pathway may require modulation of the interaction between PPARs and β-catenin.

Materials and Methods

Reagents

The chemical library used for the screen is part of the DIVERSet collection from ChemBridge. ChemBridge was also the source for FH535 and its analogues. L165041 was purchased from Calbiochem-EMD Biosciences. All other PPAR ligands and nitric oxide–donating aspirin were obtained from Cayman Chemical.

High-throughput Library Screen

Three copies of the optimized or mutated Tcf-binding element from TOPFLASH or FOPFLASH (18) driving a secreted alkaline phosphatase reporter gene were cloned into pCEP4 plasmid (Invitrogen), replacing the cytomegalovirus promoter. The plasmids were transfected into HepG2 cells, and hygromycin-resistant clones were pooled. Library screening was done at 20 μmol/L concentration in HepG2 serum-free media (19). Hits were tested in the HCT116 cell line for inhibition of TOPFLASH luciferase activity but not for inhibition of a reporter activity controlled from β-actin promoter (details of the reporter construct used in the screen and the stepwise characterization scheme for prioritization of hits is available in the Supplemental Materials).¹

Transfection and Reporter Gene Assays

TransIT-LT1 transfection reagent (Mirus) was used according to the manufacturer’s instructions. After transfection of reporter constructs (18–24 h), cells were trypsi-

¹ Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
medium, the FBS was substituted with 10 µg/mL of insulin, 5.5 µg/mL of transferrin, 35 nmol/L of sodium selenite, and 10 ng/mL of human epidermal growth factor (Life Technologies-Invitrogen). Cell viability was determined by the modified \(^3\)H-thymidine incorporation assay (24).

Briefly, cells were plated in 96-well microplates for 24 h and treated in triplicate with various concentrations of the test compound. After 48 h of compound exposure, the cells were incubated for an additional 48 h in compound-free medium. The cells were then incubated in medium containing \(^3\)H-thymidine for 24 h, washed and mixed with the scintillant in the 96-well plate. Individual wells were counted with a 96-well scintillation counter (TopCount, Packard Instruments) and the LC\(_{50}\) was calculated.

**Reverse Transcription-PCR**

Total RNAs were extracted from HCT116 cells by the RNeasy kit and cDNA was synthesized with Moloney murine leukemia virus reverse transcriptase (Invitrogen). The primer sequences for amplification were: GAPDH-F 5'-ATGATCTTGAGCTGTTG-3', GAPDH-R 5'-CTCAGACCCATGGGGAA-3', TCF-4F 5'-TTCAAGACCGCGCCCG-3', TCF-4R 5'-TTAATTTCTGATACCTGATAAAGAGGCG-3'. PCR amplification was done using Taq Polymerase (Fisher) for 20 cycles at 50 °C (GAPDH) and 57 °C (TCF-4) annealing temperatures.

**Results**

**FH535 Antagonizes β-Catenin/Tcf-Mediated Transcription**

To identify inhibitors of the Wnt/β-catenin pathway, we modified the existing TOPFLASH reporter system (18) to suit a high-throughput screen. Episomal reporter constructs containing three copies of either an optimized or mutated Tcf-binding element were stably transfected into HepG2 hepatocellular carcinoma cells expressing high levels of nuclear β-catenin. Robust reporter activity was detected in clones containing the optimized element, which was 10-fold higher than activity in clones expressing the mutated element (data not shown).

Using this cell-based reporter system, we screened a diverse library of 11,600 low molecular weight compounds for the inhibition of β-catenin/Tcf-mediated transcription. Initially, two structurally related compounds (FH525 and FH614; Fig. 1A) were found to be potent inhibitors. Thirteen additional analogues were tested and another two active compounds were identified (FH535 and FH610; Fig. 1A). The nitro group located at the para or meta position in all four compounds is critical for function. Substitution of this nitro group with any of six other functional groups (OH, ethyl, ethylcarbonate, benzamide, O-benzyl, para-tolylsulfonamide) abolishes activity. In four cell lines harboring deregulation of the Wnt/β-catenin pathway, FH535 was found to be the best β-catenin/Tcf inhibitor (Fig. 1C). FH535 and two additional compounds (FH610 and FH614) were more active than nitric oxide-donating aspirin, a NSAID that has been shown to disrupt the formation of the β-catenin/Tcf complex at the drug concentration used here (ref. 25; Fig. 1C).

**FH535 Antagonizes both PPARγ and PPARδ Activity**

The newly identified β-catenin/Tcf inhibitors also share structural similarity to the known PPARγ antagonists GW9662 and T0070907 (refs. 26, 27; Fig. 1B). All of the compounds contain the nitro group, differing mainly in the

---

*Figure 1.* Inhibitors of β-catenin/Tcf–mediated transcription are structurally similar to PPARγ antagonists. A, chemical structures of β-catenin/Tcf–mediated transcription inhibitors. B, chemical structures of the PPARγ antagonists GW9662 and T0070907. C, inhibition of β-catenin/Tcf–dependent luciferase reporter activity in cell lines harboring stable β-catenin. Treatment with vehicle (DMSO) only was used to calculate the 100% activity for each cell line. FH535 is the most effective Wnt/β-catenin inhibitor.
central amide or sulfonamide groups as well as their orientation. Because PPARγ and PPARδ have been implicated in Wnt/β-catenin pathway regulation (reviewed in ref. 8), we tested the ability of our most active compound, FH535, to antagonize these PPARs.

First, we investigated the transactivation of a reporter construct containing three copies of the acyl-CoA oxidase PPAR-response element (20). In HCT116, this reporter is active and is sensitive to the addition of PPARγ and PPARδ agonists (Fig. 2). FH535 inhibits the reporter-dependent activity driven from natural PPAR ligands found in the cellular environment (Fig. 2A) as well as from the added ligands (Fig. 2B and C). A more direct assay for PPAR activity uses PPAR fused to the gal4 DNA-binding domain. Treatment with PPARγ or PPARδ agonists causes the activation of the UAS–thymidine kinase reporter (see Materials and Methods for details) in cells cotransfected with the relevant PPAR-gal4 chimera (Fig. 3A and B). This PPAR agonist–dependent transactivation is inhibited when FH535 is present.

We next evaluated the role of PPARδ in the inhibition of β-catenin/Tcf–mediated transcription by FH535. PPARδ-null HCT116 cells are more resistant to FH535 treatment than their matched paired cells expressing the wild-type PPARδ protein (Fig. 3C). However, deletion of PPARδ is not sufficient to fully counteract FH535 inhibition, suggesting that PPARδ is not the only target by which FH535 inhibits the Wnt/β-catenin pathway. Parallel to these findings, GW9662 is unable to inhibit β-catenin/Tcf–signaling regardless of PPARα cellular levels (Fig. 3C).

FH535 Activity Does Not Require PPARγ Cys285 and PPARδ Cys248

The PPARγ antagonists GW9662 and T0070907 irreversibly modify Cys285 in the PPARγ ligand–binding site via a nucleophilic aromatic substitution of chlorine (26, 27). The same cysteine residue is essential for the activity and covalent binding of some PPARγ agonists such as 15d-PGJ2 but not for rosiglitazone (28). Among our four active compounds, only FH614 has a chemical structure capable of cysteine residue arylation (Fig. 1C). This strongly suggests that the antagonistic activity of our compounds does not require the modification of a cysteine. However, because PPARγ Cys285 and its equivalent Cys248 in PPARδ may be important for a noncovalent interaction with the compounds, we mutated this cysteine to alanine and found that these changes had no significant bearing on FH535 antagonism (Fig. 3A and B). Thus, FH535 activity does not require the same PPARγ binding residues that GW9662 uses. The use of different PPAR residues for ligand binding was also observed for the PPARγ agonists, rosiglitazone and lysophosphatidic acid (29).

Both Serum and Lysophosphatidic Acid Reduce FH535 Inhibition of the Wnt/β-Catenin Pathway

We noticed that inhibition of PPRE-dependent activity in defined medium requires lower FH535 concentrations than in serum-containing medium (Fig. 2B and C). Because a variety of fatty acids and their metabolites are secreted into plasma and are naturally present in the serum, it is likely

Figure 2. FH535 inhibits PPARγ and PPARδ transactivation in HCT116 cells. A, FH535 inhibits PPRE luciferase reporter activity. Cells were assayed in 10% FBS. B, FH535 inhibition of the PPRE reporter is suppressed by the PPARγ agonist 15d-PGJ2. Cells were assayed in serum-free medium. C, FH535 inhibition of the PPRE reporter is suppressed by the PPARδ agonist L165041. Cells were assayed in 10% FBS.
that serum contains PPAR agonists capable of suppressing the effects of FH535. The opposing relationship between FH535 and PPAR agonists with regard to PPAR transactivation (Fig. 3A and B), raises the possibility that PPAR agonists can also counteract FH535 inhibition of the Wnt/β-catenin pathway. First, we tested the effect of serum on this FH535 activity. FH535 is five times more active in defined medium than medium containing serum (Fig. 4A). Adding albumin to the defined medium reduced FH535 activity only 2-fold (data not shown). Next, 10 natural and synthetic PPAR ligands representing a broad spectrum of binding affinities were tested. Of those examined, lysophosphatidic acid was the only ligand found to reduce the inhibition activity of FH535 on the β-catenin/Tcf reporter (Fig. 4B). Lysophosphatidic acid is a pleiotropic growth factor–like lipid that mediates its effects through the activation of G protein–coupled receptors LPA1-4 and PPARγ (30). Thus, lysophosphatidic acid could either directly interfere with FH535 inhibition of PPARγ or the activation of lysophosphatidic acid receptors could indirectly suppress the activity of FH535.

**FH535 Inhibits β-Catenin and GRIP1 Recruitment to PPARγ and PPARδ**

To investigate the mechanism in which FH535 suppresses β-catenin/Tcf and PPAR-dependent transactivation, we focused on β-catenin and the coactivator GRIP1 because...
they are activators of both pathways (5, 31, 32). PPARs recruitment of these factors and the corepressors NCoR and SMRT was studied using a mammalian two-hybrid assay, in which activation of a gal4-dependent reporter is regulated by the interaction between VP16-PPARs with the gal4-transcription factor chimera. FH535 inhibits GRIP1 but not the corepressors recruitment to PPARs (Fig. 5A). A similar pattern of repressing coactivator recruitment, whereas allowing corepressor binding, was observed for GW9662 (26). However, the recruitment of β-catenin to PPARγ is inhibited by FH535 but not by GW9662 (Fig. 5B).

In this regard, GW9662 behaves like the vitamin D receptor antagonist, ZK159222, that is unable to prevent the recruitment of β-catenin to vitamin D receptors, whereas maintaining the ability to inhibit the recruitment of other coactivators (33).

Because PPAR ligand regulates the transcription of only a subset of genes, we looked for genes targeted by FH535. We focused on genes most important for Wnt/β-catenin signaling. β-Catenin levels were unaffected by FH535 (data not shown), whereas TCF4 transcription was suppressed in FH535-treated HCT116 cells (Fig. 5C).

**Figure 5.** FH535 inhibits recruitment of the coactivators GRIP1 and β-catenin to PPARα and PPARγ. A, FH535 inhibits PPARα interaction with GRIP1 but not with NCoR or SMRT. HCT116 cells were transfected with a gal4-dependent luciferase reporter, VP16-PPARα, and gal4-factor or gal4. The effects of FH535 on gal4-factor and gal4 were measured and the ratio of gal4-factor/gal4 was calculated. B, FH535 but not GW9662 inhibits the physical interaction between PPARγ and β-catenin. HCT116 cells were transfected with gal4-dependent luciferase reporter, gal4/β-catenin and either VP16-PPARγ or VP16. The effects of PPAR antagonists on gal4/β-catenin transactivation were measured in the presence or absence of 1 μmol/L of rosiglitazone. C, FH535 inhibits tcf4 transcription. The expression levels of GAPDH and Tcf4 in HCT116 cells treated with 15 μmol/L of FH535 or vehicle (DMSO) were determined by reverse transcription-PCR. Data from two independent compound treatments is shown. GAPDH served as an internal control.
Table 1. LC50 values of FH535 in different cell lines grown in 10% FBS

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell type</th>
<th>Wnt/β-catenin signaling status*</th>
<th>LC50 (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT116</td>
<td>Colon adenocarcinoma</td>
<td>Elevated, mutant β-catenin</td>
<td>&gt;30</td>
</tr>
<tr>
<td>SW48</td>
<td>Colon adenocarcinoma</td>
<td>Elevated, mutant β-catenin</td>
<td>&gt;30</td>
</tr>
<tr>
<td>RKO</td>
<td>Colon carcinoma</td>
<td>Active, mutant CDX2</td>
<td>16</td>
</tr>
<tr>
<td>LoVo</td>
<td>Colon carcinoma</td>
<td>Elevated, mutant APC</td>
<td>12</td>
</tr>
<tr>
<td>COLO205</td>
<td>Colon carcinoma</td>
<td>Elevated, mutant β-catenin and APC</td>
<td>12</td>
</tr>
<tr>
<td>IEC6</td>
<td>Immortal small intestine</td>
<td>Inactive</td>
<td>&gt;30</td>
</tr>
<tr>
<td>A427</td>
<td>Squamous lung carcinoma</td>
<td>Elevated, mutant β-catenin</td>
<td>10</td>
</tr>
<tr>
<td>HCC15</td>
<td>Lung adenocarcinoma</td>
<td>Elevated, mutant β-catenin</td>
<td>3.5</td>
</tr>
<tr>
<td>NCI-H1703</td>
<td>Squamous lung carcinoma</td>
<td>Elevated, high Dv13</td>
<td>5.5</td>
</tr>
<tr>
<td>A549</td>
<td>Large cell lung carcinoma</td>
<td>Active, high Wnt2</td>
<td>18</td>
</tr>
<tr>
<td>HepG2</td>
<td>Hepatocellular carcinoma</td>
<td>Elevated, mutant β-catenin</td>
<td>6.5</td>
</tr>
<tr>
<td>Hep3b</td>
<td>Hepatocellular carcinoma</td>
<td>Elevated, high nuclear β-catenin</td>
<td>5</td>
</tr>
<tr>
<td>Huh7</td>
<td>Hepatocellular carcinoma</td>
<td>Elevated, high nuclear β-catenin</td>
<td>15</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Primary foreskin</td>
<td>Inactive</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

NOTE: FH535 is selectively toxic to some colon, lung, and liver carcinomas expressing high or active Wnt/β-catenin pathways, but not to cells in which the Wnt/β-catenin signaling is not active.

*The levels of Wnt/β-catenin signaling (inactive, active, and elevated) in the different cell lines were determined using an optimized Tcf-binding reporter. The relevant known status of proteins responsible for Wnt/β-catenin signaling activation in the different cell lines is noted and compiled from published literature.

FH535 Is Selectively Toxic to Some Carcinomas Expressing the Wnt/β-Catenin Pathway

Inhibition of Wnt/β-catenin signaling in some cancer cell lines that overexpress this pathway was shown to be toxic. For example, deletion of the activated mutant β-catenin in SW48 colon carcinoma cells is lethal but causes only a weak growth inhibition of HCT116 cells (34, 35). Because FH535 is an effective β-catenin/Tcf inhibitor (Fig. 1C), we tested its toxicity in 12 carcinoma cell lines expressing this signaling pathway (Table 1). The LC50 of most carcinomas tested against FH535 was 5 to 15 μmol/L. At this range, β-catenin/Tcf-dependent transactivation is inhibited by >50% (Figs. 1C and 4A). Only the two colon carcinomas, SW48 and HCT116, were resistant to the compound's toxicity at concentrations of up to 30 μmol/L. This suggests that FH535 inhibition of β-catenin/Tcf-dependent transactivation does not extend to the other oncogenic functions of β-catenin essential for the survival of SW48 cells. Interestingly, FH535 is toxic to A549 and RKO cell lines that express low levels of β-catenin/Tcf-dependent activity but respond to treatment with Wnt ligands. Cells that do not express the Wnt/β-catenin pathway, the primary fibroblasts and the immortal intestine cell line IEC6, were unaffected by FH535 at concentrations of up to 30 μmol/L.

Discussion

Several groups studying the therapeutic potential of PPARγ and PPARδ ligands to suppress Wnt/β-catenin pathway both in cell culture and in vivo conditions reported conflicting results (reviewed in ref. 8). This suggests that only a specific type of PPAR modulation in a defined cellular environment could lead to the inhibition of Wnt/β-catenin pathway. Comparison of FH535 activities with other PPAR ligands provides an insight to the requirement for Wnt/β-catenin pathway inhibition.

FH535 and GW9662 share similar structure and both are antagonistic to PPARγ, but FH535 is unique in its ability to inhibit the Wnt/β-catenin pathway (Fig. 3C). One explanation for this difference is the target specificity of the compounds. FH535 antagonizes both PPARγ and PPARδ whereas GW9662 is specific for PPARγ. GW9662 is unable to reduce Tcf/β-catenin transactivation in cells expressing or lacking the PPARδ gene (Fig. 3C). In contrast, FH535 maintains partial antagonistic activity even in PPARδ-deficient cells (Fig. 3C). Thus, the inability of GW9662 to antagonize PPARδ cannot be the only reason for the difference between the two compounds, and suggests that they might also differ in the ability to inhibit the Wnt/β-catenin pathway via PPARγ.

GW9662 requires Cys285 to covalently bind PPARγ (26), whereas FH535’s antagonistic activity does not depend on this cysteine residue (Fig. 3A). Similarly, the PPARγ agonist 15ΔPGJ2, but not rosiglitazone, use Cys285 for binding and transactivation (28). The nature of the interactions between the PPARγ ligands and specific residues in the PPARγ-binding domain leads to selectivity in coactivator recruitment. For example, recruitment of GRIP1 and SRC1 coactivators by 15ΔPGJ2 is superior compared with troglitazone, an analogue of rosiglitazone (31). Thus, lysophosphatic acid, in contrast to the other PPAR agonists we tested (Fig. 3B), may direct the recruitment of specific coactivators that contribute to Wnt/β-catenin signaling regulation and are the targets of FH535 inhibition. Coactivator recruitment is believed to be a crucial step in PPAR-targeted gene activation because it is important for chromatin remodeling and for interaction with the basic transcription machinery (36). This suggests that the differences between FH535 and GW9662, with regard to binding PPARγ and β-catenin recruitment, may form the basis for the compounds’ different abilities to regulate β-catenin/Tcf-dependent genes.

Mol Cancer Ther 2008;7(3). March 2008

Downloaded from mct.aacrjournals.org on September 7, 2017. © 2008 American Association for Cancer Research.
The ability of PPARγ to bind β-catenin and regulate β-catenin/Tcf activity is shared with other nuclear receptors like the orphan nuclear receptor LRH1 and the androgen receptor. LRH1 was suggested to serve as a direct coactivator for Tcf/β-catenin transactivation from the cyclin D1 promoter (37). Androgen receptor can sequester β-catenin from Tcf4 or promote β-catenin/Tcf4 interaction depending on the biological system analyzed (38, 39). The direct binding of androgen receptor to β-catenin and Tcf4 is thought to mediate the regulation of Wnt signaling. Like the androgen receptor, PPARγ interacts with β-catenin and is found in a complex with Tcf (5, 40). Thus, it is conceivable that FH535 inhibits a complex containing PPARγ β-catenin and Tcf/Lef proteins, depriving the complex bound to Tcf/Lef DNA sites from trans-activating. FH535 may achieve this by preventing the recruitment of GRIP1 to this complex because GRIP1 transactivates both PPAR and LRH1-RE site in the cyclin E1 promoter (37).

FH535 shows some functional similarity to R-etodolac, a stereoisomer of the NSAID etodolac. Unlike FH535, R-etodolac is a weak activator of the PPARγ-dependent reporter (16). However, in the presence of a strong PPARγ agonist, R-etodolac blocks the recruitment of the coactivator PBP by PPARγ. This antagonistic function of R-etodolac was suggested to explain its inhibition of the Wnt/β-catenin pathway (40). Diclofenac and indomethacin are also NSAIDs capable of both Wnt/β-catenin pathway inhibition and antagonizing strong activation of PPARγ (41, 42). All three compounds have been shown to bind and activate PPARγ. Thus, R-etodolac, diclofenac, and indomethacin are PPARγ ligands and may inhibit the Wnt/β-catenin pathway via their function as antagonists of PPARγ. Natural PPARγ antagonists in the cancer cell environment could provide the necessary switch from a weakly agonistic to antagonistic function of these three compounds. The same natural PPARγ agonists can be expected to have the opposite effect on a true PPARγ antagonist's ability to suppress the Wnt/β-catenin pathway. This was observed for FH535 with the addition of either serum or lysophosphatidic acid (Fig. 4A and B).

FH535 inhibits Wnt/β-catenin signaling in cell lines that are resistant or sensitive to the toxic effect of FH535 (Fig. 1C; Table 1). FH535's effect on cell viability is complex and depends on several factors. First, inhibition of Wnt/β-catenin signaling is not toxic to all cell lines with elevated Wnt signaling (34, 35). Additionally, PPAR antagonists have general toxicity to some cells. This toxicity is independent of the ability to inhibit the Wnt/β-catenin signaling pathway because GW9662 and T0070907 are toxic to some cancer cell lines (43). Also, the effect of FH535 depends on the production and cellular concentration of PPAR agonists that antagonize its activity (Fig. 4B).

In this article, we show that FH535 is a tool to study the cross-interaction between the Wnt/β-catenin and the PPAR signaling pathways. FH535 is a more potent Wnt/β-catenin inhibitor than NO-aspirin (Fig. 1C), a leading experimental chemopreventive compound against colon cancer that inhibits intestinal tumors in APCmin mice (17). We expect that therapeutic concentration of FH535 can be achieved in mice because T0070907 was shown to reduce the metastasis of injected cancerous cells in mice (44) and GW9662 suppressed mice obesity induced by a high-fat diet (45).

Acknowledgments

We thank Sondra Goehle and Kevin Schutz for technical assistance.

References


A small-molecule inhibitor of Tcf/β-catenin signaling down-regulates PPAR γ and PPARδ activities

Shlomo Handeli and Julian A. Simon

Mol Cancer Ther 2008;7:521-529.

Updated version
Access the most recent version of this article at:
http://mct.aacrjournals.org/content/7/3/521

Cited articles
This article cites 45 articles, 20 of which you can access for free at:
http://mct.aacrjournals.org/content/7/3/521.full#ref-list-1

Citing articles
This article has been cited by 10 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/7/3/521.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.