The Frizzled-related sFRP2 Gene Is a Target of Thyroid Hormone α1 and Activates β-Catenin Signaling in Mouse Intestine*ST

Elsa Kress1, Amelie Rezza2, Julien Nadjar, Jacques Samarut, and Michélène Plateroti3

From the Université de Lyon, Université Claude Bernard Lyon 1, Ecole Normale Supérieure de Lyon, INRA, CNRS, Institut de Génomique Fonctionnelle de Lyon, 69364 Lyon, France

The thyroid hormone receptor TRα1 regulates intestinal development and homeostasis by controlling epithelial proliferation in the crypts. This involves positive control of the Wnt/β-catenin pathway. To further investigate the effect of thyroid hormone-TRα1 signaling on the intestinal epithelium proliferating compartment, we performed a comparative transcription profile analysis on laser microdissected crypt cells recovered from wild type animals with normal or perturbed hormonal status, as well as from TR knock-out mice. Statistical analysis and an in silico approach allowed us to identify 179 differentially regulated genes and to group them into organized functional networks. We focused on the “cell cycle/cell proliferation” network and, in particular, on the Frizzled-related protein sFRP2, whose expression was greatly increased in response to thyroid hormone-TRα1 signaling. Indeed, sFRP2 stabilizes β-catenin, activates its target genes, and enhances cell proliferation. In this conclusion, we note these results, in conjunction with our previous results, indicate a complex interplay between TRα1 and components of the Wnt/β-catenin pathway. Moreover, we describe in this study a novel mechanism of action of sFRP2, responsible for the activation of β-catenin signaling.

The thyroid hormones (TH), T3 and T4, control cell proliferation, cell differentiation, and apoptosis depending on tissue targets (1). This is well illustrated during TH-dependent amphibian metamorphosis (2). The action of TH is mediated by T3 binding to nuclear receptors (TRs), encoded by the TRα and TRβ genes (3). The TRs activate or repress transcription of target genes by binding to specific DNA sequences named thyroid hormone-responsive elements (TRE) (1). In some organs of the gastrointestinal tract, TH-TRs stimulate cell proliferation (4–8).

The intestinal epithelium is characterized by continuous and rapid cell renewal, fuelled by adult stem cells located in the crypts of Lieberkühn (9). Epithelial cells acquire differentiated phenotypes during their migration up to the villi, where they eventually die and are shed into the lumen. In the mouse, the whole process lasts 3–4 days (9, 10). This continuous cell renewal is regulated by several intrinsic (i.e. transcription factors) and extrinsic (i.e. growth factors and hormones) components. A complex interplay between different signaling pathways maintains epithelial homeostasis (11, 12). We recently showed that the TRα1 receptor controls the proliferation of intestinal epithelial progenitors (8). The mechanism behind this effect involves direct transcriptional regulation of the Ctnnb1 gene, which encodes β-catenin, the intracellular mediator of the canonical Wnt pathway (13).

Canonical Wnt is activated when Wnt proteins bind to the Frizzled receptors, allowing stabilization and nuclear translocation of β-catenin. β-Catenin then binds to the TCF/LEF family of transcription factors to regulate the expression of Wnt target genes (13). Because some of these factors control intestinal progenitor cell proliferation (12, 13), this pathway is thought to be a key regulator of intestinal homeostasis.

To analyze the TH-responsive genes in the intestinal epithelium, we used global comparative transcription profiling of laser microdissected intestinal crypt cells. This approach allowed us to define in detail the cross-talk between genes and signaling pathways involved in the control of epithelial cell homeostasis. Moreover, we were able to extend our previous results regarding the control of β-catenin expression by TH. In fact, we identified a new target of TRα1, the secreted Frizzled-related protein sFRP2, which in these cells behaves as a positive regulator of β-catenin stabilization and signaling.

EXPERIMENTAL PROCEDURES

Animal Treatment and Tissue Preparation—For microdissection, we used TRα1−/− (14), TRβ−/−/− (15), and wild type animals, housed and maintained with approval from the animal experimental committee of the Ecole Normale Superieure de Lyon (Lyon, France), and in accordance with European legisla-

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1 The on-line version of this article (available at http://www.jbc.org) contains supplemental Materials and Methods, Figs. S1–S8, and Table S1.

2 To whom correspondence should be addressed: Institut de Génomique Fonctionnelle de Lyon, Ecole Normale Supérieure de Lyon, 46 Allée d’Italie, 69364 Lyon Cedex 07, France. Tel.: 33-472728536; Fax: 33-472728080; E-mail: Michela.Plateroti@ens-lyon.fr.

3 The abbreviations used are: TH, thyroid hormone; WT, wild type; TRE, thyroid hormone-responsive element; RT-QPCR, reverse transcription-quantitative PCR; TR, thyroid hormone receptor; BrdUr, bromodeoxyuridine; GFP, green fluorescent protein; PTU, propylthiouracil; IP, immunoprecipitation; T3, triiodothyronine; T4, thyroxine.
Thyroid Hormone and Wnt

Isolation of Villus-Crypt Epithelial Fractions—The sequential isolation of mouse small intestinal epithelial cells along the villus-crypt axis has been described previously and validated (16). 1-Month-old mice were maintained under a standard chow diet and either untreated or TH-injected (a single IP injection). They were euthanized 24 h after the injection. TH status was confirmed by measuring the circulating levels of free T3 and T4 (Bio Merieux).

Laser Capture Microdissection and GeneChip Analysis—Tissue-Tek-embedded intestine fragments were prepared for LCM by using the protocol described previously (17). Details for RNA extraction, labeling, and hybridization can be found in the supplemental Materials and Methods.

Statistics—For pairwise comparisons (WT-PTU-treated versus WT-Control; WT-TH-injected versus WT-Control; WT-TH versus WT-PTU; TRα0/0 versus WT-Control; TRβ0/0 versus WT-Control), we used Affymetrix Microarray Suite software 5.0 and two-tailed Student’s T-Test (Zoe software). We also applied the analysis of variance for multiple comparisons. More details can be found in the supplemental “Materials and Methods.

Ingenuity Pathway Analysis—The associations between the genes were further evaluated using the Ingenuity Pathways Analysis software (Ingenuity Systems). See also the supplemental Materials and Methods.

Primary Culture of Intestine Epithelial Cells—Intestinal epithelial primary cultures were derived from 4- to 6-day-old neonatal mice, using the protocol and culture conditions described previously (8). Either 2 × 10−7 M T3 or the vehicle alone was added to the culture medium for the indicated length of time. For proliferation studies, 10 μM BrdUrd was added to the culture medium during an overnight incubation. Recombinant sFRP2, Wnt3a, chimeric Fz4, and chimeric Fz7 (R & D Systems) were added to the culture medium for 24 h, at the indicated concentrations. In blocking experiments, 1 μg/ml of anti-sFRP2 (Santa Cruz Biotechnology) or anti-GFP (Roche Applied Science) antibodies were added to the culture medium for 24 h. After 4 days in culture, cells were washed twice with phosphate-buffered saline and frozen at −80 °C prior to being used for RNA or protein extraction, or they were fixed in 2% paraformaldehyde for immunofluorescence.

RNA Preparation and Analysis—RNA was extracted from primary cultures with Absolutely RNA nanoprep kit (Stratagene), and from epithelial fractions with total RNA and protein isolation kit (Macherey-Nagel). Reverse transcription was performed using 1 μg of RNA and the Sprint PowerScript Pre-Primed SingleShots with random hexamer primers (Clontech). For RNA recovery by laser microdissection, 1 ng of RNA was retro-transcribed. See the supplemental Materials and Methods for details of quantitative PCR.

Immunostaining and Western Blot—Immunolabeling for β-catenin (Santa Cruz Biotechnology) and BrdUrd (Roche Applied Science) was performed on 2% paraformaldehyde-fixed cell cultures. Staining for β-catenin was also performed on 5-μm paraffin sections. Secondary fluorescent antibodies were obtained from The Jackson Laboratories. For fluorescence (Zeiss Axioplan) or confocal microscopy (Zeiss Axiophot), nuclei were stained by Hoechst or propidium iodide, respectively.

Whole proteins from the intestine or from fractionated epithelial cells were extracted using the total RNA and protein isolation kit (Macherey-Nagel). Proteins from cell cultures were obtained by adding the SDS-loading buffer directly to the culture dish. The culture medium was concentrated with centrifugal filter devices (Centricon, Millipore). Proteins were separated and analyzed as described previously (8). We used the following primary antibodies: anti-sFRP2 (Santa Cruz Biotechnology), anti-β-catenin (Santa Cruz Biotechnology), anti-activated β-catenin (Upstate), anti-actin (Sigma), anti-Dishevelled 1 (Santa Cruz Biotechnology), and anti-phospho-GSK3β (Ser-9; Cell Signaling).

Chromatin Immunoprecipitation—The chromatin immunoprecipitation study was performed on collagenase-dispase separated epithelial fragments from 3- to 6-day-old mouse intestine as described previously (8). For conventional PCR, we used the Eurobio Taq (Eurobio). Oligonucleotides are listed in the supplemental Fig. S5B. All amplicons were sequenced.

RESULTS

Crypt Cell Isolation and Microarray Data—Crypt cells were from wild type (WT) animals with normal or perturbed TH status, as well as from TRα and TRβ knock-out mice (14, 15). The supplemental Fig. S1 summarizes the approach of laser microdissection. Statistic methods were used to identify the differentially expressed genes illustrated in the supplementary Table S1.

Fig. 1 shows examples of validation by RT-QPCR on RNA recovered from microdissected cells. Fig. 1, A and B, displays Ccnb1 (cyclin B1) and Mad2L1 (mitotic arrest deficient homolog-like 1) genes, which encode regulators of cell cycle progression and mitotic checkpoint, respectively (18, 19). Similar results obtained by microarray and by RT-QPCR approaches are illustrated in supplemental Fig. S2.

For a finer in silico study, we used the software Ingenuity Pathway Analysis, and defined groups of functions and canonical pathways represented in the set of input genes (supplemental Fig. S3). Finally, we used the gene network tool, which links genes and/or proteins based on a knowledge base. The highest score corresponded to the network “cell cycle/cell proliferation,” which contained 35 input genes (Fig. 1C). This network gives good evidence of the global response to the TH-mediated proliferative stimulus in crypt cells. It is composed of two major
Thyroid Hormone and Wnt

nodes. One is Fos, which is up-regulated by TH treatment, coherent with its positive control of cell proliferation (20). The second is Ctnmb1, encoding β-catenin, already described as a TH-Tra1 target (8). An interesting result was the positive relationship between the Frizzled-related protein sFRP2 and β-catenin, in agreement with work described previously (21).

Sfrp2 Is a Target Gene of TH in Vivo and in Vitro—We focused on the Sfrp2 gene because its expression was highly stimulated upon treatment with TH, and it participates in the Wnt/β-catenin pathway. Moreover, the action of sFRP2 on canonical Wnt has been described in contradictory terms, either repression or activation (21–25). Our goal was to clarify its role in intestinal epithelial progenitors.

First, we validated sFRP2 differential expression depending on TH status or on TR genotype in vivo. Fig. 2A shows that sFRP2 mRNA levels in WT crypts were lower in hypothyroid conditions and were stimulated in hyperthyroid conditions compared with the control condition. Moreover, they decreased in TRα0/0 and increased in TRβ−/− compared with WT control crypts. It is worth noting that TRβ−/− mice are congenitally hyperthyroid (15). We also quantified sFRP2 mRNA and protein in the intestines of TRα and TRβ mutant mice with altered TH status to evaluate whether TH responsiveness depended on a specific TR subtype (supplemental Fig. S4, A and B). WT and TRβ−/− animals both showed decreased sFRP2 mRNA and protein expression under hypothyroid conditions and an induction of sFRP2 after TH treatment. In TRα0/0 mice, sFRP2 expression was unchanged by alteration of TH levels. Slight differences in the results reported in Fig. 2A and supplemental Fig. S4A are probably because of the use of isolated crypt cells in the first case and the whole mucosa in the second.

Finally, we quantified by Western blot sFRP2 protein levels in fractionated villus-enriched or crypt-enriched cells from the villus-crypt axis (16) (supplemental scheme Fig. S5). sFRP2 protein was present at comparable levels along the villus-crypt axis in control animals (Fig. 2B, lanes 1–4). However, TH injection increased the protein specifically in the two last fractions, corresponding to crypt-associated progenitors (Fig. 2B, lanes 3′ and 4′ versus lanes 3 and 4). It is worth pointing out that TRα1 is the only TR receptor expressed and exclusively present in crypt cells. This observation was made both in this study and previously (8). Altogether, these data demonstrated that the regulation of sFRP2 expression by TH depended specifically on the TRα receptor.

To analyze whether the regulation of sFRP2 by TH was epithelial cell-autonomous, we used intestinal epithelium primary cultures maintained in the absence or presence of T3 for 24 h (Fig. 2C). In WT cells, sFRP2 mRNA levels were clearly and significantly increased by T3 treatment. We obtained a similar result by short term T3 treatment (6 h, Fig. 2D), strongly suggesting that this gene might be a direct target of TRα1. The mRNA levels of sFRP2 in TRα0/0 cells maintained in control condition were significantly decreased compared with WT control cells. As expected, the up-regulation of sFRP2 mRNA by T3 was abolished in cells from TRα0/0 mutant mice (Fig. 2C).

FIGURE 1. Validation of microarray data by RT-QPCR on RNA from intestinal crypts. A and B, differentially regulated genes. We used a multiplex method to simultaneously quantify genes of interest and internal control (3684) mRNAs. Values (mean ± S.D., n = 3) represent fold change, after normalization to the WT control condition. WT-C, wild type control; WT-PTU, wild type PTU-treated; WT-TH, wild type TH-injected. **, p < 0.01, compared with WT-C condition; $$, p < 0.01, compared with WT-PTU and TRα0/0 conditions, by Student’s t test.

C, molecular relationships between transcripts in the identified network “cell cycle/cell proliferation” by Ingenuity Pathway Analysis. This network had the highest significant score. Genes or gene products are represented as nodes, and connections between genes are supported by information in the Ingenuity Pathways Knowledge Base. The color indicates up-regulation (red) or down-regulation (green). Different shapes of nodes represent different functional classes of gene products (rhomboid, enzymes; oval, transcription factors; triangles, kinases; trapeziums, transport and carrier proteins; and circles, others). *, validated. $, nonvalidated.
Characterization of a TRE in the Promoter of the sFRP2 Gene—
Using an in silico approach, we found a putative TRE located at 
−924 bp from the transcription start site (supplemental Fig. S6).
To check whether this TRE is a binding site for TR-1, we carried out 
chromatin IP assays on freshly isolated intestinal epithelium. 
Sonicated chromatin was incubated with anti-TR-1, anti-TR-β1, 
or preimmune serum. The DNA precipitated and in starting inputs was 
analyzed by PCR (Fig. 3). Using primers that amplified a fragment of DNA 
comprising the sFRP2-TRE, we showed the presence of a band only in 
samples incubated with the anti-TR-1 antibody. A similar result was 
also obtained for the positive control Ctnnb1-TRE (8). We also 
quantified by QPCR the DNA precipitated by the TR-1 antibody, 
expressed as a percentage of the starting input. We obtained a value of 
6.4 ± 0.3% (n = 4) for sFRP2-TRE and 5.8 ± 0.4% (n = 4) for Ctnnb1-TRE. Negative controls included Sfrp2 
promoter regions located 1 and 3 kb from the TRE, and the 
TH-insensitive 36B4 gene. The binding of TR-1 to sFRP2-TRE was also 
demonstrated in vitro by gel shift analysis (supplemental Fig. S7). Altogether, these data clearly demonstrated that 
TR-1 binds sFRP2-TRE in intestinal epithelial cells both 
in vitro and in vivo.

TH-stimulated sFRP2 Stabilizes β-Catenin through Frizzled 
and Promotes Cell Proliferation—sFRP2 action on Wnt/β-cate-



FIGURE 3. Molecular analysis of the thyroid hormone-responsive element present in the Sfrp2 gene, by in vivo chromatin immunoprecipitation. Study by PCR of the DNA purified from the different samples before and after chromatin IP. The picture is representative of two independent experiments. Indicated on the right part of each panel is the fragment amplified on the Sfrp2, Ctnnb1, and 36B4 genes. C, preimmune serum; TR-1, anti-TRα1; TRβ1, anti-TRβ1; SI, starting input; Ctrl PCR, negative control for PCR mix.

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FIGURE 2. In vivo and in vitro regulation of the Sfrp2 gene. A, validation of microarray data by RT-QPCR on RNA from intestinal crypts. Histograms illustrate mean ± S.D., n = 3. *, p < 0.05, and **, p < 0.01, compared with WT-C condition; $, p < 0.05, and $$, p < 0.01, compared with WT-PTU and TRα0/0 conditions by the Student’s t test. B, representative Western blot analysis of sFRP2 in epithelial cells fractionated from the villus-crypt axis. The picture is representative of four independent experiments. 1–4, fractions from WT control animals; 1′–4′, fractions from WT animals injected with TH. 50 μg of protein per lane were separated on gel; 20 ng of sFRP2 (lane Ctrl) was included as positive control. Histograms in the lower panel (mean ± S.D., n = 4) summarize densitometry analyses (by ImageQuant) from four independent experiments. Data are normalized to the amount of actin in each sample. Statistical analysis was conducted using the Student’s t test. **, p < 0.01, compared with the preceding fractions of the same experimental condition; $, p < 0.01, compared with the corresponding untreated fraction. C and D, Sfrp2 gene regulation by T3 in primary cultures by RT-QPCR analysis. Cells were treated with T3 during 24 h (C) or 6 h (D). Histograms illustrate mean ± S.D. (n = 6) from three independent experiments, each conducted in duplicate. **, p < 0.01 by the Student’s t test; $, p < 0.05 compared with WT control by the Student’s t test.

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−924 bp from the transcription start site (supplemental Fig. S6). 
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Increased levels of β-catenin targets depend on increased availability of β-catenin (13). Because the levels of β-catenin mRNA were not changed by sFRP2 treatment in vitro (Fig. 4B), we focused on protein analysis and in particular on the amount of total as well as nonphosphorylated stabilized β-catenin. For this we used a specific antibody recognizing the N-terminal part of the protein only when not phosphorylated at residues Ser-37 and Thr-41. Wnt signals specifically increase the levels of dephosphorylated β-catenin as detected with this antibody (28, 29). Fig. 4C illustrates the results of Western blot analysis, showing that T₃ or sFRP2 treatment up-regulated the level of both total and stabilized β-catenin. It is worth noting that T₃ treatment induced a clear-cut increase of sFRP2 in culture medium (Fig. 4D), suggesting that activation of β-catenin targets might depend on T₃-stimulated expression of sFRP2. To verify this hypothesis we co-treated the cells with T₃ and with an anti-sFRP2 antibody. This blocking treatment specifically blunted the stimulation of c-Myc (Fig. 4E) and cyclin D1 (not shown) mRNA expression by T₃, whereas an anti-GFP antibody was ineffective. Control cells treated only with the anti-sFRP2 antibody showed unchanged levels of β-catenin targets compared with untreated cells (not shown). Finally, T₃ was still able to induce β-catenin mRNA expression, even in the presence of the anti-sFRP2 antibody (Fig. 4B). We also checked for intracellular signaling molecules such as Dishevelled 1 (Dvl1) and Ser-9-phospho-Gsk3β expression by Western blot. Interestingly, T₃ or sFRP2 treatment in vitro causes an increase in phosphorylated Dvl1 as well as Ser-9-phospho-GSK3β compared with the control (supplemental Fig. S8A). Moreover, the increase was similar to that obtained by treating the cells with Wnt3a (supplemental Fig. S8A). These different treatments did not affect the mRNA levels of Dvl1 and GSK3β (not shown).

To determine the mechanism by which sFRP2 stimulated β-catenin signaling, we focused on Frizzled receptors (Fz). Our transcription profile data showed that intestinal crypts expressed Fz4 and Fz7, which can both transduce Wnt signal (30, 31). We then analyzed the effect of soluble chimeric Ch-Fz4 and Ch-Fz7, which act as Wnt inhibitors (32). Fig. 5A illustrates the expression levels of c-Myc mRNA in epithelial cells maintained in different culture conditions. As expected, treatment with either sFRP2 or Wnt3a significantly increased the level of c-Myc mRNA compared with control cells, whereas each chimeric Fz was ineffective. By co-treating the cells with each chimeric Fz and sFRP2 or each chimeric Fz, the up-regulation of c-Myc mRNA expression was significantly blunted. A similar result was obtained in cells co-treated with T₃ and each chimeric Fz (Fig. 5B). These data clearly show the involvement of Fz receptors in transducing the positive effect of recombinant or T₃-stimulated sFRP2 on β-catenin.

The consequence of β-catenin stabilization in intestinal epithelial progenitors is nuclear translocation and activation
incorporating BrdUrd greatly and significantly increased. Finally, most of the cells displayed nuclear co-staining for BrdUrd and β-catenin (Fig. 6, H and L versus D). When an antibody against sFRP2 was added to T₃-treated cells, the number of cells displaying nuclear β-catenin and BrdUrd were significantly reduced to control levels (Fig. 6, Q and R). These data clearly show that the Sfrp2 gene, transcriptionally induced by TRα1, acts as a positive activator of β-catenin signaling by stabilizing it and by increasing cell proliferation of the intestinal epithelial progenitors in vitro.

To analyze whether TH treatment induced β-catenin nuclear translocation in vivo, we perturbed the TH status of WT animals. Immunohistochemical analysis of intestinal sections showed an induction of β-catenin expression upon TH treatment (Fig. 7, I–L versus A–D). This was accompanied by a clear expression of β-catenin in some nuclei of crypt cells. These data were further confirmed by Western blot performed on protein extracts from the villus-crypt fractions. As reported in Fig. 7M and in agreement with others (13), the activated β-catenin was mainly expressed by the crypt fraction (Fig. 7, M lane and histogram 4). When the animals were injected with TH, the levels of activated β-catenin were greatly and significantly increased in the crypt fraction (Fig. 7M, compare lane and histograms 4 versus 4). It is worth noting that sFRP2 was also significantly up-regulated in this same cellular fraction (Fig. 2B), as well as Dvl1 and Ser-9-phospho-Gsk3β (supplemental Fig. S8B). These data strongly suggest that by increasing the expression of sFRP2, TH can activate the β-catenin signaling in vivo as well as in vitro.

**DISCUSSION**

The thyroid hormones regulate intestinal development. This has previously been described in detail for amphibian metamorphosis (2). In mouse models lacking the expression of TRs, we showed that the Trα gene controls intestinal development during maturation at weaning as well as intestinal homeostasis in adulthood. More precisely, TH-TRα activates the proliferation of intestinal progenitors (7, 8, 33). One mechanism involves positive control of the β-catenin signaling. The current work extended this finding.

To globally characterize genes and signaling pathways regulated by TH-TRα1 in intestinal epithelial progenitors, we used a comparative transcription profile approach on laser microdissected crypt cells. This allowed us to compile a comprehensive list of differentially regulated genes. Some of these were already known to be TH targets, including Idk3α and Apobec1 in the liver (34) and Bub1b and Ccnal1 in intestine, during TH-dependent amphibian metamorphosis (35). In agreement with other studies, we showed a similar number of genes that were up- or down-regulated and different patterns of TH-mediated regulation (1, 34, 36).

In this study we focused on the Sfrp2 gene, which we identified as a new target gene of TH-TRα1. It likely undergoes direct regulation by T₃ through the TRE characterized in its promoter region. In fact, this is the unique responsive element found within the promoter environment. It is composed of two direct repeats separated by eight nucleotides, which is quite uncom-
Thyroid Hormone and Wnt

The functional link between TH-TRs and canonical Wnt has been the focus of previous studies (8, 38, 39), which show that depending on cell type TH can either activate or repress components of the Wnt pathway. Indeed, in some organs, TH induced cell differentiation (40), although in others, it induces cell proliferation (5). We showed that in highly proliferating progenitors of crypts, TH-TRα1 induced the expression of the Ctnnb1 gene, which encodes β-catenin. This in turn activated its targets cyclins D1 and D2 as well as c-Myc (8), all positive regulators of cell proliferation (41– 43). The nuclear action of β-catenin depends on its stabilization (13). Our previous data showed the up-regulation of β-catenin and its molecular targets, but we did not define how β-catenin was stabilized. We describe here another component of the Wnt pathway as a direct target of the TH-TRα1. It is the Frizzled-related sFRP2 that, in the crypt cells, behaves as an activator of the β-catenin. Altogether, in the intestinal epithelial progenitors, TH act simultaneously on Ctnnb1 and Sfrp2 genes to increase and stabilize the levels of the β-catenin and finally to stimulate the cell proliferation.

Since their discovery, sFRP proteins have been considered competitors of canonical Wnt, because of their sequence homology with membrane Frizzled receptors (22, 23). However, several papers have suggested a potential role for sFRP2 in stabilizing β-catenin (21, 24, 25). A study on metanephric development also showed that sFRP2 blocked sFRP1-dependent inhibition of Wnt (44). However, in this system, sFRP2 did not act directly on Wnt. As it is increasingly recognized in signaling systems, the molecular and cellular contexts for signal transmission may be crucial in determining the final outcome (45, 46). Our results in vitro excluded a functional interference between sFRP2 and canonical Wnt3a ligand and clearly demonstrated a positive action of sFRP2 on β-catenin. Moreover, in accord with recent reports (47– 49), our results showed that its action implicated Fz receptors. This is the first study describing a functional interaction between sFRP2 and Fz receptors resulting in positive regulation of β-catenin signaling. It is tempting to speculate that sFRP2, by binding to Fz, activates β-catenin and intestinal progenitors proliferation through the classical Wnt pathway (13). However, we cannot exclude an action involving other signaling pathways (50) as well as noncanonical Wnt (13). It is worth noting the current lack of methodology for directly modifying gene expression in intestinal epithelial primary cultures. Therefore, new strategies and tools will need to be developed to test our hypothesis and to define the mechanisms of this functional interaction.

Several reports have indicated that mutant TRs are involved in various cancers (51– 53). Other data showed correlations between altered levels of TH and human breast and colon cancers (54, 55). Finally, it has been shown that the mutation (56) or aberrant expression (57) of TRs is associated with gastrointestinal tumors. However, there is no conclusive evidence that their proliferative action is linked to tumorigenesis. Here we described the complex interplay between TH-TRα1 and other signaling pathways, key regulators of intestinal epithelial progenitor homeostasis. By focusing on Wnt, we demonstrated a new modality of β-catenin stabilization by sFRP2. Given the key role of canonical Wnt in intestinal homeostasis, our work opens a new perspective in the study of TRα1 as a potential inducer of cell transformation leading to tumorigenesis.
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