Adenomatous Polyposis Coli Control of C-terminal Binding Protein-1 Stability Regulates Expression of Intestinal Retinol Dehydrogenases*

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Mutations in the human adenomatous polyposis coli (APC) gene are thought to initiate colorectal tumorigenesis. The tumor suppressor function of APC is attributed primarily to its ability to regulate the WNT pathway by targeting the destruction of β-catenin. We report here a novel role for APC in regulating degradation of the transcriptional co-repressor C-terminal-binding protein-1 (CtBP1) through a proteasome-dependent process. Further, CtBP1 suppresses the expression of intestinal retinol dehydrogenases, which are required for retinoic acid production and intestinal differentiation. In support of a role for CtBP1 in initiation of colorectal cancer, adenomas taken from individuals with familial adenomatous polyposis contain high levels of CtBP1 protein in comparison with matched, uninvolved tissue. The relationship between APC and CtBP1 is conserved between humans and zebrafish and provides a mechanistic model explaining APC control of intestinal retinoic acid biosynthesis.

Germline mutations in the adenomatous polyposis coli (APC)2,3 tumor suppressor invariably result in familial adenomatous polyposis coli (FAP), a syndrome characterized by early onset colorectal cancer (1). The mechanism by which APC mutations cause colon tumorigenesis is attributed primarily to its role in negatively regulating canonical WNT signaling (2, 3). In this role, APC functions by targeting the transcriptional co-activator β-catenin for intracellular degradation through a proteasome-dependent pathway, thereby limiting its ability to associate with T cell factor/lymphoid enhancer factor nuclear transcription factors. Current evidence indicates that following APC mutation, β-catenin accumulates and translocates into the nucleus, where it partners with T cell factor/lymphoid enhancer factors to drive a program of cellular proliferation. As evidence for a genetic relationship between APC and WNT signaling, some studies cite the existence of rare, β-catenin-activating mutations in colon adenocarcinomas (4, 5). Importantly, however, these mutations do not appear to fully recapitulate the clinical phenotypes associated with APC mutation (6). This discrepancy raises the possibility of additional, β-catenin-independent functions for APC.

A number of reports suggest that the functions of APC are not limited to its well established role in regulating canonical WNT signaling. For example, APC is reported to bind to microtubules, to regulate asymmetric cell division in Drosophila male germline stem cells, and to promote proper T-cell differentiation in mice (7–11). Further, we recently demonstrated that sporadic human colorectal carcinomas lack retinol dehydrogenases and that introduction of APC into human colon carcinoma cells lines induced the expression of the retinol dehydrogenase DHRS9 in a β-catenin-independent manner (12). In addition, apc<sup>micr</sup> zebrafish lack expression of intestinal enzymes, such as rdh11, that are required for retinoic acid production. Injection of apc<sup>micr</sup> zebrafish embryos with mRNA encoding rdh11 or treatment with exogenous RA partially rescued markers of intestinal differentiation in apc<sup>micr</sup> embryos (13). Although these findings support the notion that APC contributes to intestinal differentiation by stimulating retinoic acid production, the mechanism underlying this regulation remains undefined.

In this respect, Hamada and Bienz (14) recently provided evidence for a direct binding interaction between APC and the transcriptional co-repressor CtBP1 that occurs in Drosophila melanogaster and in human cells. CtBP1 plays a critical role in regulating gene expression patterns throughout development and in oncogenesis (15). CtBP1 regulates target gene transcription, in part, via recruitment of histone deacetylases involved in gene silencing (16, 17). In addition, CtBP1 co-purifies with components of histone-modifying complexes and factors such as LSD1, a reported H3K4 histone demethylase, thereby suggesting that CtBP1 may act to coordinate histone modifications that regulate gene expression (18).

Given the role of CtBP1 in transcriptional repression and its physical interaction with APC, we considered a model wherein...
APC promotes RA biosynthesis by relieving CtBP1-mediated suppression of retinol dehydrogenase (RDH) expression. Here, we found that CtBP1 levels are high in human FAP adenomas when compared with uninvolved tissues. Reintroduction of APC into human colon carcinoma cell lines caused a proteasome-dependent degradation of CtBP1. APC-mediated destruction of CtBP1 was paralleled by increased expression of the retinol dehydrogenase, DHR9. This increase was recapitulated by siRNA-mediated knockdown of CtBP1. Finally, our studies showed that apcmcr zebrafish express abnormally high levels of Ctbp1 protein. Restoration of Apc or targeted knockdown of Ctbp1 in apcmcr zebrafish embryos restored expression of rdh11, as well as markers of intestinal differentiation. These findings support a genetic model wherein APC controls retinoic acid biosynthesis and intestinal differentiation, in part by negatively regulating the levels of CtBP1.

**EXPERIMENTAL PROCEDURES**

**Western Blotting and Antibody**—Human colon adenomas and matched uninvolved tissue were harvested in a buffer containing 25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM CaCl2, 1% Triton X-100, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 1 mg/ml pepstatin A, and 1 mg/ml phenanthroline. Zebrafish embryos were collected at 54 or 72 hpf and homogenized in SDS lysis buffer (63 mM Tris-HCl, pH 6.8, 10% glycerol, 5% β-mercaptoethanol, 3.5% sodium dodecyl sulfate) and 1X protease inhibitor mixture (Sigma). All lysates were boiled for 10 min and then centrifuged at 14,000 × g for 10 min. Protein lysates were quantified using the DC protein assay (Bio-Rad). Equal amounts of protein from each sample were fractionated through a 4–12% gradient NuPAGE gels using the MES buffer system (Invitrogen) and transferred to a nitrocellulose membrane for immunoblotting. The following primary antibodies were used: mouse anti-CtBP1 (BD Biosciences), rabbit anti-CtBP (Santa Cruz Biotechnology), rabbit anti-β-catenin (Sigma), mouse anti-β-actin (Novus biologicals), and mouse anti-vinculin (Sigma).

*Zebrafish Stocks and Embryo Culture*—Wild-type and apcmcr Danio rerio (zebrafish) were maintained on a 14-h:10-h light:dark cycle. apcmcr zebrafish were a kind gift of Drs. Anna Pavlina-Haramis and Hans Clevers. Fertilized embryos were collected following natural spawnsings and allowed to develop at 28.5 °C. All embryos were raised in 0.003% phenylthiourea to inhibit pigment formation (19).

*Whole Mount in Situ Hybridizations*—Zebrafish embryos were fixed in sucrose-buffered 4% paraformaldehyde, rinsed in PBS, dehydrated in methanol, and stored at −20 °C. Digoxigenin-labeled riboprobes for rdh11, trypsin, and intestinal fatty acid-binding protein i-fabp were generated as reported previously (20). Whole mount in situ hybridizations were carried out as described (20). Embryos were cleared in 70% glycerol in PBS and photographed using an Olympus DP12 digital camera.

*Quantitative RT-PCR*—Total RNA was harvested using TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. cDNA was synthesized from 1 μg of total RNA using SuperScript III (Invitrogen). PCR was performed using the Roche LightCycler instrument and software, version 3.5 (Roche Diagnostics). Primers for DHR9 were reported previously (12). Primers for zebrafish rdh11 and trypsin were reported previously (13). Primers for E-cadherin and i-fabp were as follows: E-cadherin: forward, 5’-TGCCCCAGAAATGAA AAAGG-3’; reverse, 5’-GTGTATGTTGCAATGCGTTT-3’; i-fabp: forward, 5’-ATGACCTTCAACGGGACCTGGA-3’; reverse, 5’-TTAACGCCCTTTGAAATCCTC-3’.

PCR reactions were performed in duplicate using the LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics). PCR conditions were as follows: 35 cycles of amplification with 10-s denaturation at 95 °C, 5-s annealing at 58 °C, and 10-s extension at 72 °C. A template-free negative control was included in each experiment.

*siRNA Knock-out of CtBP1 and β-Catenin*—For silencing of CtBP1, the sequences of the small interfering RNAs were as follows: sense, 5’-GGG AGG ACC UGG AGU UdTdG-3’; antisense, 5’-dGdTCCC UCC UGG ACC UCU UCAA-3’. For silencing of β-catenin, the sequence of the small interfering RNAs were as follows: sense, 5’-AAU GCU UGG UUC ACC AGU GGA TT-3’; antisense, 5’-UCU AGU GGA CAA AGC AUU TT-3’. The sequence for the control siRNA was as follows: sense, 5’-AGA CAG ACA GAG CTT-3’; antisense, 5’-GCC UAU CUG UCU GUC UTT-3’. Transfection experiments were performed using Lipofectamine2000 (Invitrogen) according to the manufacturer’s recommendations. Following transfection with 50 or 100 nM siRNA, cells were incubated for 24–48 h and then harvested for Western blotting analysis or RT-PCR. In some cases, after transfection, cells were treated with the proteasome inhibitor MG-132 (20 μM) or a vehicle control (Me2SO) for 10–20 h prior to harvesting.

*Immunofluorescence*—Staining of human tissues was performed on 7-μm-thick, paraaffin-embedded, formalin-fixed sections. Sections were deparaffinized in xylene and rehydrated in graded alcohols and then permeabilized for 15 min in 0.1% Triton X-100 in PBS. Antigen retrieval was performed by boiling for 20 min in 10 mM sodium citrate buffer, pH 6.0, followed by cooling for 20 min at room temperature. Sections were then incubated in blocking buffer (2% goat serum and 1% bovine serum albumin, and 5 mM glycine in 1× PBS) for 1 h at room temperature and subsequently incubated in wash buffer (0.2% goat serum, 0.1% bovine serum albumin, and 5 mM glycine in 1× PBS) for 1 h at room temperature. Primary antibody (mouse anti-CtBP1, BD Biosciences, diluted 1:100 in PBS) was applied overnight at 4 °C. Sections were rinsed twice in PBS and then incubated overnight at 4 °C in secondary antibody (goat anti-mouse Alexa 488, Molecular Probes; dilution 1:250) and the nuclear stain TO-PRO-3 (Molecular Probes; dilution 1:2000). Sections were rinsed in water, and coverslips were applied using ProLong Gold antifade reagent (Molecular Probes). Fluorescent images were collected using an Olympus FluoView FV300 confocal laser scanning microscope.

*Retrovirus Infections and Luciferase Assays*—Portions of APC were cloned into the pLent6/UbC/V5-DEST lentiviral expression vector using the Gateway system (Invitrogen). Expression vectors were transfected with ViraPower packaging mix into 293FT cells using Lipofectamine according to the manufacturer’s protocol (Invitrogen). 72 h after transfection, virus containing medium was collected, filtered, and transferred to HT29...
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cells at an approximate multiplicity of infection of 1.0. 48 h following infection, total RNA was collected using TRIzol reagent (Invitrogen), and total protein was harvested as described above. For luciferase assays, luciferase values were analyzed using a Dual-Luciferase assay system (Promega). Transfection efficiencies were normalized by dividing the firefly luciferase activity by the Renilla luciferase activity for each sample. All experiments were performed at least three times.

Morpholino and Microinjection Experiment—Antisense morpholino oligonucleotides were obtained from Gene Tools, LLC. The ctbp1MO splicing blocking morpholino (5'-TCC TTA TGA CGA TAC CTG CCT ATG G-3') and control morpholino (5'-CCT TTT ACC TCA GTT ACA ATT TAT A-3') were solubilized to a final concentration of 1.0 mM in 1M pholino (5CtBP1 protein in comparison with control treated cells (Fig. 2). RT-PCR analysis revealed that the CtBP1 siRNA effectively knocked down the level of CtBP1 protein in comparison with cells with induction of proteasome inhibitor MG-132 (20µM). Western blot analysis of whole cell lysates from cells treated with or without ZnCl2 revealed that APC induction resulted in decreased levels of CtBP1 protein in comparison with cells with induction of β-galactosidase (Fig. 1A). Further, when cells were treated with the proteasome inhibitor MG-132 (20 µM) following APC induction, CtBP1 levels accumulated (Fig. 1A).

Given the well described role for APC in regulating the WNT/β-catenin pathway, we considered the possibility that reduction of CtBP1 levels following APC induction was dependent on β-catenin. To test this, we transfected two different colon tumor cell lines, HCT116 and HT29, with control or β-catenin-targeted siRNAs. Western blot analyses revealed that although this approach effectively knocked down β-catenin levels, the levels of CtBP1 protein remained unchanged (Fig. 1B). RT-PCR for DHR95 expression following β-catenin knockdown in parallel cultures showed no change in the expression level of DHR95 (Fig. 1C). Functional knockdown of β-catenin was confirmed by cotransfection with a TOP-FLASH reporter construct. In each case, knockdown of β-catenin decreased activation of the reporter TOP-FLASH approximately 4-fold. The control siRNA showed no effect on TOP-FLASH expression (data not shown).

Since CtBP1 levels appeared dependent on APC and the proteasome, but independent of β-catenin, we sought to determine whether the repression of RDHs in APC mutant human colon tumor cells occurs via CtBP1. Thus, we transfected control and CtBP1 directed siRNAs into three colon carcinoma cell lines that carry APC mutations (DLD-1, SW480, HT29). Western blot analysis revealed that the CtBP1 siRNA effectively knocked down CtBP1 protein in comparison with control treated cells (Fig. 2A).

In contrast to knockdown of β-catenin, however, RT-PCR analysis indicated robust induction of DHR95 transcription following CtBP1 knockdown in each of the cell lines tested (Fig. 2B).

As an additional approach to testing whether APC controls the levels of CtBP1 and DHR95, we prepared two deletion constructs of APC for expression in APC-deficient colon tumor cell lines via retroviral transduction. The first construct, APC955–2075 (amino acids 955–2075), contains all of the sites described as necessary for binding CtBP1 (14) (Fig. 3A). The second, APC1–985, contains primarily the N terminus of APC (amino acids 1–985) and lacks all reported CtBP1 binding motifs (Fig. 3A). Western blot analysis of lysates from HT29 and DLD-1 transfected with the second construct revealed no induction of CtBP1 levels following adenoviral infection (data not shown). To test whether CtBP1 levels were regulated by the APC tumor suppressor in this context, we utilized the engineered human colon carcinoma cell lines, HT29-APC and HT29-β-galactosidase. These cell lines express either wild-type APC or β-galactosidase upon the addition of ZnCl2, respectively (21). Western blot analysis of lysates from cells treated with or without ZnCl2 revealed that APC induction resulted in decreased levels of CtBP1 protein in comparison with cells with induction of β-galactosidase (Fig. 1A). Further, when cells were treated with the proteasome inhibitor MG-132 (20 µM) following APC induction, CtBP1 levels accumulated (Fig. 1A).

RESULTS

Our previous studies have shown that induction of APC in the colorectal carcinoma cell line HT29 stimulated increased expression of DHR95 (12). To determine whether CtBP1 protein levels are regulated by the APC tumor suppressor in this context, we utilized the engineered human colon carcinoma cell lines, HT29-APC and HT29-β-galactosidase. These cell lines express either wild-type APC or β-galactosidase upon the addition of ZnCl2, respectively (21). Western blot analysis of whole cell lysates from cells treated with or without ZnCl2 revealed that APC induction resulted in decreased levels of CtBP1 protein in comparison with cells with induction of β-galactosidase (Fig. 1A). Further, when cells were treated with the proteasome inhibitor MG-132 (20 µM) following APC induction, CtBP1 levels accumulated (Fig. 1A).

The control siRNA showed no effect on TOP-FLASH expression, whereas CtBP1 knockdown in each of the cell lines tested (Fig. 2A). Western blot analysis of lysates from HT29 cells treated with Me2SO (vehicle) or MG132 at 30 h after the addition of ZnCl2 revealed that APC induction resulted in decreased levels of CtBP1 protein in comparison with cells with induction of β-galactosidase (Fig. 1A). Further, when cells were treated with the proteasome inhibitor MG-132 (20 µM) following APC induction, CtBP1 levels accumulated (Fig. 1A).

In contrast to knockdown of β-catenin, however, RT-PCR analysis indicated robust induction of DHR95 transcription following CtBP1 knockdown in each of the cell lines tested (Fig. 2B).

As an additional approach to testing whether APC controls the levels of CtBP1 and DHR95, we prepared two deletion constructs of APC for expression in APC-deficient colon tumor cell lines via retroviral transduction. The first construct, APC955–2075 (amino acids 955–2075), contains all of the sites described as necessary for binding CtBP1 (14) (Fig. 3A). The second, APC1–985, contains primarily the N terminus of APC (amino acids 1–985) and lacks all reported CtBP1 binding motifs (Fig. 3A). Western blot analysis of lysates from HT29...
cells infected with lentivirus expressing each protein revealed that introduction of APC955–2075 effectively reduced CtBP1 levels and that expression of APC1–985 had no effect (Fig. 3B). As expected, H9252-catenin levels were also decreased by introduction of the APC955–2075 construct (Fig. 3B). RT-PCR analysis of DHRS9 transcript levels in this same experiment indicated that concomitant with reducing CtBP1 levels, APC955–2075, but not APC1–985, induced DHRS9 transcript levels 4.3-fold (Fig. 3C).

Given that APC appears to regulate CtBP1 levels in colon carcinoma cell lines, we next asked whether adenomas taken from FAP patients would accordingly harbor higher levels of CtBP1 when compared with matched normal tissue (Fig. 4B). Furthermore, in 10 samples from FAP patients, we analyzed DHRS9 levels by RT-PCR and determined that 8 of 10 adenomas expressed reduced levels of DHRS9 in comparison with matched uninvolved tissues (Fig. 4C).

The findings in human cell lines and FAP adenomas suggest a relationship between APC and CtBP1. Recent studies have reported that homozygous apc mutant and morphant zebrafish embryos harbor a number of developmental defects consistent with loss of retinoic acid including failed pectoral fin development, underdeveloped jaws, and pancreatic and intestinal differentiation defects (12, 13, 20). In addition, adult zebrafish carrying heterozygous apc mutations develop spontaneous adenomas in the liver, pancreas, and intestine (22). We, therefore, examined the relationship between Apc and Ctbp1 in vivo using apcmcr zebrafish. When compared with wild-type siblings, apcmcr harbored increased H9252-catenin levels when compared with wild-type siblings (Fig. 5A). To determine whether Ctbp1 protein levels were dependent on Apc, we sought to restore Apc function in mutant zebrafish by injecting mRNA encoding APC955–2075, the region that, in human cells, caused degradation of CtBP1 (14). Similar to that seen in human cells above, APC955–2075 caused a strong reduction of Ctbp1 protein levels (Fig. 5B). As expected, H9252-catenin levels were also decreased following injection of APC955–2075 (Fig. 5B). In contrast to APC955–2075, injection of APC1–985 had no effect on Ctbp1 or β-catenin levels (Fig. 5B).
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![Image](https://example.com/image.png)

FIGURE 4. Human FAP adenomas express high levels of Ctbp1. A, total cellular protein was harvested from human FAP colon polyps (P1, P2, P3, etc.) or matched normal tissue (N1, N2, N3, etc.) and immunoblotted with the indicated antibodies. P4a, P4b, and P4c indicate three different polyps from the same patient. Vinc, vinculin. B, immunofluorescence was performed on histological sections from grossly uninvolved tissue or FAP colon polyps (adenoma) using a CtBP1-specific antibody (red) and counterstained with TOPRO3 to reveal nuclei (blue). The same sections where then stained with hematoxylin and eosin (H&E). C, quantitative RT-PCR with primers specific for DHR59 was performed using total RNA harvested from adenomatous or matched normal tissue of 10 FAP patients. Fold decrease was calculated as the total number of DHR59 transcripts in FAP adenomas divided by the total number of DHR59 transcripts in matched normal tissue. Error bars indicate standard deviation.

In parallel injections, quantitative RT-PCR revealed that expression of the DHR59 homolog in zebrafish, rdh1l, was induced in *apcmcr* following injection of APC<sup>955–2075</sup>. This was also the case for markers of pancreas (trypsin) and intestinal (i-fabp) differentiation (Fig. 5C). Again, injection of APC<sup>1–985</sup> had no effect (data not shown).

The above findings suggested that intestinal differentiation in *apcmcr* zebrafish was repressed by the accumulation of either β-catenin or Ctbp1. To further examine this issue, we first attempted to reduce levels of β-catenin. As antisense morpholino constructs directed against β-catenin showed severe toxicity prior to gut development (data not shown), we reduced levels of β-catenin by treatment with the selective COX2 inhibitor NS-398. Consistent with our previous finding of elevated COX-2 in *apcmcr* zebrafish (23) and recent studies showing PGE2-induced activation of β-catenin (24, 25), treatment of *apcmcr* embryos with NS-398 effectively reduced the levels of β-catenin (Fig. 6A). However, RT-PCR analysis revealed that this reduction in β-catenin protein levels failed to induce the expression of rdh1l, i-fabp, and trypsin (Fig. 6B). This suggested suppression of these markers by increased levels of Ctbp.

To address this possibility, we knocked down Ctbp1 by injecting a Ctbp1-targeted antisense morpholino oligonucleotide that specifically blocked splicing of the Ctbp1 transcript. Injection of the Ctbp1-targeted antisense morpholino resulted in substantial reduction in Ctbp1 protein as determined by Western blot analysis (Fig. 7A). Injection of a control antisense morpholino caused no change in Ctbp1 levels (Fig. 7A). As seen in human cell lines, knockdown of Ctbp1 showed no effect on β-catenin or actin protein levels (Fig. 7A). Having established that the Ctbp1 morpholino effectively reduced Ctbp1 splicing (data not shown), we examined rdh1l expression in *apcmcr* injected with the Ctbp1 antisense morpholino. As observed in human cells, this analysis revealed that in comparison with control-injected embryos, knockdown of Ctbp1 rescued rdh1l expression in a significant proportion of *apcmcr* embryos (Fig. 7, B and C). Further, we observed restoration of trypsin and i-fabp expression in a similar fraction of *apcmcr* injected with Ctbp1 morpholino (Fig. 7, B and C).

DISCUSSION

In this report, we employ human cell lines and tissues, as well as a vertebrate model system to expose a novel role for APC in regulating intracellular levels of the transcriptional co-repressor Ctbp1. Our studies also indicate that Ctbp1 dictates the expression of retinoic acid biosynthetic enzymes, as well as intestinal differentiation. The ability of APC to regulate Ctbp1 occurs independently of β-catenin and relies upon degradation...
mediated by the proteasome. Our findings are consistent with a model wherein APC regulates the stability of both β-catenin (14) and CtBP1. This allows simultaneous negative regulation of Wnt signaling while stimulating retinoic acid biosynthesis during intestinal cell differentiation. Evidence for this model includes: i) expression of wild-type APC in APC mutant human cells or zebrafish rescues expression of RA biosynthetic enzymes such as DHRS9 and rdhl; ii) knockdown of CtBP1 in APC-deficient human cells and in apcmcr zebrafish restores expression of DHRS9 and rdhl, respectively; iii) injection of a ctbp1 morpholino in apcmcr zebrafish rescues RA-deficient differentiation defects including the expression of the markers i-fabp and trypsin; iv) siRNA against β-catenin in human cells had no effect on CtBP1 levels, and v) human colon polyps from FAP patients harbored increased CtBP1 and decreased DHRS9.

Our data indicate that reintroduction of wild-type APC into APC-deficient human colon tumor cells or injection of wild-type APC into apcmcr zebrafish results in decreased ctbp1 protein levels and that in human cells, this decrease was blocked by inhibition of the proteasome. It appears, therefore, that APC control of both CtBP1 and β-catenin shares common features. In both cases, APC is reported to bind these proteins directly, and in both cases, APC inhibits the accumulation of these two transcriptional regulators. Second, APC regulation of CtBP1 and β-catenin protein levels is dependent upon the proteasome (26–28). Several reports indicate that phosphorylation of β-catenin by the APC complex is required for proteasomal degradation (26,28). Likewise, recent studies indicate that CtBP1 is destroyed by the proteasome in cells responding to stimuli such as UV irradiation and that phosphorylation and ubiquitination of CtBP1 are required for proteolysis to occur (28,29). Thus, although current models assign to APC a role in controlling intestinal cell proliferation and differentiation by regulating the transcriptional co-activator β-catenin, our results assign to APC a simultaneous role in contributing to intestinal cell differentiation by regulating the transcriptional co-repressor CtBP1. These results support the hypothesis that in the setting of APC mutations, both β-catenin and CtBP1 accumulate, with each contributing to impaired intestinal cell differentiation. Importantly, this model may reconcile apparent clinical incongruities indicating that colon adenocarcinomas bearing APC mutations differ from those carrying only β-catenin-activating mutations (6). For example, lesions carrying stabilizing β-catenin mutations may retain retinoic acid production, thereby limiting tumor growth.

Although APC appears to regulate both β-catenin and CtBP1 similarly, levels of CtBP1 do not appear to be dependent on β-catenin. This is indicated by the finding that siRNA knockdown of β-catenin showed no corresponding effect on CtBP1 levels. The regulation of CtBP1 by APC is, therefore, consistent...
with recent reports detailing a β-catenin-independent role for APC in controlling retinoic acid production in humans and in zebrafish (12, 13, 20). In addition, these findings are consistent with reports describing roles for APC that are considered non-canonical with respect to its classical role in controlling β-catenin (7–11). For example, APC appears important in stabilization of microtubule polymerization, thereby helping to guide the formation of microtubule networks (8, 9). In addition, APC plays a role in regulating asymmetric cell division in male germ line stem cells by orienting mitotic spindles during stem cell division (10). Finally, -cell differentiation in the mouse requires β-catenin-independent actions of APC (11).

CtBP1 is a well described transcriptional co-repressor that represses gene expression, in part, by recruiting histone deacetylases to gene promoters (16, 17). In addition, tagged purification of CtBP1 proteins has recently revealed that these proteins assemble into large chromatin-modifying complexes (18). Further, a recent report has implicated APC in targeting of CTBP1 to sites of repression as a WNT antagonist (30). These findings, coupled with our report that APC controls CtBP1 levels, suggest that APC may act to direct intestinal cell differentiation by regulating the stability of chromatin remodeling complexes. Interestingly, Jette et al. (12) reported the regulation of human DHRS9 by the intestinal-specific transcription factor CDX2. CDX2, however, is present in adenomas and carcinomas bearing APC mutations (31). The accumulation of CtBP1, which suppresses RDH expression, could explain the failed activation of DHRS9 in FAP tissues despite the presence of CDX2.

Studies in Drosophila and Xenopus suggest that CtBP can act, via Brinker and xTcf-3, to antagonize Wg/WNT signaling (32, 33). In addition, many studies cite constitutive WNT signaling as a driving force in colon tumorigenesis (2, 3). Paradoxically, our findings suggest that loss of APC, a negative regulator of the WNT pathway, results in high CtBP1 levels in human colonic adenomas. However, CtBP1 has been reported to both activate and repress gene transcription in a context-specific manner (34, 35). Further, murine embryonic knock-out of CtBP2 results in decreased expression of the Wnt3a target gene, brachyury, suggesting that CtBP2 may serve in vivo as an activator of this Wnt target gene (36). It appears, therefore, that CtBP has diverse functions that are defined by multiple contributing factors.

In conclusion, our results suggest that accumulation of CtBP1 following APC mutation represses intestinal cell differentiation and that elimination of CtBP1 promotes a program of differentiation. Further, these data support the existence of this pathway in zebrafish and humans and provide evidence for CtBP1 dysregulation in human adenomas, coincident with a loss of RA biosynthetic capabilities due to the absence of RDH expression. These results draw attention to the necessity of continued research into non-canonical roles for APC and suggest that inhibition of canonical WNT signaling alone may not effectively restore intestinal differentiation in APC-deficient tissues.

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