Human DNA methyltransferase gene \(DNMT1\) is regulated by the APC pathway

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Introduction

The methylation status of DNA and the machinery responsible for it have been implicated in both the initiation and progression of cancer (1). The primary enzyme responsible for adding methyl groups to 5 position of cytosine in a CG dinucleotide motif is DNA (cytosine-5) methyltransferase (DNMT1). Cancer cell lines and tumor biopsies show increased levels of DNMT1 protein and activity (2,3). It has also been demonstrated that promoter region hypermethylation can inhibit the transcription of target genes, including the tumor suppressors p14ARF and p16 INK4a (4,5) and BRCA1 (6). Recent evidence that DNMT1 is a critical mediator in APC-mutation triggered colorectal tumorigenesis has already been put forth by others (22,23). Laird et al. showed that mice with a homologous mutation in APC (24) developed spontaneous intestinal tumors like those in FAP colorectal cancer patients, and when crossed with mice with reduced expression of methyltransferase, the number of tumors and the degree of genetic instability were decreased (22). When the amount of cytoplasmic DNMT1 protein was further reduced using the suicide substrate 5-aza-deoxycytidine, polyp number was additionally decreased.

In this paper we test the hypothesis that APC/β catenin pathway regulates DNMT1 expression in human cancer cells, thus providing an explanation for the genetic interaction observed in the mouse model. We report here that mutations in the tumor suppressor Apc gene increase the transcriptional activation of DNMT1 promoter and mRNA levels in human cancer cells. We further show that antisense oligonucleotides to \(DNMT1\) mRNA can inhibit anchorage-independent growth of these APC-mutated cells, thus supporting the hypothesis that DNMT1 is a critical mediator in APC-mutation triggered colorectal tumorigenesis. Our results might support use of DNMT1 inhibitors in treatment of FAP.

Abbreviations: APC, adenomatous polyposis coli; CAT, chloramphenicol acetyltransferase; CRC, colorectal cancer; \(DNMT1\), DNA (cytosine-5) methyltransferase 1; DNTCF, dominant negative \(N\)-terminal mutant T-cell factor; FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer; Lef, lymphocyte enhancer factor; TCF, T-cell factor.

Materials and methods

Cell culture

The HT-29 Apc–/– human colon carcinoma cells were obtained from the American Type Culture Collection and were maintained in McCoy’s 5A...
medium (Gibco BRL) supplemented with 10% fetal calf serum (Colorado Serum Company) and 0.292 mg/ml glutamine. HT29/APC cells, a stable HT-29 transfected expressing a wild-type APC cDNA under the direction of a metallothionein promoter, and HT29/Gal, a control transfected expressing the bacterial β-galactosidase gene under the direction of the same promoter, were kindly gifted of Drs K. Vogelstein and M. Szyf (25). APC or βGal expression was induced in the respective transfecants by adding 100 μM ZnCl2 to the culture medium.

To determine cell growth kinetics, 50 000 HT29/Gal or HT29/APC cells per well were plated in six well dishes in standard conditions and treated with 100 μM ZnCl2 the following day. Cells were harvested after 0, 6, 12, 24, and 48 h of induction by trypsin and counted. Experiments were performed in triplicate.

To establish the effect of growth inhibition on DNMT1 expression, two methods of cell stasis were used. First, proliferating HT29/APC cells were subjected to serum starvation (1% fetal calf serum as opposed to 10%) for 3 days. Cells were then treated with 100 μM of ZnCl2 for 24 h, harvested and total RNA extracted for northern analysis. Second, cell cycle inhibition involved transient transfection of a constitutively expressed human wildtype p53 tumor suppressor gene, a kind gift of Dr Branton (26), into HT29/APC cells. Cells were harvested after 48 h and total RNA extracted for northern analysis.

To investigate the effect of wildtype APC on ecotopic DNMT1 transcription, transient transfections were performed into HT29/APC of a DNMT1 expression construct (pSMT3-Met, which contains a metallothionein promoter, full length DNMT1 (27) in pcDNAHis vector (Invitrogen)). Transfection efficiency and translational control was ascertained by cotransfection of a CMV–GFP cDNA in the same vector, and blotting with anti-GFP antibodies (sc-8334, Santa Cruz Biotechnology, Santa Cruz, CA). Cells were observed for GFP expression, harvested, and proteins extracted and resolved. Western blotting against the Xpress tag of the vector according to the manufacturer’s instructions allowed for specific visualization of the exogenous DNMT1 expression.

Antisense oligonucleotide treatments were performed in serum-reduced media (OptiMEM, Gibco BRL) before changing the media back to McCoy’s 5A. For anchorage-independent growth assays, treated HT-29 cells were mixed with enriched media (McCoy’s 5A containing an additional 10% of fetal calf serum) before adding the agar. For all experiments, cells were maintained at 37°C, 95% O2, 5% CO2.

**CAT reporter assays**

Cells were plated in six-well plates at a density of 300 000/well. Transfections of reporter constructs took place the following day. CAT reporter assays were performed as has been previously described (28). Briefly, a 0.8 kb genomic fragment containing the 5′ DNMT1 minimal promoter was excised with XhoI and BamHI and ligated to a bacterial chloramphenicol acetyltransferase reporter construct (pCAT, Promega) to create pMet-P1-ΔGal (29), Figure 1A). Using standard calcium-mediated transfer protocols (28), 5 μg of pMet-P1-ΔGal/CAT reporter was transfected in triplicate either alone or in consort with plasmids containing an inducible wild type Apc or lacZ cassette (5 μg each, Figure 1B, pSAR-MT-APC and pSAR-MT-βGal respectively, generous gifts from Drs B. Vogelstein and K. Kinzler, Johns Hopkins University (25)) into HT-29 human colon carcinoma cells. Additional reporter activation experiments were performed with the same inducible constructs stably transfected into HT-29 cells (HT29APC and HT29Gal). In both cases, production of full length APC protein was induced with 100 μM ZnCl2.

Forty-eight hours after transfection, cells were harvested and total cellular extracts were isolated. Fifty μl of the extract was used per assay. DNMT1 promoter-driven CAT gene activation was measured by the enzymatic transfer of tritiated acetyl groups from [3H] acetlycoenzyme A to chloramphenicol. The latter was extracted and 3H counted.

**Adenoviral infection and expression**

To demonstrate a dependence of DNMT1 transcription on the β catenin/TCF pathway in human colon cancer cells, 300 000 HT-29 cells were plated in standard conditions. These cells were then infected the following day with 3–300 MOI of an adenovirus expressing either β-galactosidase or dominant-negative human TCF4 (AdβGal or AdDNCTF4, respectively, generous gifts from Drs B. Vogelstein and K. Kinzler, Johns Hopkins University (25)). Virus stocks were propagated in HEK293 cells to high titers, and then purified in CsCl gradients (18). Cells were harvested 18 h after infection and total RNA was processed for northern blotting.

**DNMT1 antisense treatment and anchorage-independent growth assays**

The DNMT1 antisense used in this study was previously characterized (11,14). HT-29 cells were plated at a density of 50 000 cells per well in six well dishes 18 h before treatment. Lipofectin (6.25 μl/ml final concentration, Gibco BRL) was mixed with 100 μl of serum-reduced OptiMEM media (Gibco BRL) for 45 min at room temperature. Two hundred nM (final concentration) of MG88S (5′-fluorescein-tagged) or MG208 4 × 4 hybrid 2′-O-methylphosphorothioate antisense oligonucleotide (14) (5′-AACGCGACCGGTCCTCC-3′ and 5′-AACGATCAGGACCTTGTC-3′, respectively, OligoEtc., Wilsonville, OR; underlined bases are 2′-O-methyl modified) was added and incubated for an additional 15 min. The volume was brought to 2 ml and the oligo mixture was layered onto seeded HT-29 cells. Transfection of the oligo from the agarose plug took place over 4 h at 37°C before the media was replaced with normal McCoy’s 5A. Antisense treatment was repeated once. Treated cells were trypsinized, and 3000 cells/well were counted and mixed with 3 ml of enriched media and 1 ml of warm (65°C) 1.5% agar and plated onto six-well plates. One ml of standard McCoy’s media was brought to 2 ml and the oligo mixture was layered onto the top of the gel matrix and colonies were allowed to grow at 37°C for 21 days. Digital images were captured with a CCD camera (XC-77, Sony) through a Nikon 55 mm lens (Nikon) to a MCID-M4 image analysis system (Imaging Research, St Catharines, ON). As a control, 300 cells per well were grown in standard conditions to ensure that treated cells were still viable.

**Northern and western blot analyses**

Cells were lysed directly on the culture plate with 1 ml of Trizol (Gibco BRL) and total RNA prepared as per the manufacturer’s instructions. Ten μg of RNA were separated in a 1% denaturing agarose gel, transferred to nitrocellulose (Hybond N+, Amersham Pharmacia Biotech) and crosslinked with 120 μl of UV radiation (Stratalinker 2400, Stratagene). The northern blots were prehybridized in a phosphate-based cocktail (5 ml 1 M sodium phosphate, pH 6.8, 5 ml dH2O, 20 μl 0.5 M EDTA, 1 ml 2 mg/ml herring sperm, 0.7 g SDS) for 1 h at 68°C, and then were hybridized with a 32P-labelled BamHI–EcoRI cDNA fragment of DNMT1 (Accession number NM_001379, GenBank). Membranes were washed 2×15 min Solution A (3 ml 2 mg/ml herring sperm, 25 g SDS, 20 ml 1 M NaPO4 pH 6.8, 1 ml 0.5 M EDTA) at 65°C, 4 ×10 min Solution B (5 g SDS, 20 ml 1 M NaPO4 pH 6.8, 1 ml 0.5 M EDTA) at 65°C and exposed to XAR film (Eastman Kodak). Blots were stripped off the DNMT1 probe and rebuhybridized to an 18S RNA-specific 32P-labelled oligonucleotide and exposed again to a film. Films were scanned and the DNMT1 signal was quantified on a MCID-M4 image analysis system (Imaging Research, St Catharines, ON).

**Western blot analysis**

To study expression of full and mutated APC protein was performed using the method described by Smith et al. (30). Proteins (100 μg) from whole cell lysates were separated on 3% agarose/10% SDS, blotted to Hybond P+ membranes (Amersham). Membranes were blocked in 10% non-fat milk in TBST (Tris-buffered saline + 0.1% Tween-20) for 1 h at room temperature and incubated with 1 μg/ml in 5% milk/ TBST of a mouse monoclonal antibody against APC (OP44, Oncogene Research Products, San Diego, CA) overnight at 4°C. An HRP-conjugated goat anti-mouse secondary was used for 1 h at room temperature and the signals were visualized with enhanced chemiluminescence using the standard ECL kit from Amersham.

**Results**

**Wild type APC expression suppresses the activity of the human DNMT1 promoter in a transient transfection assay**

To examine the possibility that increased activity of the DNMT1 promoter is a downstream consequence of mutations in the APC/β-catenin/TCF axis, we utilized a previously characterized reporter construct bearing the bacterial CAT gene under the direction of the 5′ minimal promoter of the human DNMT1 gene (10) (Figure 1A). When transiently transfected into HT-29/ACP colon carcinoma cells, the 0.8 kb HindIII–XhoI minimal human DNMT1 promoter stimulates the expression of CAT >14-fold over the basal activity of the promoterless construct. Zinc induction of the wildtype, full-length APC protein inhibited the DNMT1-driven CAT activity by >76% (Figure 1C; P = 0.011). To ensure that the results were not an artefact of the specific stable APC transfectant, we transiently cotransfected either the Zn-inducible wildtype Apc gene or control β-Gal vector along with pMet-P1-ΔXH-CAT reporter constructs into HT-29 cells. While induction of full length APC reduced DNMT1-dependent CAT activity by 85% (Figure 1D; P = 0.00016), the β-Gal control plasmid had no effect. These data are consistent with the hypothesis that induction of DNMT1 promoter is a downstream consequence of disrupted APC/β-catenin/TCF transcription pathway in...
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\textbf{Fig. 1.} Full-length APC suppresses \textit{DNMT1} mRNA levels in HT-29 CRC cells. Either a promoterless CAT construct (pCAT) or a \textit{DNMT1} 5' minimal promoter CAT construct (\textit{pMet-P1-\Delta XH-CAT}) (A) were transfected in triplicate into HT-29, HT29APC or HT29\textbeta-Gal cells. (B) The physical map of the APC/\textbeta-Gal expression vector. (C-D) CAT activity was measured 2 days after transfection as described in Materials and methods. The graphs depict the average of three transfections/SEM (C). \textit{pMet-P1-\Delta XH-CAT} was transiently transfected into cells harboring a stable (C) or transient (D) APC (pSAR-MT-APC) or \textbeta-galactosidase (pSAR-MT-\textbeta-Gal) expression vector.

CRC, and that the inability of truncated APC protein to cause \textbeta catenin degradation may result in augmented DNMT1 expression.

\textit{Full-length APC suppresses DNMT1 mRNA levels in HT-29 CRC cells}

Since the mutated APC pathway results in stimulation of \textit{DNMT1} promoter activity in a transient transfection reporter assay, we determined whether APC could modulate the expression of the endogenous mRNA in HT-29 cells. HT29APC cells were induced to express wild type APC and \textit{DNMT1} mRNA was quantified by northern blot analysis. The \textit{DNMT1} signal was normalized to the 18S tRNA signal (Figure 2B). As a control we subjected HT29\textbeta-Gal transfectants to the same treatment. Expression of wild type APC following Zn induction of HT29APC cells was verified by a western blot analysis (Figure 3B). Our results reveal that after a 9 h exposure to 100 \mu M ZnCl\textsubscript{2}, HT29APC Zn-induced cells show 50\% diminished levels of \textit{DNMT1} mRNA relative to that of HT29\textbeta-Gal cells (Figure 2A). Continued 40\% suppression of \textit{DNMT1} transcription was seen at 12 h following wild type APC induction. Repeated experiments yielded similar results. Later time points of Zn induction were not analyzed for \textit{DNMT1} message as global mRNA degradation was evident (data not shown). This may have been a consequence of increasing cell detachment or apoptotic DNA cleavage.

\textit{Wildtype APC inhibits cell proliferation}

\textit{DNMT1} inhibition has been previously shown to inhibit the proliferation of human cancer cells \textit{in vitro} (10,11). Here we show that the reduction in \textit{DNMT1} mRNA following APC induction coincides with inhibition of cell growth. HT29APC colon carcinoma cells supplemented with 100 \mu M ZnCl\textsubscript{2} to induce full length APC (Figure 3B) rapidly reach a plateau in proliferation (Figure 3A) only 6 h after initiation of Zn induction. Further expression of wildtype APC for 2 days inhibited cell growth by 67\%, while \textbeta-galactosidase induction in control cells had little effect on proliferation at either time.
Morin et al. have previously shown that the growth plateau following wildtype APC reconstitution persists for at least 5 days and is the result of apoptosis (25).

Because of the links shown between DNMT1 and elements of cell cycle regulation (31) and DNA replication (32), we sought to discover whether the reduction in DNMT1 expression was a result of the either transcription regulation or the growth effects of differing APC status. We first inhibited HT29APC cell proliferation by limiting exposure to fetal calf serum to one-tenth of its concentration in normal media. We found that this results in cell growth inhibition without overt cell death (data not shown). No differences were seen in the level of DNMT1 transcription for the serum starved cells versus the untreated counterparts (Figure 4A). Serum deprived cells were then treated with ZnCl2 to induce wildtype APC. Full-length APC protein expression results in a 61% reduction of DNMT1 mRNA (Figure 4D) in serum deprived HT29APC cells.

We then investigated whether the tumor suppressor p53 could modulate the expression of DNMT1, and whether wildtype APC reconstitution was additive. The parent HT-29 cell line harbors mutant p53 (33). Transient transfection of a human wildtype p53 expression vector resulted in a one-third reduction of DNMT1 message, to almost 34% of that with p53 expression alone, was effected by induction of wildtype APC (Figure 4B,D). These results indicate that while proliferation status is a factor in the degree of expression of DNMT1 in certain contexts, so too does APC have a role in the transcriptional activation of this methyltransferase protein.

Dominant-negative TCF inhibits the transcription of DNMT1

To further test the hypothesis that DNMT1 is one of the downstream effectors of APC/β-catenin/TCF signalling, we took advantage of an N-terminal deletion dominant negative mutant of the human transcription factor TCF4 (DNTCF4 (34)). This dominant-negative mutant has been previously shown to suppress transcription of APC/β-catenin/TCF regulated sequences. We infected HT-29 cells with either an adenovirus expressing this N-terminal mutant or a control adenovirus expressing β-Gal at the indicated MOI. RNA was extracted from the cells 48 h after initiation of infection and was subjected to a northern blot analysis. As seen in Figure 5, DNMT1 mRNA levels are diminished with increasing expression of DNTCF4.

To further ensure that reduction of DNMT1 was mediated through TCF activation in cis, we performed transfections of a DNMT1 expression construct that lacks the 5’ DNMT1 promoter, driven instead by a CMV promoter lacking TCF consensus sites. In HT29APC cells, activation of APC by Zn had no effect on the expression of the exogenous DNMT1 gene (Figure 3F). Transfection and expression efficiency was
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\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig4}
\caption{Reduction of \textit{DNMT1} is not solely a result of growth inhibition. (A) HT29APC cells were arrested by serum starvation (SS) or human wildtype p53 expression (p53) or left in normal proliferating conditions (untreated, UT). Total RNA was resolved and probed with a radiolabeled \textit{DNMT1} probe (upper panel) and 18S (lower panel). (B) Serum starved and p53 transfected HT29APC cells were treated with Zn to induce full-length APC. Total RNA was resolved and probed with similar probes as in (A). (C–D) Quantification of northers in A and B, respectively. \textit{DNMT1} mRNA signal is normalized to 18S rRNA signal. (E) Western blot of p53 treated cells. p53 protein is indicated, arrowheads indicate degradation products of exogenous p53 and mutant p53 isoforms. (F) Western blot showing \textit{DNMT1} expression driven by a CMV promoter is not affected by wildtype APC induction. CMV–GFP serves as a transfection/expression control.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig5}
\caption{Inhibition of TCF transcription decreases \textit{DNMT1} mRNA. HT-29 cells were treated with increasing multiplicities of infection of an adenovirus expression vector harboring either a dominant negative human TCF4 or a \(\beta\)-galactosidase cassette. Total RNA was extracted and subjected to northern blot analysis with either a radiolabeled \textit{DNMT1} probe (A, upper panel) or 18S rRNA (A, bottom panel). Quantification is depicted in (B) showing \textit{DNMT1}/18S ratio.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig6}
\caption{DNMT1 antisense inhibition of colony formation

To test whether increased \textit{DNMT1} expression plays a causal role in the pathway leading from APC mutation to cellular transformation, we determined whether an antisense knockdown of \textit{DNMT1} in HT-29 cells inhibited the transformed properties of these cells using anchorage-independent growth as an indicator of tumorigenicity (35). We have previously designed and demonstrated the efficacy of \textit{DNMT1} antisense oligonucleotides for decreasing cell line tumorigenicity \textit{ex vivo} and \textit{in vivo} (12). MG88 is a human \textit{DNMT1} antisense that has previously been shown to inhibit \textit{DNMT1} mRNA at an EC\textsubscript{50} of 80 nM (11,14). Three daily treatments of HT-29 cells with 200 nM doses of either MG88F or the mismatched control MG208 were performed. Colonies were visible in the soft agar matrix after 7–10 days, and were quantified after 21 days in agar. Digital images representative of triplicate wells are shown in Figure 6A–B. HT-29 cells treated with MG88F lose the ability to grow in a semi-solid medium, with visible colony number being reduced in MG88F-treated cells by 89% as compared with control treatment (Figure 6C). Figure 6D shows intracellular localization of the fluorescein-tagged MG88F with intense accumulation in the nucleus 4 h after transfection on Day 1. Treated cells were also plated on plastic cell culture plates to insure that viable contact dependent growth in standard conditions was not inhibited (data not shown).

\section*{Discussion}

The APC mutation-triggered pathway plays an important role in both hereditary and nonhereditary colon carcinoma but the mechanisms initiated by this pathway that are critical for cellular transformation remain to be clarified. The HT-29 colorectal cancer cell line and derivatives thereof were used for these experiments since like the majority of CRC cell lines, HT-29 cells lack the full-length APC protein (36), instead expressing truncated forms of APC that lack the \(\beta\)-catenin-binding domain. To investigate the role of APC in normal epithelia, or the lack of it in CRC, stable transfection of the
cotransfection of wild type APC suppresses $DNMT1$ promoter driven CAT activity in transient transfection assays. Third, induction of wild type APC in HT29 cells leads to suppression of $DNMT1$ mRNA levels that is at least partially independent of the cell proliferation inhibition caused by full length APC, and fourth, ectopic expression of a dominant negative mutant of TCF4 suppresses $DNMT1$ mRNA.

Additionally, our data indicate that this upregulation is critical for the transformation process triggered by APC since knockdown of $DNMT1$ levels by antisense oligonucleotides reduce the tumorigenicity of these cells.

These data provide an explanation to the previously observed genetic linkage between the APC mutation and $DNMT1$. In those data, abrogation of the $dnmt1$ gene within a murine model of FAP with a homologous mutation in $Apc$ ($Apc^{Min/+}$) resulted in fewer spontaneous intestinal polyps. Further reduction of DNA methyltransferase burden by treating these mice with the $DNMT1$ suicide substrate 5-aza-2′-deoxycytidine, inhibited polyp load to an even greater extent. In a recent study, it has been shown that $Min$ mice that are hypomorphic for $DNMT1$ expression are protected from colon carcinoma. The observation that $DNMT1$ expression is induced by APC mutation provides a possible explanation for this linkage.

$DNMT1$ is responsible for maintaining the DNA methylation pattern during DNA replication. It has been previously proposed that $DNMT1$ overexpression might be triggering cellular transformation by methylation-dependent and independent mechanisms (8). It has been shown that increased $DNMT1$ activity can lead to hypermethylation of CG islands in both cell culture and in vivo (38). Hypermethylation of CG islands and silencing of tumor suppressor genes is frequently observed in cancer and has been shown to be involved in the silencing of tumor suppressor genes (8). Herman et al. have shown this to be the case for the majority of sporadic CRC tumors with microsatellite instability, indicating hypermethylation of the mismatch repair gene $hMLH1$ promoter (39). Others have shown this hypermethylation of mismatch repair genes as well (40). There appears to be a positive feedback relationship between APC and its methylation, where in both sporadic and inherited CRC, mutations in $Apc$ lead to increases in $DNMT1$, which in turn often causes hypermethylation of $Apc$ (7). Evidence exists to indicate an association between either methylation or DNA methyltransferase and CRC. Focal hypermethylation of promoter regions in $hMLH1$, a mismatch repair gene, has been shown in replication error-prone tumors of both HNPCC and sporadic CRC patients, and as such, a decrease in hMLH1 protein may result (39–41). This lack of mismatch repair leads to genomic instability. In addition, $DNMT1$ can also suppress tumor suppressors such as p21 directly by a mechanism that does not involve DNA methylation (13,42). It is possible that both mechanisms are involved in cellular transformation in response to APC mutation in CRC.

An open question is how does the APC-TCF pathway regulate $DNMT1$ expression? APC suppresses the transcription factors TCF/LEF whereas mutant APC is not inhibitory. While the promoter element of $DNMT1$ investigated here lacks consensus TCF/LeF binding sites (43), it is possible that TCF is binding to the DNA at nonconsensus sites, or that activated TCF is an intermediate transcription factor, upregulating another signalling molecule which in turn increases the transcription of $DNMT1$. AP-1 activity has previously been shown to be induced by activation of TCF/LEF in colorectal cancer cell lines (44). As AP-1 is a ubiquitous transcription factor,
with several putative sites within the DNMT1 promoter region, we tested this latter hypothesis. No AP-1 binding was found, however, in the minimal promoter region used in our experiment (data not shown), in agreement with previous results that demonstrated the presence of AP-1 responsive elements outside the 5’ minimal promoter region (29). Though the exact mechanisms by which mutations in APC affect the expression of DNMT1 are not yet clear, our data indicate that reconstitution of wildtype APC protein quickly diminishes the transcription of DNMT1 mRNA. Within nine hours of wildtype APC induction in stably transduced HT-29APC cells, DNMT1 mRNA levels were abrogated to at least half of those in the control HT-29βGal cells. The rapid elimination of DNMT1 message following full-length APC production indicates either transcriptional inhibition or augmented mRNA degradation. However, given that mutant APC has already been shown to be a transcriptional activator through decreased β-catenin elimination, and the effect that APC has on DNMT1 promoter activity in transient transfection assays, it is likely that DNMT1 transcription is upregulated by the APC mutation. Further experiments are required to identify the cis acting sequences in the DNMT1 promoter that respond to the APC/β-catenin/TCF pathway.

If augmented expression of DNMT1 is a key element in the tumorigenesis of APC mutated CRC, then it may be possible to suppress the neoplastic activity by inhibiting the production of DNMT1. We have previously developed modified oligonucleotides that are antisense to DNMT1, and shown their efficacy both in vitro and in vivo with the non-small cell lung carcinoma A549 cell line (12). These oligos bind to DNMT1 mRNA and the heterodimer is degraded by RNase H. Antisense oligos developed to target DNMT1 are now in clinical trials for both renal carcinoma and head and neck squamous cell cancer, but have yet to be tried as a treatment for colon cancer. Our data suggest that this treatment might be relevant for APC-mutated CRC.

A variety of cancers in addition to CRC have abnormal β-catenin/TCF-driven transcription and these cancers may result from increased production of DNMT1 (45–51). As the majority of HNPPC patients exhibit genetic mutations in the APC/β-catenin/TCF pathway, familial history of colorectal cancer could provide reason to screen for such mutations. Therefore, along with treating CRC patients, the possibility exists to employ DNMT1 antisense therapies to mutation-positive individuals in a prophylactic paradigm, and move treatment to the presymptomatic stage.

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