



Published in final edited form as:

Cancer Biol Ther. 2009 January ; 8(1): 1–7.

Epigenetic downregulation of the DNA repair gene *MED1/MBD4* in colorectal and ovarian cancer

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Abstract

MED1 is a base excision repair enzyme that interacts with the mismatch repair protein MLH1 and maintains genomic integrity by binding methylated DNA and repairing spontaneous deamination events. MED1 mutations have been associated with microsatellite instability and accelerated colorectal cancer (CRC) tumorigenesis. We propose that promoter methylation may serve as an alternative epigenetic mechanism for MED1 gene suppression during sporadic CRC tumorigenesis. Methylation status of the MED1 promoter was investigated in a panel of ovarian and colorectal cancer cell lines. The MED1 promoter region was sequenced following bisulfite treatment and sequence analysis identified a CpG island within the MED1 promoter which is frequently and preferentially methylated ($\geq 50\%$) in ovarian and colorectal cancer cell lines with low/reduced MED1 expression. In vitro reversal of methylation restored MED1 expression. In colorectal cancer patients, when MED1 methylation was present, both tumor and matched mucosa were affected equally (mean frequency of methylation 24%) and there was no correlation between methylation and tumor stage. Patients without history of CRC showed significantly lower frequency of methylation (mean 14%, $p < 0.05$). Decreased MED1 transcript levels were observed in matched normal mucosa when compared to controls (median fold difference 8.0). Additional decreased expression was seen between mucosa and matched tumor (median fold decrease 4.4). Thus, MED1 promoter methylation and gene silencing occur in sporadic CRC patients and represent an early event in CRC tumorigenesis. Detection of MED1 methylation and gene suppression in normal colon mucosa may contribute to identifying patients at higher risk of developing CRC during screening procedures.

Keywords

MED1; colorectal cancer; ovarian cancer; methylation

INTRODUCTION

Tumors of epithelial cell origin, or carcinomas, comprise approximately 85% of all diagnosed human cancers. Two of the most frequent carcinomas are colorectal adenocarcinoma and epithelial ovarian cancer. The Centers for Disease Control report colorectal cancer (CRC) as

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the second leading cancer cause of death.¹ It is the third most common cancer in both men and women in the United States. In 2007 it is estimated that over 150,000 cases of CRC will be diagnosed and over 52,000 deaths will be attributed to the disease in an estimated US population of 301.6 million. Ovarian cancer also plays a significant role in cancer deaths for women. Nearly 15,000 deaths from ovarian cancer were recorded for the year 2003 with an estimated US female population of 147.7 million and it is predicted to be the fifth leading cause of cancer death in women for 2007 with over 22,000 new cases predicted to be diagnosed with an estimated US female population of 152.9 million.²

The causes of both CRC and ovarian cancer are multifactorial but inactivating mutations in tumor suppressor genes such as *MLH1* and *BRCA1* leading to tumorigenesis are well established.^{3,4} Epigenetic modifications of DNA have also become an important mechanism in tumorigenesis. Epigenetic changes are heritable changes that affect DNA expression without changing the DNA sequence *per se*. Epigenetic changes include DNA methylation, histone modifications, and chromatin remodeling that can either activate or silence genes. When present, these changes result in altered chromatin structure and a subsequent silencing of genes due to the resulting condensed chromatin.⁵ In this article, we use the term epigenetic downregulation to refer to the silencing effects of DNA methylation. Importantly, gene inactivation through DNA methylation has been implicated in the development of cancer.⁶ This epigenetic modification of the genome generally occurs at CpG islands located in gene promoters. DNA methylation is associated with alterations of the chromatin structure which ultimately leads to transcriptional silencing of the gene. Although promoter methylation may be physiologic as seen with imprinted genes and the inactive X chromosome,^{7,8} it is more frequently associated with tumor suppressor genes and the development of cancers including renal, hematologic, ovarian, and colorectal.⁸⁻¹⁰

The importance of DNA methylation with subsequent gene silencing in CRC is well described.¹¹⁻¹⁶ Methylation of multiple tumor suppressor genes has been identified in CRC as part of a “CpG island methylator phenotype”.¹³ The DNA mismatch repair (MMR) gene *MLH1* is one of several genes observed to be methylated in CRC but has also been shown to be methylated in ovarian cancer.¹⁷ *MLH1* methylation and silencing has specifically been associated with the development of microsatellite instability (MSI) in sporadic CRC.^{11,16}

The base excision repair (BER) enzyme *MED1*, also known as *MBD4*, has been shown to interact with the MMR protein *MLH1* and bind methylated DNA.^{18,19} In addition to BER, *MED1* plays multiple functions in cellular processes including DNA damage response, apoptosis, and transcriptional repression.²⁰⁻²⁴ The protein structure of *MED1* reveals an amino-terminal 5-methylcytosine binding domain (MBD) and a carboxy terminus with DNA *N*-glycosylase and *MLH1* complex formation capabilities.¹⁹ Specifically, *MED1* acts as a thymine and uracil glycosylase that recognizes and repairs G:T and G:U mismatches originating from spontaneous deamination events of 5-methylcytosine and cytosine at CpG sites.^{18,25} If not repaired, these mismatches would cause CpG to CpA or CpG to TpG transition mutations.^{23,26}

MED1 mutations that affect its BER function have been described in CRC. Mutations observed in MSI+ CRC result in a truncated protein predicted to retain the MBD but not the glycosylase domain.^{27,28} The known mutations in *MED1* have been related to MSI+ tumors only. Alternative mechanisms of gene inactivation, such as silencing by promoter methylation, have not been described thus far for *MED1*. By analogy with other tumor suppressor genes of the caretaker type, we propose promoter methylation as a plausible mechanism leading to *MED1* gene silencing in sporadic colorectal cancers. The specific comparison to *MLH1* is based on the interactions between the two proteins as well as the caretaker roles these two proteins play in CRC.^{11,19,29} Methylation of the *MED1* promoter may potentially lead to

decreased gene expression causing an increase in CpG site mutations, decreased apoptosis and accelerated tumorigenesis similar to murine knockout models.³⁰ Here we report identification of CpG islands within the *MED1* promoter and increased methylation with reduced expression in both CRC and ovarian cancer.

MATERIALS AND METHODS

Cell lines

A panel of colorectal cancer cell lines (SW403, DLD1, CaCo2, SW480, HT29, RKO, SW948, HCT116, and WiDr) and a panel of ovarian cancer cell lines (OVCAR-2, OVCAR-3, OVCAR-4, OVCAR-8, OVCAR-10, and OVCAR-13) were obtained from ATCC and cultured according to their guidelines. Cells were incubated in a humidified atmosphere at 37°C with 5% CO₂.

Cloning of the *MED1* promoter CpG island

A human genomic lambda bacteriophage library (Stratagene) was screened with an exon 1 probe derived from *MED1* cDNA, as previously described.³¹ Clones that hybridized to the probe were identified, subcloned, and sequenced. The *MED1* gene sequence was later confirmed by comparison with human genome project data. Location of the CpG sites was done with the program Map of the Genetics Computer Group (GCG) Software Package (Wisconsin Package Version 9.1).

Clinical specimens

Patient samples were procured from the Department of Surgery at UAB, following IRB-approved methods and informed consent. Thirty-nine human tissue specimens were collected at time of surgery. Samples included matched normal colon mucosa and tumor from each patient. Five specimens had synchronous colon adenomas that were also evaluated. Three patients had only adenomatous disease and no colorectal cancer was identified. Tissues were macrodissected, snap frozen in liquid nitrogen, and stored at -80°C. Normal colonic mucosa was obtained in the operating room from fresh, surgically resected colon specimens. Mucosa was harvested from the clinically negative margin of the specimens and macro-dissected from the colonic basement membrane prior to the preservation process. Tumor and polyp tissue were also harvested in the operating room from the clinical specimens. The tumors were grossly incised to obtain tissue. The amount of stroma tissue was not quantified in our specimens; it is known that colon adenocarcinomas are constituted by a majority of epithelial cells. Patients' age ranged from 25 to 87 years old (mean age 61.0, median age 66). The group included 54% males and 46% females. Eighty percent of the patients were Caucasian and 20% were African-American (Table 1). Control tissue of normal colon mucosa was obtained from six patients with no history of CRC or polyps (Table 1).

Identification and analysis of *MED1* CpG island methylation

DNA was extracted from ovarian cancer cell lines, colorectal cancer cell lines, and clinical specimens using AquaPure Genomic DNA Isolation Kit (Bio-Rad, Hercules, CA). DNA from all specimens was subjected to a bisulfite reaction³² using the EZ DNA Methylation kit following the manufacturer's recommendations (Zymo Research, Orange, CA). Products from the bisulfite reactions were subjected to PCR using the following primers designed to amplify a region of the *MED1* promoter upon bisulfite conversion: 5'-GTTATTTTATAAGTTATTTTGGTTATT-3' and 5'-CACCAATCAAATCCATTCTC-3'. PCR reactions contained 1x PCR buffer (Denville Scientific, Inc, Metuchen, NJ), 0.5mM dNTP mix, 0.5μM of each primer, and 0.04U/μL of Choice Taq Blue DNA Polymerase (Denville Scientific). After an initial denaturation step of 95°C for five minutes, DNA was amplified

through 35 cycles of 30 seconds denaturing at 95°C, 30 seconds annealing at 54°C, and 30 seconds extension at 72°C. The products were resolved on a 1.6% agarose gel in TAE buffer and visualized by UV light after staining with ethidium bromide. PCR products were purified using PureLink PCR Purification Kit (Invitrogen, Carlsbad, CA). After purification, amplified products of the promoter region of *MED1* were subcloned into pGEM-T Easy or pCR 2.1 vectors (the latter using TOPO TA cloning kit, Invitrogen), to produce PCR fragment libraries of mucosa and tumor for each matched specimen. Individual inserts were PCR amplified using M13 primers flanking the polylinker sites vectors to verify a size-appropriate insert. Correct size inserts were identified as described above and plasmids were directly sequenced using M13 (-20) reverse primer.

***MED1* promoter demethylation in ovarian and CRC cell lines**

OVCAR-4 and DLD-1 cells were grown to confluence, split 1:4 and treated with a 5µM final concentration of the demethylating agent 5-azacytidine in culture media (Sigma, St. Louis, MO) for five days.³³ Every 24 hours the culture media was changed and supplemented with fresh medium containing 5-azacytidine. During the final 12 hours, cells were also treated with 500 nM trichostatin A (TSA) (Sigma) suspended in 100% EtOH. An equivalent amount of 100% EtOH was added to the control cells. Cells were viable at the end of the treatment cycle. DNA and RNA were extracted from both treated and control cell lines. Cytotoxicity assays for 5-azacytidine and TSA were not performed.

***MED1* Gene Expression**

RNA was extracted from specimens using Trizol reagent (Invitrogen) according to manufacturer's protocol. cDNA was synthesized using High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). For mRNA expression analysis by quantitative real time PCR, TaqMan® Gene Expression assays (Applied Biosystems, Foster City, CA) were used for *MED1* (Hs00187498_m1) and ribosomal *S9* (forward-5'-ATCCGCCAGCGCCATA-3', reverse-5'-TCAATGTGCTTCTGGGAATCC-3', and probe-5'-6FAMAGCAGGTGGTGAACATCCCGTCCTTTAMRA-3'). Twenty-five microliter reactions contained 1x TaqMan® Universal PCR Master Mix, 1x expression assay, and 2µL of cDNA. *S9* was used as the endogenous control for gene expression in specimens.³⁴ A cDNA control stock was made using equal amounts of RNA from the six control patients: 500 ng of RNA from each patient were combined and cDNA was synthesized as described. This stock solution was used as a normal *MED1* expression value for colon mucosa. *MED1* and *S9* expression were determined by measuring PCR product fluorescence compared to cycle number to determine C_T values. Relative *MED1* expression for each tissue sample ($\Delta C_T = MED1 C_T - S9 C_T$) was calculated so that matched specimens could be compared. The change in *MED1* expression ($\Delta MED1$) between normal and tumor specimens was calculated with the formula ($\Delta MED1 = 2^{\Delta Normal C_T - \Delta Tumor C_T}$). The change in *MED1* expression was also evaluated between control mucosa and morphologically normal colon mucosa from cancer patients using a similar equation ($\Delta MED1 = 2^{\Delta Control C_T - \Delta Normal C_T}$). All gene expression studies were performed in triplicate and repeated.

***MED1* mutational analysis**

Primers were designed to amplify exon 3 of *MED1* including an A₁₀ track representing codons 310–313 and three A₆ tracks representing codons 247–248, 280–282, and 327–329. DNA was amplified using *MED1* primers forward-5'-AAAGGTTAGAAAGCCCAAAGG-3' and reverse 5'-GCAAATGTTCTTTCTTTCCA-3' following previously described conditions,²⁸ purified with PureLink PCR Purification Kit (Invitrogen) and sequenced. Colorectal cancer cell line HCT116 was used as a positive control for screening of mutations at the A₁₀ motif due to its previously described point deletion at this site.^{27,28}

Statistical analysis

SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL) was used for statistical analysis of patient data. Patient age, relative *MED1* expression, and change in *MED1* expression were given as medians (range) or means (standard deviation). Differences between control individual and cancer patients were compared using a t-test, Fischer's exact test, or chi-squared test, as appropriate. Correlations for *MED1* expression, methylation status, cancer stage, age, race, tumor histology and tumor location were performed using Spearman's rho. Survival was evaluated using Kaplan-Meier plots. Significance was set to $p < 0.05$.

RESULTS

Analysis of *MED1* gene expression using quantitative real time-PCR

MED1 expression was compared in normal mucosa and matched tumor from individual cancer patients to identify changes in gene expression associated with the development of sporadic CRC. Colorectal adenocarcinoma tissue showed decreased *MED1* expression when compared to matched normal colon mucosa (Fig. 1). The median change in expression was -8.00 fold ($+0.84$ to -52.71). Following the paradigm of the adenoma-carcinoma sequence, we evaluated gene expression in adenomatous tissue to identify when these changes occur in CRC tumorigenesis. *MED1* expression was decreased in colorectal adenomas ($n = 8$) when compared to matched normal mucosa. The median change in adenoma expression was -4.44 fold (-1.29 to -7.49). *MED1* suppression was not limited to adenomatous tissue and tumor. A decrease in *MED1* expression was also observed in normal mucosa from the cancer specimens when compared to normal mucosa from control patients (Fig. 2). The median change observed was -3.9 fold ($+3.87$ to -30.55). *MED1* expression consistently decreased in colorectal cancer specimens as they progressed from normal mucosa to the invasive carcinoma phenotype.

Evaluation of clinical specimens for previously described *MED1* mutations associated with MSI+ tumors

To account for alternative mechanisms of reduced gene expression, DNA from clinical specimens and CRC cell lines DLD1 and HCT116 were evaluated for previously described *MED1* mutations in exon 3. Four poly-adenine sites have been documented as known sites for frameshift mutations resulting in a prematurely truncated protein.^{27,28} *MED1* mutational analysis of all 39 CRC patients revealed no mutations in the four poly-A mutational hotspots within exon 3 of the *MED1* gene (data not shown). The positive control CRC cell line HCT116 did show a loss of a single adenine in the A_{10} sequence as previously described.²⁸

Identification of *MED1* gene promoter CpG islands

In order to isolate the promoter region of *MED1*, a probe derived from exon 1 of *MED1* was used to screen a human genomic library. Several clones were isolated and subcloned into smaller fragments. Subcloned fragments hybridizing with the probe were sequenced, which allowed assembling of the tentative promoter region of the gene. In order to determine whether the promoter region of *MED1* may contain a CpG island, we mapped the CpG sites by sequence analysis. Sequences were later confirmed by comparison with the Human Genome Project. Sequence analysis revealed a concentration of approximately 74 CpG sites centered around the exon 1 transcription start site comprising a putative CpG island within the promoter (Fig. 3).

Identification of *MED1* promoter methylation

In the absence of mutations, promoter methylation was evaluated as a cause of *MED1* downregulation in our clinical specimens. We focused on the 22 CpG sites most proximal to the transcription start site. To determine whether these CpG sites in the proximal region of the

MED1 promoter were methylated, genomic DNA was first treated with sodium bisulfite, a reaction that exclusively converts unmethylated cytosines into uracils. Methylated cytosines are protected from the reaction allowing identification of methylated sites.³² Analysis of DNA methylation in the *MED1* promoter from our six ovarian cell lines, nine colorectal cell lines and 39 patient specimens revealed preferential methylation of CpG sites 14 and 16 of the 22 sites evaluated from the *MED1* promoter region. DLD1 and OVCAR-4 were the only cell lines that consistently showed methylation of the *MED1* promoter. Methylation was not restricted to tumor tissue in patients with CRC. When methylation was present in clinical subjects, methylation was observed in both normal mucosa and matched colorectal adenocarcinoma (Fig. 4). Methylation of the *MED1* promoter was consistently detected in both cell lines and clinical specimens.

Frequency of methylation in cell lines and clinical specimens

In order to derive semiquantitative measurements of the extent of DNA methylation of the *MED1* promoter, PCR fragments after bisulfite treatment were subcloned in plasmids and used to transform bacteria. Independent bacterial colonies (ranging in number from 5 to 26) from each sample were sequenced, thus allowing an estimation of the frequency of methylation. Frequency of methylation revealed a wide range in cell lines and patients. Promoter methylation in ovarian cell lines ranged from 22% to 75%, and in colon cancer clinical specimens ranged from 0% to 100%. The average frequency of methylation in ovarian cell lines was 50% (\pm 18%) and in colon cancer patients was 24% (\pm 19%). Colon mucosa from the six control patients with no history of cancer was evaluated and consistently showed low frequency of methylation. The average frequency of methylation for these control patients was 14% (\pm 9%). When frequency of methylation was compared between cancer patients and control patients by t-test, these frequencies were significantly different ($p < 0.001$).

Evaluation of demethylation on *MED1* gene expression

As mentioned, *MED1* methylation was detected both in cell lines and in clinical specimens. To determine the effect of this epigenetic change on the *MED1* transcription, experiments were conducted to demethylate the promoter and subsequently evaluate gene expression. Cells were treated with the demethylating agent 5-azacytidine (AZA) and the potent histone deacetylase inhibitor trichostatin A (TSA). The addition of TSA has been shown to act synergistically with DNA demethylating agents in reactivating gene expression.³⁵ Sequencing of ovarian cell line OVCAR-4 and CRC cell line DLD-1 had previously revealed methylation at sites 14 and 16. Treatment of these two cell lines with AZA and TSA resulted in a 2.5 and 7.8 fold increase, respectively, in *MED1* expression when compared to baseline levels.

Statistical analysis

Correlations were performed in CRC patients between *MED1* expression, frequency of methylation, and clinico-pathological variables, such as age, sex, tumor stage, race, anatomic location, lymph node status, and survival. Statistical analysis revealed a trend towards more advanced tumor stage and decreased *MED1* expression, though this did not reach statistical significance ($p = 0.081$). Frequency of methylation negatively correlated with patient age ($p < 0.05$). No correlation with tumor location was detected.

DISCUSSION

Our findings demonstrate that expression of the tumor suppressor gene *MED1* is decreased in sporadic colorectal cancer and that it can be attributed to promoter methylation. We suggest that *MED1* is suppressed in a large proportion of sporadic colorectal cancers in the absence of known *MED1* mutations. Additionally, there is a significantly lower incidence of *MED1* promoter methylation in normal control patients when compared to patients with colorectal

cancer. Our *in vitro* studies show that reversal of *MED1* methylation leads to increased gene expression, suggesting that methylation does contribute to gene suppression. The importance of these findings stem from the fact that *MED1* methylation and gene suppression seem to develop in normal colon mucosa prior to the development of the adenoma-carcinoma sequence. The epigenetic regulation of the tumor suppressor gene *MED1* thus seems to be associated with tumor initiation rather than tumor progression. This is corroborated by the lack of a significant decrease in *MED1* expression correlated to more advanced tumor stages that would imply a role in tumor progression. Gene suppression can be detected in the normal colon mucosa of tumor-bearing patients as well as in polyps and carcinomas from matched specimens.

MED1 promoter methylation observed in tumor tissue is also present in adjacent, matched normal mucosa. This phenomenon of methylated tumor suppressor genes in tumor and adjacent normal mucosa has previously been reported in sporadic CRC and may reflect a “field effect” involving the mucosal lining of CRC patients. Epigenetic changes have been implicated as an early event in CRC tumorigenesis.^{12,36} We propose that a similar mechanism involving *MED1* plays a role in sporadic CRC tumor initiation. The resulting CRC patient decrease in *MED1* gene expression can be readily detected in the associated normal mucosa when compared to control samples. Normal colonic mucosa *MED1* gene methylation and suppression has also been reported for the *MLH1* promoter.³⁷ The 56% increase in the amount of *MED1* promoter methylation observed in mucosa from cancer patients further supports the role of promoter methylation as a plausible cause of *MED1* suppression in these patients.

Our *in vitro* studies demonstrated the reversibility of *MED1* promoter methylation restoring *MED1* gene expression, also in concordance with the *MLH1* methylation reports.³⁸ The reversibility of *MED1* expression in ovarian and CRC cell lines suggests that observed promoter methylation does affect gene expression in at least two different types of epithelial cancers. If tissue methylation causes an initial drop in *MED1* expression within normal mucosa, it poses the question as to what additional changes are necessary to induce the downregulation of *MED1* expression detected in carcinomas. As suggested by Jones and Laird, promoter methylation in *MED1* may require a second “hit” to see the ultimate degree of gene suppression observed in tumors. We speculate that methylation may trigger the initial decrease in gene expression and this is followed by mutations or LOH causing greater gene silencing and the development of cancer. An initial decrease in *MED1* expression is seen when DNA from normal mucosa is methylated. This process could enable subsequent genetic changes that would further decrease *MED1* expression, as described in polyps and carcinomas.³⁹

The importance of decreased *MED1* in CRC tumorigenesis has been demonstrated in mouse models with *MED1/MBD4* targeted gene inactivation.^{30,40} Mice with *MED1* knockout in the setting of an *Apc*^{Min/+} background had a significantly reduced survival and increased CRC tumor burden compared to control mice.^{30,40} Work from our and other laboratories suggests an additional role for *MED1* inactivation in tumorigenesis.^{20,21,41} These studies suggest that decreased *MED1* protein levels have an inhibitory effect on apoptosis. These concepts support our findings. The early changes that we have observed in *MED1* expression in colon mucosa from cancer patients suggest that either decreased genomic surveillance, decreased apoptosis, or both, could lead to initiation of tumorigenesis.

A high frequency of *MED1* methylation in cancer patients and the ability to demethylate and restore gene expression in cell lines does implicate a role for methylation in the silencing of this putative tumor suppressor gene. Further, comparison of frequently methylated DNA from CRC patients with DNA from non-diseased patients and their consistently low frequency of methylation status suggest that hypermethylation of the *MED1* promoter is an abnormal occurrence in CRC tumorigenesis. The significance of this finding is that it appears to be an

early occurrence in the development of CRC similar to other genes involved in the development of sporadic colorectal cancer.¹²

In conclusion, two observations suggest that *MED1* promoter methylation and gene suppression are important in CRC tumorigenesis. First, methylation occurs in cancer patients at higher frequencies than those found in patients without cancer. Second, and perhaps most importantly, *MED1* suppression seems to occur first at the level of the normal mucosa. The suppression of *MED1* follows the adenoma-carcinoma sequence and is observed in normal mucosa, adenomas, and adenocarcinoma of the colon suggesting an early and detectable change. Suppression of *MED1* expression and early detection of promoter methylation may necessitate a more frequent and rigorous screening repertoire in this population of patients leading to earlier detection of malignant changes.

Acknowledgments

We thank S. Cortellino and M. Sannai for critical reading of the manuscript; P. Barth and J. Doss for help with DNA sequencing; and R. Sonlin for secretarial assistance. We thank the following core facilities and personnel at the Fox Chase Cancer Center: Cell Culture (S. Howard), Sequencing (A. Cywinski), and the Fannie E. Rippel Biotechnology Facility (P. Do, G. Miller and A. Yeung). This work was supported by NIH grants CA78412 and CA06927, the Robert E. Reed Gastrointestinal Oncology Research Foundation and an appropriation from the Commonwealth of Pennsylvania to the Fox Chase Cancer Center.

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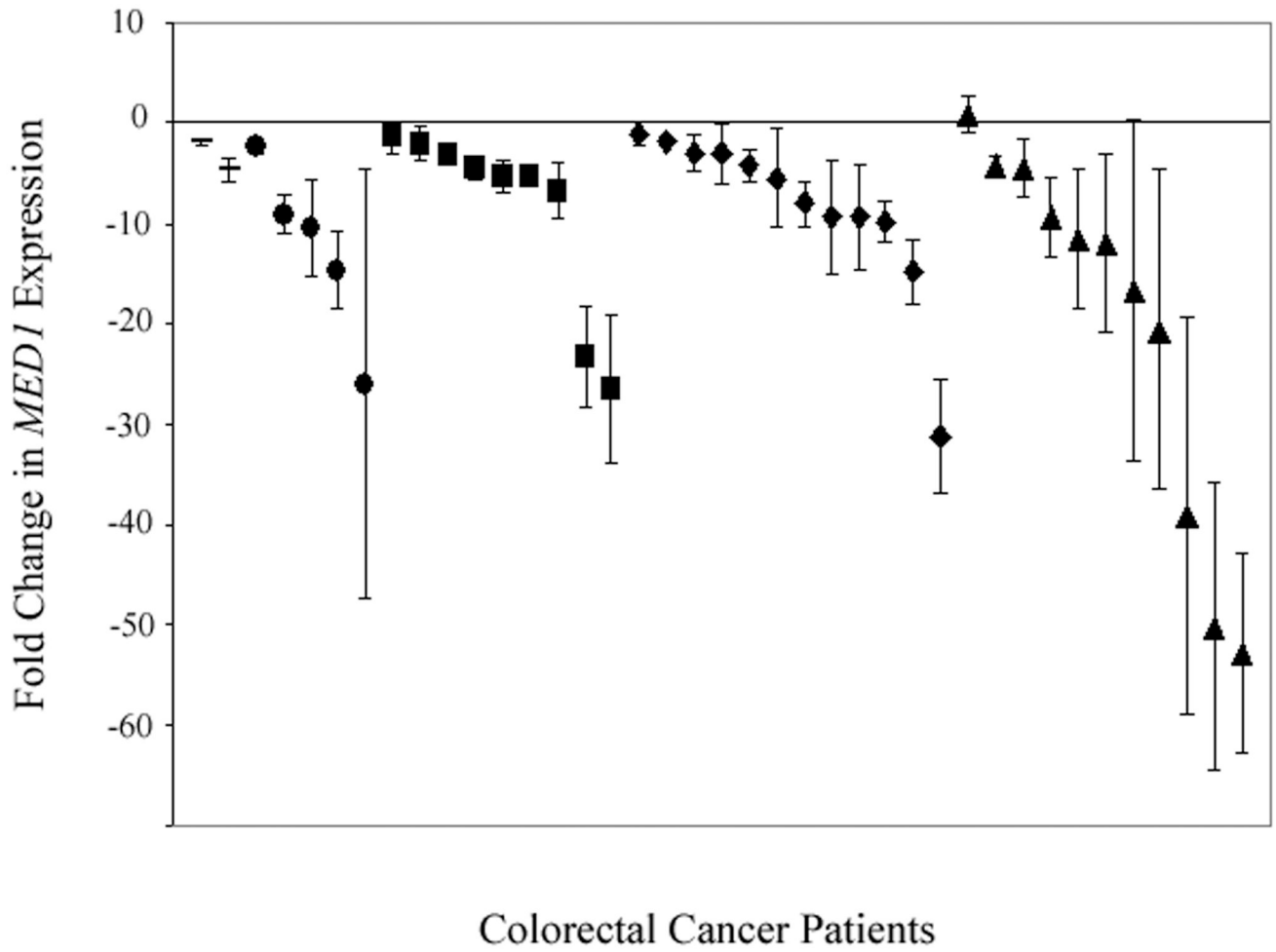


Figure 1.

Relative expression of *MED1* in adenomatous polyp or colorectal cancer specimens (N=39) compared to *MED1* expression in matched normal mucosa from the same individual. (□ = polyp, ● = stage I, ■ = stage II, ◆ = stage III, ▲ = stage IV)

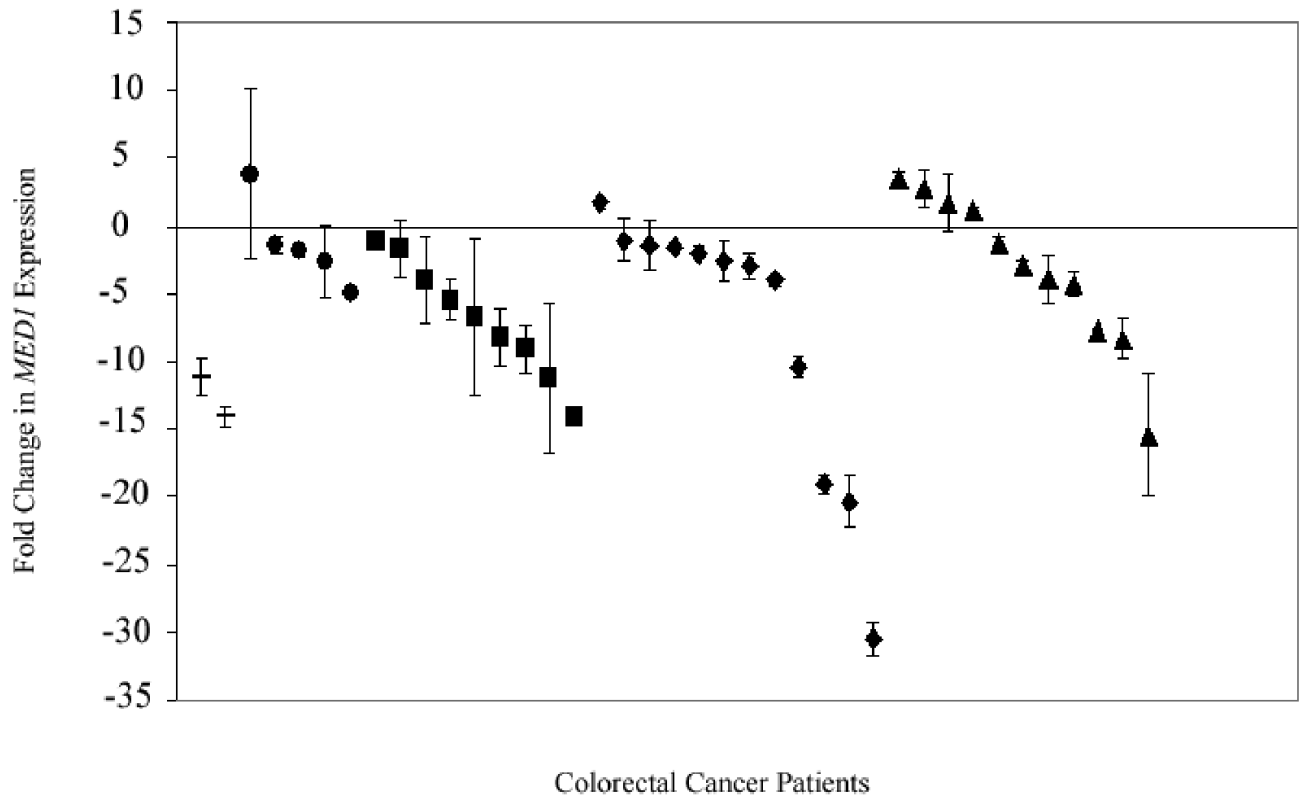


Figure 2. Relative expression of *MED1* in morphologically normal mucosa from patients with polyps or cancer (N=39) compared to *MED1* expression in mucosa from non-cancer control patients. (- = polyp, ● = stage I, ■ = stage II, ◆ = stage III, ▲ = stage IV)

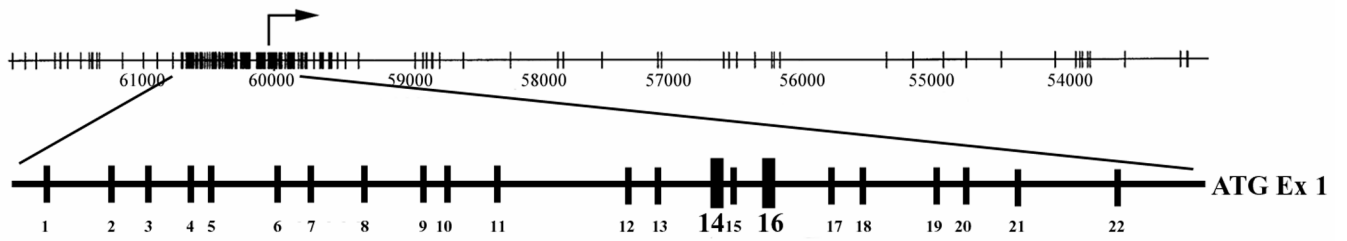


Figure 3.
Schematic of *MED1* promoter with transcription start site indicated by rightward arrow.
Enlarged sequence represents amplified region of promoter with vertical hash marks signifying individual CpG sites. Preferentially methylated sites 14 and 16 are in bold.

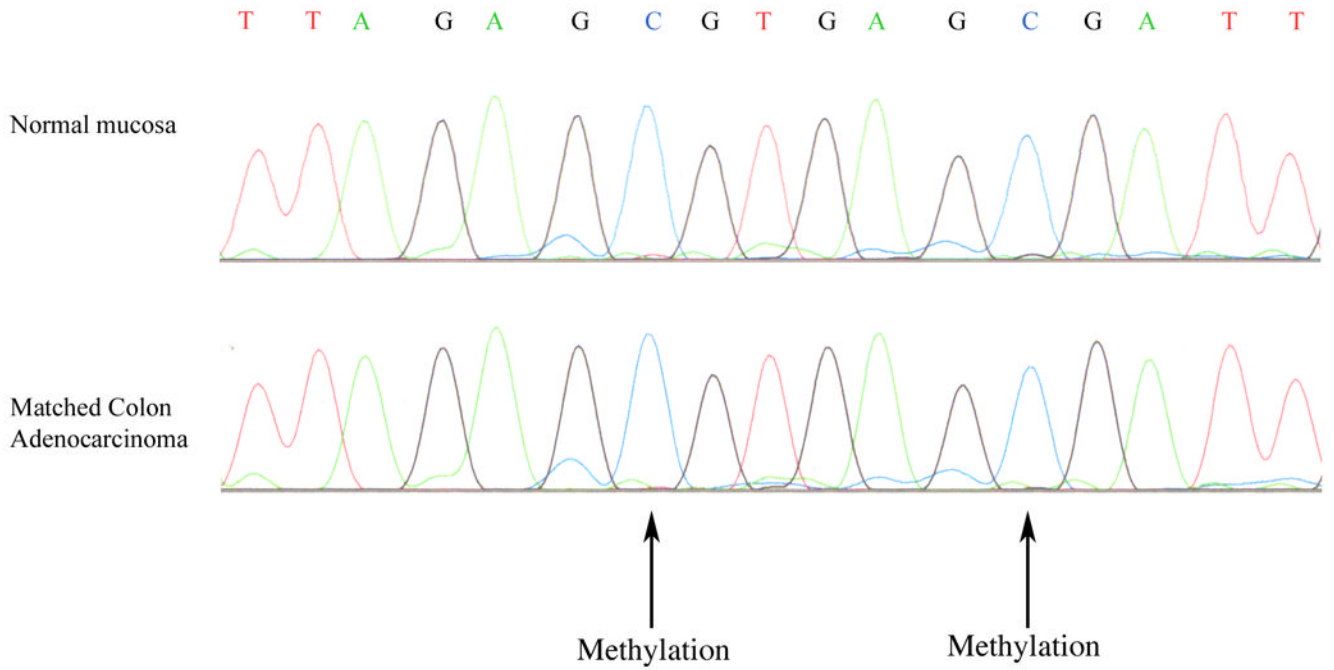


Figure 4. Examples of methylation of the *MED1* promoter in normal colon mucosa and matched colon adenocarcinoma. Methylation of CpG sites 14 and 16 (arrows) is indicated by preservation of cytosine nucleotides in PCR sequences of both normal mucosa and tumor from the same patient.

Table 1

Demographics of control and adenoma/CRC patients

	Control individuals		Adenoma/CRC patients	
Age	<40	1	<40	7
	40–60	3	40–60	8
	60–80	2	60–80	21
	>80	0	>80	3
	N=	6	N=	39
		16.7%		100.0%
Sex	Male	3	Male	21
	Female	3	Female	18
	N=	6	N=	39
		50.0%		100.0%
Race	Caucasian	5	Caucasian	31
	African American	1	African American	8
	N=	6	N=	39
		83.3%		100.0%
		16.7%		79.5%
		100.0%		20.5%
				100.0%
Stage				
			0	2
			I	5
			II	9
			III	12
			IV	11
			N=	39
				5.1%
				12.8%
				23.1%
				30.8%
				28.2%
				100.0%
Tumor Location				
			Ascending Colon	17
			Transverse Colon	1
			Descending Colon	7
			Rectum	11
			n/a	3
			N=	39
				43.6%
				2.6%
				17.9%
				28.2%
				7.7%
				100.0%