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Regulation of h*MSH2* and h*MLH1* expression in the human colon cancer cell line SW1116 by DNA methyltransferase 1

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Abstract

Aberrant DNA methylation is now recognized as an important epigenetic alteration occurring early in human cancer. To directly study the role of DNA methyltransferase 1 (DNMT1) in the regulation of expression of tumor-related genes in human colon cancer cells, we stably transfected expression constructs containing sense or antisense DNMT1 into the human colon cancer cell line, SW1116. The expression level of mismatch repair genes (MMR), human mut-L homologue 1 (h*MLH1*) and human Mut S homologue 2 (h*MSH2*), was monitored by real-time RT-PCR. The methylation status of h*MLH1* and h*MSH2* promoters was determined by bisulfite modification and methylation-specific PCR (MSP). The protein levels of DNMT1, h*MSH2* and h*MLH1* were determined by Western analysis. The results show that DNMT1 protein expression was increased or decreased in transfected cell lines containing sense or antisense DNMT1 constructs, respectively. In cells expressing the sense DNMT1 construct, the expression of h*MLH1* and h*MSH2* was down-regulated through hypermethylation of their respective promoters. Furthermore, antisense DNMT1 expression induced promoter demethylation and up-regulated transcription of h*MSH2* ($P<0.05$) and h*MLH1* ($P=0.064$) in SW1116 cells.
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1. Introduction

Aberrant DNA methylation is now considered an important epigenetic alteration that is intimately involved in the development of human tumors. Aberrant methylation of promoter CpG islands (CGI) in tumor suppressor (TS) genes correlates with the loss of expression of TS genes in cancer cell lines and primary tumors [1,2]. In this regard, aberrant DNA methylation has been implicated to play a role in the development of colon cancer in both mouse and human tumor model systems [3–6].

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Two different pathways for tumor development have been described in colorectal carcinoma: the chromosomic instability, raised by TS genes and proto-oncogene alterations, and the microsatellite instability (MSI), caused by alterations in DNA mismatch repair genes (MMR). Loss of expression and promoter methylation in the major MMR genes, h*MLH1* and h*MSH2*, occurs frequently in primary tumors and cell lines of colorectal cancer with MSI.

Evidence of activation of silenced genes can be pursued by documenting the relationship between 'molecular switches', DNA-demethylating agents and the genes which are turned on by these agents [7]. DNMT1, the major DNA methylating enzyme in mammalian cells, recognizes hemi-methylated CpG dinucleotides in mammalian DNA and catalyzes the transfer of methyl groups to cytosine residues in newly synthesized DNA. While hemi-methylated DNA is the preferred substrate, DNMT1 can also carry out de novo methylation in vitro and in vivo [8,9]. The demethylation of h*MLH1* by 5-aza-2′-deoxycytidine (5-aza-dC), a inhibitor of DNMT1 [10], has been shown to induce re-expression of h*MLH1*. This re-expression of h*MLH1* is accompanied by a decrease in h*MLH1* gene promoter methylation [11].

The first inhibitor directed against the DNMT1 mRNA, an antisense oligonucleotide was described by Fournel. The treatment of cells with DNMT1 antisense oligonucleotides results in the loss of DNMT11 protein, demethylation of the promoter of the tumor suppressor gene *CDKN2A*, and re-expression of *CDKN2A* mRNA [12]. However, expression of antisense oligonucleotides by Fournel is transient and therefore does not address the effect of constitutive expression of DNMT1. Furthermore, to our knowledge no prior studies have addressed the effect of antisense DNMT1 expression on the expression of tumor-related genes in gastrointestinal (GI) cancer.

To further identify the etiological association of MMR gene expression and DNMT1 in human colon cancer, we studied the transcription and promoter methylation status of h*MLH1* and h*MSH2* genes in the colon cancer cell line, SW1116 transfected with antisense or sense DNMT1 constructs. The data from this study show forced expression of DNMT1 regulates the expression of h*MSH2* and h*MLH1* directly through the promoter methylation in SW1116.

2. Methods

2.1. Cell culture

The colon cancer-derived cell line SW1116 was maintained by serial passage in RPMI 1640 containing 10% heat-inactivated FCS, 100 U/ml penicillin and 100 μg/ml streptomycin, and incubated at 37 °C, 5% CO_2–95% air using the standard tissue culture incubators as described previously [13]. The cells were plated as 10^6 cells per 100 mm dish.

2.2. Stable transfection of recombinant DNMT1 expression constructs

The recombinant constructs developed by Vertino et al. containing sense DNMT1 (called HMT) and antisense DNMT1 (called TMH) were used in this experiment. These constructs contain a *Xba* I-*Dra* I fragment in a full-length cDNA encoding DNMT1 [14], which was subcloned into *Bam* HI site of pCMVneo vector [15]. pCMVneo was used as a vector control in all stable transfections. SW1116 cells were grown to a density of 8×10^3 cells/ml in 60 mm dish in RPMI 1640. Thereafter, the different constructs (1 μM) were transfected into the cells using the cationic liposome Lipofectamine (2 μg/μl; Invitrogen, Carlsbad, CA, USA) according to manufacturers instructions. After incubating 16 h, the liposome/DNA complexes were removed and RPMI 1640 containing 10% FCS was added to the cells. Eight hours later, the cells were transfected a second time and incubated for another 16 h. For stable transfections, cells were selected in 400 μg of G418 (Invitrogen) per ml for 4 weeks. G418-resistant cells were maintained in selective medium. Transfected cells were washed with phosphate-buffered saline (PBS) prior to extracting DNA, RNA and protein.

2.3. Detection of transfected genes by RT-PCR

To evaluate the efficiency of the transfection, a bacterial neomycin resistance (Neo) gene [16] contained in the original DNMT1 constructs along with constructs pCMVneo-HMT containing sense DNMT1, pCMVneo-THM containing antisense DNMT1, pCMVneo and mock transfected was monitored using RT-PCR. The total cellular RNA was extracted and purified with TRIzol Reagent (Invitrogen). RNA was re-suspended in DEPC-treated water and quantitated by OD 260/280. An agarose gel using

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