Identification of a p53 Response Element in the Promoter Region of the hMSH2 Gene Required for Expression in A2780 Ovarian Cancer Cells*

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C. Terry Warnick‡, Bashar Dabbas‡, Clyde D. Ford§, and Kevin A. Strait§§

From the Departments of ³Medicine and ³Pathology, Cancer Research Laboratory, LDS Hospital, Salt Lake City, Utah 84143

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Defects in the human MSH2 mismatch repair system have been implicated in cellular mutagenesis, tumorigenesis, and chemotherapeutic resistance. The current studies characterized the 5′ upstream proximal promoter region of the hMSH2 gene using transient transfection of A2780 ovarian cancer cells. Serial deletions of a 1.88-kb fragment of the proximal promoter region of the hMSH2 gene revealed that promoter activity was restricted to the first −281 bp. Targeted deletions within this −281 bp region coupled with specific sequence mutagenesis identified a response element for the p53 tumor suppressor protein located between −242 and −222 bp. The −242 hMSH2 p53 element is configured as a direct tandem repeat palindrome with 80% homology to the p53 consensus binding sequence. Co-transfection of an hMSH2 reporter and p53 expression vector into the p53-null cell line SK-OV-3 produced 10-fold enhanced transcription, which was lost when the −242 to −222 p53 binding site was mutated. These results clearly demonstrate the presence of a previously unidentified p53 response element in the hMSH2 proximal promoter. Its location at −242 bp upstream of the start site of transcription is distinct from two previously reported p53 sites at −447 and −416, which transactivate in Saos-2 cells (Scherer, S. J., Maier, S. M., Seifert, M., Hanselmann, R. G., Zang, K. D., Muller-Hermelin, H. K., Angel, P., Welter, C., and Schartl, M. (2000) J. Biol. Chem. 275, 37469–37473). Finally, in sharp contrast to their activity in Saos-2 cells, deletion of the −447 and −416 sites in A2780 cells had no effect on hMSH2 promoter activity. Thus, it appears that p53 regulates hMSH2 expression through multiple cell type-specific DNA response elements.

In humans, the product of the MSH2 gene is a protein belonging to the DNA mismatch repair (MMR)1 system. The MMR proteins play a critical role in maintaining the fidelity of the cellular genome by correcting errors in base pairing introduced into the newly synthesized “daughter strand,” during DNA replication (1). MMR proteins were first identified in Streptococcus pneumoniae (2) and subsequently characterized in Escherichia coli as the MutS, MutL, and MutH proteins (3). The hMSH2 protein forms the core of the human homologue to the bacterial MutS protein via dimerization with two other members of the MMR family, hMSH3 (4) and hMSH6 (5). hMSH2 and hMSH6 dimers form the hMutSo complex responsible for the initial recognition and targeting of mismatched nucleotides (1).

Defects in the hMSH2 gene have been implicated in the genesis of a number of malignancies of the gastrointestinal, gynecological, and genitourinary tracts (6). In the case of colorectal cancers, hereditary non-polyposis colorectal cancer (HNPCC) syndrome has been shown to have greater than 90% germline mutation of the MMR genes, a large percentage of which are directly attributable to mutations of hMSH2 (7). In a majority of the other cancers associated with MMR defects, mutations arise spontaneously in somatic cells, as is the case in ovarian cancers where recent data indicate that defective MMR proteins are present in ~20% of sporadic tumors (8).

The loss of MMR proteins in tumor cells is also associated with resistance to certain DNA adduct-producing chemotherapeutic agents exemplified by the platinum-based compound, cisplatin. It has been suggested that the loss of MMR proteins in these cells leads to chemotherapeutic resistance via an inability of the cell to link DNA damage to an apoptotic signaling pathway. Support for a link between the MMR system and chemotherapeutic-induced apoptosis comes from the following. 1) The hMSH2 protein MutS complex is responsible for the recognition and binding of cisplatin DNA-adducts (9, and 2) reintroduction of the hMSH2 gene via chromosome transfer into hMSH2-deficient cisplatin-resistant cells lines produces chemotherapeutic re-sensitization (10). The current hypothesis is that loss of MMR genes leads to diminished DNA repair, an increase in resistance to chemotherapeutic agents, and a loss of apoptotic signaling pathways, eventually leading to widespread genome instability and ultimately mutations in other genes that are directly linked to tumorigenesis.

Like the MMR system, the p53 tumor suppressor protein also plays an important role in maintaining the fidelity of the cellular genome. As such, p53 mutations occur in a number of cancers (11). In ovarian cancers, more than 50% of advanced stage tumors show a loss of p53 function (12). Whether this is a primary event in tumorigenesis or secondary to instability in the cellular genome is an area of active investigation. p53 has been shown to respond to a variety of mutagenic factors such as chemotherapeutic agents, ionizing radiation, and nucleotide depletion, all of which result in cellular injury via DNA damage (13), as well as down-regulating the expression of a number of cellular oncogenes (14).
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several mechanisms of action have been proposed for the p53 protein, the most extensively studied is its ability to regulate transcription of target genes through direct binding to specific DNA sequences.

Because of its primary function as a DNA-binding transcription factor, the p53 protein resides primarily within the nucleus of the cell. Transcriptionally active DNA-bound p53 protein exists as a tetramer (15). Tetramers of p53 occur via binding of two pairs of p53 dimers to specific target sequences of DNA (p53 response elements) located in the regulatory regions of genes. These p53 response elements generally consist of two tandem repeats of a sequence homologous to the consensus p53 binding sequence, 5'-PuPuPuC(A/T)(A/T)GPyPyPy-3' (16). p53 response elements have been characterized in several genes including p21, GADD45, MDM2, and bax (17), which are activated by p53 following cellular injury and, in turn, lead to cell cycle arrest, DNA repair, or apoptosis.

Recent studies point to a cooperative interaction between p53 and the MMR proteins with respect to activation of apoptosis after exposure of cells to mutagenic agents (18). The point of overlap between the p53 and MMR systems may be located in the hMSH2 gene promoter region, which has been recently cloned and sequenced (19, 20). Electromobility shift experiments have identified two putative p53 palindromic binding sequences located at −447 bp and −416 bp upstream of the start site of transcription (20). In Saos-2 cells the p53 binding sites were capable of mediating p53 induction of the hMSH2 promoter only in the presence of a co-activator such as ionizing radiation or co-transfected c-Jun (21). The requirement for additional transcription factors for activity may reside in the fact that unlike the p53 consensus palindromic sequence that is arranged as two direct tandem repeats, the −447 and −416 sites each comprise only a single palindromic sequence and are separated by 23 bp. What affect this spacing has on dimer/dimer formation of the p53 protein and the ability of these sequences to function as a transcriptional activator in other cell types is presently unknown. Adding additional uncertainty to the role of p53 in hMSH2 expression is a report by Iwahashi et al. (19) in which serial deletions of the hMSH2 promoter region were transfected into NIH3T3 cells. In NIH3T3 cells maximal transcriptional activity was present in as little as −298 bp of the hMSH2 promoter. Thus, it would appear that at least in NIH3T3 cells the p53 sites at −416 and −447 are not required for transcriptional activity of the hMSH2 promoter. Unfortunately, Iwashi et al. (19) did not further characterize the hMSH2 proximal promoter region to determine what elements were functionally active in the −298-bp region of the promoter.

Therefore, in the present series of studies we sought to clarify the role of p53 in hMSH2 expression in ovarian cancer by rigorously characterizing the proximal promoter region of the hMSH2 gene.

We began our investigation with a series of 5′ deletion mut-ants of the hMSH2 promoter region utilizing transient transfection of A2780 ovarian cancer cells. Our data indicate that similar to NIH3T3 cells, in A2780 cells deletion of the previously identified p53 binding sequences at −447 and −416 had no effect on hMSH2 promoter activity. However, a comprehensive mutational analysis of the hMSH2 proximal promoter has identified a new p53 response element motif, located between −242 and −222 bp upstream of the start site of transcription. This new p53 site (−242 hMSH2) has an 80% sequence homology to the tandem repeat p53 palindromic consensus binding sequence. Co-transfection of the p53-null SK-OV-3 ovarian cancer cell line with −281 hMSH2-pGL3 and a p53 expression construct indicates that the −242 hMSH2 sequence is capable of functioning as a p53 response element.

EXPERIMENTAL PROCEDURES

Cell Culture—Human ovarian cancer cell lines were obtained from the European Collection of Cell Cultures (A2780) (ECACC, Salisbury, UK) and the American Type Culture Collection (SK-OV-3; ATCC, Manassas, VA). A2780 cells were cultured in RPMI 1640 and the SK-OV-3 cells in McCoy’s 5A medium, both supplemented with 10% fetal bovine serum. Cultures were periodically tested to ensure they remained free of mycoplasm infection during the course of the experiments.

Preparation of Paraffin Cell Blocks—A2780 cells in culture were trypsinized and removed from the culture dishes upon dilution with two volumes of medium + 10% fetal bovine serum. The resulting cell suspension was centrifuged at 150 × g for 5 min and the pellet was washed once in culture medium to remove the excess trypsin and recentrifuged. The final pellet was then resuspended in 0.25 ml of medium. To this suspension 0.5 ml of human plasma was added immediately followed by a similar volume of thromboplastin as previously described (22). The mixture was agitated for 2 min until coagulation occurred. At this point, 10 ml of 10% buffered-formalin was added, and the coagulated cells gently rocked for 2 min. The clotted sample of cells was then wrapped in “perm” paper, secured in a cassette, placed in a VIP tissue processor (SAKURA), and allowed to process overnight. The following day, the processed sample was embedded in a paraffin block and 4-micron sections were cut and mounted on slides.

Immunohistochemistry—A2780 cells were immunostained for p53 and hMSH2 using an immunoperoxidase procedure as previously described (23). Slides were stained using the horseradish peroxidase (HRP), LSAB2 System (DAKO). Briefly, mouse monoclonal antibodies against human p53 (DAKO) or hMSH2 (BD PharMingen) were used as the primary antibody. The secondary antibody was a biotinylated goat anti-mouse (DAKO) antibody. After washing the sections, a streptavidin-HRP incubation was carried out. Finally, the samples were counterstained with hematoxylin (DAKO), dehydrated, and mounted with coverslips.

hMSH2 Luciferase Reporter Constructs—1880 bp of the hMSH2 upstream/promoter region were amplified from human genomic DNA by the polymerase chain reaction. A high fidelity Taq DNA polymerase with proofreadability capabilities (Platinum Taq, Life Technologies, Inc.) was utilized to minimize potential PCR errors. hMSH2-specific oligomeric primers were synthesized (Life Technologies, Inc.); a 3′-MSH2 primer 5′-ATAT/GACTTT/GTCGAAACCTCCTACCTCCTG3′ and the 5′-MSH2 primer 5′-ATAT/GCTAGG/GGACCTGGCTACTGCAACCTC-3′ using the previously published sequence for the 5′ upstream/promoter region of the hMSH2 gene (19). The underlined sequence in each primer corresponds to sequences derived from the 3′- and 5′-ends of the hMSH2 promoter region. For cloning purposes a SacI/HindIII site was added to the 3′-MSH2 primer and an NheI site to the 5′-MSH2 primer (shown in parentheses). The 4-bp sequence ATAT was added immediately adjacent to both restriction enzyme sites to aid in restriction enzyme digestion of the final PCR product. The final PCR product was digested with NheI/HindIII, gel-isolated, and subcloned into the multiple cloning site of the pGL3-Basic Vector (Promega) immediately upstream of the luciferase reporter gene. The hMSH2 containing pGL3 constructs were sequenced on an ABI 3700 capillary sequencer to ensure authenticity.

Deletion Constructs—Serial deletions of the 5′-end of the −1880-bp hMSH2 promoter region were generated in separate reactions using the following restriction enzymes to shorten the 5′-end of the promoter: Smal (−952 bp), SacI (−281 bp), SphI (−225 bp), and SacII (−157 bp) (Fig. 2A). Following restriction enzyme digestion of the −1880 bp hMSH2-pGL3 vector with which the enzymes above was not performed, the ends were polished blunt using T4 polymerase, and the fragments (sizes shown in parentheses next to the restriction enzymes above), excised by digestion of their 3′-ends with HindIII. The 5′-shortened fragments were subsequently gel-isolated, and re-ligated into the Smal/HindIII sites of the pGL3 Basic Vector.

Response Element Mutation—Mutation of the p53 site in the hMSH2 proximal promoter region was accomplished using the Quick-Change Mutagenesis Kit (Stratagene). Complementary 45-mer primers were synthesized, containing a 15-bp mutant p53 sequence flanked by 15 bp of wild-type sequence on either side of the p53 site of the hMSH2 upstream promoter region. The sequence in bold type from the hMSH2 p53 response element (−242 to −222) 5′-GACATCCCGGCCGACCAT-GGCC-3′ containing both of the palindromic core binding elements was replaced with the 15-bp EcoRI linker sequence 5′-ATAAAGATTCC-
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CATAA-3' to generate a p53 mutant, –281(mut) hMSH2-pGL3, construct.

Transient Transfection Assays—DNA constructs were transiently transfected into cells using LipofectAMINE Plus Reagent (Life Technologies, Inc.). Twenty-four hours prior to transfection, cells were subcultured onto 60-mm plates so that they would be at 50–60% confluence the following day. Transfections were optimized for the amounts of DNA, PLUS reagent, and LipofectAMINE per 60-mm culture dish as follows: 0.6 μg of hMSH2-pGL3 construct, 0.1 μg of pRL-TK vector (Promega), 2.4 μl of PLUS Reagent, and 3.6 μl of LipofectAMINE reagent. The above reagents and DNA constructs were mixed and incubated with the cells overnight (18 h) according to the manufacturer’s protocol. The following day, the culture medium was replaced, and the cells were placed back into a 37 °C, 95% O2, 5% CO2 incubator for an additional 24 h.

Luciferase Assay—Cells were harvested from the plates, following a single wash of phosphate-buffered saline to remove residual medium in 400 μl of Passive Lysis Buffer (Promega) by scraping. Cell lysates were frozen at –20 °C to ensure complete lysis of the cells. Luciferase activity in the cell lysates was determined using the Dual-Luciferase reporter assay system (Promega) to allow sequential determination in the same sample of both the firefly luciferase activity from the hMSH2 constructs and the transfection efficiency from the Renilla luciferase activity, the pRL-TK vector. All assays were carried out in a single sample luminometer (DIGENE Diagnostics) model DRC-1. All reported firefly luciferase values were normalized for transfection efficiency using the pRL-TK, Renilla luciferase value. Statistically significant differences in promoter activity of the various hMSH2-pGL3 constructs were determined by analysis of variance.

RESULTS

Prior to initiating transient transfection studies of the hMSH2 promoter region, we first sought to identify a suitable ovarian cancer cell line that expressed both the hMSH2 and p53 genes. We examined the distribution of the p53 and hMSH2 mismatch repair proteins in the ovarian cell line, A2780 (Fig. 1). The photomicrographs show the immunohistochemical staining of A2780 cells for p53 (A) and hMSH2 (B) proteins. Consistent with reports in the literature that A2780 cells contain the wild-type p53 gene and express the p53 protein (24), we demonstrated positive immunostaining for p53 protein in ~25–30% of the cells (Fig. 1A). Staining was localized to the nuclei, consistent with the role of p53 as a DNA-binding transcription factor. Similar immunostaining for hMSH2 occurred in roughly 80% of the cells with the stain again confined primarily to the nucleus (Fig. 1B). Finally, as a control, A2780 cells were immunostained for another member of the MMR system, hMLH1. Staining for hMLH1 resulted in intense nuclear staining in greater than 99% of the A2780 cells (data not shown). Therefore, the lack of p53 and hMSH2 staining of a portion of the cells present on each slide was not caused from an artifact of the tissue preparation or staining procedure, but more likely the result of differences in the expression of these genes at various points in the cell cycle.

Having established that the A2780 cell line is capable of expressing both the p53 and hMSH2 genes, we turned our attention to investigating the regulation of the hMSH2 promoter region. Analysis of the hMSH2 proximal promoter region began with a PCR-based isolation of the hMSH2 5’ flanking sequence. Cloning the 5’ upstream proximal promoter region of the hMSH2 gene was accomplished using specific primers based on the published sequence (19), as described in detail under “Experimental Procedures.” The cloning exercise resulted in the isolation of the transcription initiation site for the hMSH2 gene along with 1.88 kb of the 5’ upstream promoter sequence. A series of 5’ deletion mutants of the 1.88-kb hMSH2 promoter fragment were generated (Fig. 2A) for transfection into A2780 cells in order to characterize the hMSH2 proximal promoter through identification of DNA response elements (Fig. 2B). Transfection of the full ~1.88-kb hMSH2 promoter fragment contained within the pGL3 luciferase reporter vector produced luciferase expression significantly higher (p < 0.01) than that seen with the pGL3 luciferase vector alone (Fig. 2B). Deletion of the hMSH2 proximal promoter to ~951 bp produced no significant change in promoter activity from that seen with the full-length ~1.88-kb construct. Further deletion down to ~281 bp produced a significant (p < 0.05) 2.5-fold rise in promoter activity compared with the ~1.88-kb or ~951-bp fragments (Fig. 2B). Finally, deletion to ~157 bp of the hMSH2 promoter resulted in a significant (p < 0.01) 95% reduction in promoter activity to levels one-twentieth of those seen with the ~281 hMSH2 fragment. Though drastically reduced from the activity seen in the other constructs, the ~157 hMSH2 fragment still retained significant (p < 0.05) promoter activity (3.5-fold) when compared with the empty pGL3 vector. Therefore, for the purposes of these studies, the low levels of promoter activity inherent in the ~157 hMSH2 fragment served as our minimal (basal) promoter. Of note, the anticipated fall in promoter activity upon deletion of the previously identified p53 sites at ~447 and ~416 (20) in the ~281 hMSH2 construct was not observed. Thus, it appears that the p53 sites previously identified in Saos-2 cells are either masked or non-functional in A2780 ovarian cancer cells.

From the data presented in Fig. 2, enhanced transcriptional...
activity was confined to a region of the hMSH2 promoter between −281 and −157 bp upstream of the start site of transcription. Analysis of this 124-bp region for potential enhancer elements yielded several candidate sequences, the majority of which had previously been identified (19, 20) with one notable exception. Our analysis identified a potential enhancer sequence that had not been previously reported; a sequence with the minimal 157-bp promoter. Therefore, we cannot exclude the possibility of an unknown enhancer element located within this same 56-bp region. In an attempt to produce a more restricted mutation of the −242 hMSH2 site, we generated a site-specific mutation within the −157-bp hMSH2 fragment. The mutated construct (−281(mut)) contained the 15-bp p53 site, the sequence of the consensus binding site (bases; −CTAGGCGCAGGCATG−3′) in place of the wild type p53 palindromic binding sites 5′-CTAGGCCAGGCGATG-3′ (bases; −239 to −225) in the −281 hMSH2 reporter construct. Both the −281(wt) and −281(mut) constructs were subsequently transfected into A2780 cells (Fig. 5). As previously observed, the −281(wt) construct showed enhanced hMSH2 promoter activity 35–40 times that seen with the minimal −157-bp hMSH2 fragment. Mutating the 15-bp palindromic p53 binding sequence within the hMSH2 promoter (−281(mut)) resulted in a 97% reduction in promoter activity. Indeed, mutation of the 15-bp p53 site within the −281 hMSH2 construct resulted in promoter activity statistically indistinguishable from the minimal −157 hMSH2 fragment.

Having identified the presence of a regulatory sequence within the hMSH2 promoter region, with significant homology to the p53 consensus sequence we sought to establish that p53 was capable of transactivating hMSH2 expression through this element. We co-transfected SK-OV-3 cells, which lack endoge-
nous p53 (25), simultaneously with a p53 expression vector and our hMSH2-pGL3 reporter vector. The constructs consisted of an expression vector containing the human p53 coding sequence (pORF-hp53) and either the $2^{281}$ (wt) or $2^{281}$ (mut) hMSH2 reporter vector (Fig. 6). Transfection of SK-OV-3 cells with the $2^{281}$ (wt) hMSH2 construct and the empty pORF expression vector produced low basal levels of transcription (Fig. 6). Co-transfection with the p53-expressing (p53-pORF) vector produced a significant ($p < 0.01$) 10-fold induction of the $2^{281}$ hMSH2 promoter when compared with the activity in the absence of co-transfected p53. When the $2^{281}$ (mut) hMSH2 construct containing the mutated p53 site was co-transfected with a p53 expression vector, promoter activity was induced by only 1.8-fold with overall promoter activity the same as that observed for the $2^{281}$ (wt) construct in the absence of co-transfected p53. We interpret these results as indicating that the observed 10-fold induction in the wild-type $2^{281}$ hMSH2 enhancer element. These co-transfection experiments provide convincing evidence that the $2^{242}$ to $2^{222}$ sequence within the hMSH2 proximal promoter region functions as a p53 response element in ovarian cancer cells and is capable of conferring p53-mediated regulation of hMSH2 expression.

FIG. 4. Deletion of a region within the hMSH2 promoter containing the $2^{242}$ to $2^{222}$ bp sequence. A2780 cells were transiently transfected with a series of 5’ deletion mutants of the hMSH2 promoter to examine the effects of the loss of a region containing a sequence homologous to the consensus p53 binding site ($2^{225}$ hMSH2-pGL3). Schematic representations of the constructs are shown at left. The data are expressed as relative luciferase activity. Data are the mean ± S.E. of two independent transfection experiments of four plates each ($n = 8$).

FIG. 5. Mutation of the p53 site ($2^{242}$ to $2^{222}$) within the hMSH2 promoter. A2780 cells were transiently transfected with the following hMSH2 promoter constructs: wild type, 5’-ttaggggcagcaagctg-3’ ($2^{281}$ (wt) hMSH2-pGL3) or mutated containing a non-p53 binding EcoRI linker sequence, 5’-ttagaattctagcataa-3’ ($2^{281}$ (mut) hMSH2-pGL3). Schematic representations of the constructs are shown at left. Data are expressed as relative luciferase activity. Results represent the mean ± S.E. of two independent transfection experiments of four plates each ($n = 8$).

DISCUSSION

A thorough analysis and characterization of the 5’ upstream proximal promoter region of the hMSH2 gene is critical to our understanding how the expression of this gene is regulated. A better understanding of the mechanisms responsible for regulation of hMSH2 expression may shed light on the role the hMSH2 protein plays in stabilizing the cellular genome. Additionally, the identification of regulatory elements and their associated transcription factors may also provide important clues toward understanding the association between the loss of hMSH2 expression, tumorigenesis, and chemotherapeutic resistance.

The present series of studies serves to expand our understanding of the complex mechanisms involved in the regulation of hMSH2 expression by providing convincing evidence of the presence of a p53 response element ($2^{242}$ to $2^{222}$) in the hMSH2 proximal promoter region. The $2^{242}$ hMSH2 p53 element identified in the present studies has 100% homology to the p53 consensus sequence within the core palindromic (C(A/T)(A/T)G) binding sites and an overall homology of 80% (Fig. 3). When compared to p53 elements from other genes (Fig. 7) the $2^{242}$ hMSH2 element shows a similar tandem repeat structure with divergence from the p53 consensus sequence confined to the less conserved flanking purine and pyrimidine sequences. Similar to two previously identified p53 binding sites IGFBP3 A and MCK, the $2^{242}$ hMSH2 site has an additional base (cytosine) separating the tandem repeat sites (Fig. 7). What potential effect, if any, this additional spacing has on the affinity or specificity of p53 for this sequence will be discussed.
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FIG. 6. Activation of the hMSH2 promoter by co-transfection with a p53 expression vector. p53-null SK-OV-3 cells were transfected with either the −281(wt) hMSH2−pGL3 or −281(mut) hMSH2−pGL3 in the presence or absence of the human p53 expression construct, pORF−hp53. A plus sign (+) indicates the presence and a minus sign (−) the absence of a particular construct in the transfection assay. Data are expressed as relative luciferase activity, mean ± S.E. of two independent transfection experiments of four plates each (n = 8).

That p53 acts as a regulator of the hMSH2 gene is consistent with its role as a tumor suppressor in maintaining the cellular genome. The MSH2 protein functions in concert with its dimer partner MSH6, to identify and bind to mismatched DNA base pairs. MutS binding is the first step in the mismatch base excision repair pathway to correct the newly synthesized DNA daughter strand following replication of the cellular genome in anticipation of cell division. Because the hMSH2 protein forms the core of the human MutS mismatch repair complexes, its regulation by the tumor suppressor protein p53 would be consistent with p53's role in maintaining genomic integrity following DNA replication.

Direct regulation of hMSH2 expression by p53, as demonstrated in Figs. 5 and 6 of this study, has broad implications pertaining to genome instability in p53-negative cancers. As previously mentioned, −50% of all cancers are p53-negative. Because our data indicate p53 is critical to the expression of the hMSH2 gene there is a high likelihood that many of these same cancer cells will also be deficient in mismatch repair activity. Under this scenario the absence of p53 in these cells would produce a dual effect. First, it would result in hMSH2 deficiencies rendering the cell unable to repair damage introduced into the cellular genome through normal DNA replication or as a result of DNA-damaging chemotherapeutic agents. Second, the absence of p53 would compound the lack of mismatch repair by also impairing the cells ability to undergo apoptosis; instead, these cells would continue to divide producing further instability in the cellular genome via the introduction of additional genetic mutations. Eventually, the widespread genomic instability produced in these p53-negative, mismatch repair-deficient cells would result in additional mutations to other oncogenes or oncosuppressor genes, potentially producing a more aggressive cancer that is resistant to chemotherapeutic agents.

Clearly additional studies of the relationship between mismatch repair activity and p53 in cancer cells are required to gain a better understanding of their role in cancer genetics and chemotherapeutic resistance.

The regulation of hMSH2 expression by p53 in ovarian cancer cells in the present study is strikingly different from the regulation in osteosarcoma Saos-2 cells recently described by Scherer et al. (21). Gel-retardation studies by this same laboratory have identified the presence of two p53 binding sequences within the hMSH2 promoter region at −447 and −416 bp upstream of the start site of transcription (20). In the present study, deletion of this region of the hMSH2 gene in our reporter construct (−281 hMSH2−pGL3) did not produce the anticipated reduction in promoter activity (Fig. 2). In fact, the opposite response to deletion of this region occurred (promoter activity increased ~2.5-fold) indicating the possible presence of a transcriptional repressor element in this region. The present findings (as little as −281 bp of the hMSH2 proximal promoter region are sufficient for full activity in A2780 cells (Fig. 2)) are in agreement with previously published transfection data in NIH 3T3 cells where full hMSH2 promoter activity was contained within the first 296 bp (19). Thus, it appears that in both NIH 3T3 and A2780 cells the p53 elements at −447 and −416 are not transcriptionally active under the conditions employed.

One potential explanation for the tissue-specific activity of the previously identified −447 and −416 hMSH2 p53 elements, compared with the present −242 hMSH2 element, may relate to differences in their structures. The consensus p53 element consists of two tandem palindromic binding sites. Each palindromic binding site can be further subdivided into two half-sites, with each half-site capable of binding a single p53 protein. Thus, the consensus p53 sequence consists of four half-sites arranged as two tandem palindromes. Studies indicate that four p53 proteins make up an active transcriptional stimulator through binding to each of the four half-sites. The p53 proteins bind as pairs (dimers) with both pairs of proteins in turn interacting; a complex termed a dimer of dimers (26). The close proximity of the two palindromic binding sites as a direct tandem repeat allows the pairs of p53 dimers to interact. The p53 response element described in the current study (−242 hMSH2) also consists of two tandem repeat palindromic binding sites, homologous to the p53 consensus site. Thus, we would anticipate that binding of p53 to the −242 hMSH2 element would result in dimer/dimer interactions similar to those observed with the consensus binding site. In contrast, the previously identified sites in the hMSH2 gene at −447 and −416 each consist of only a single p53 palindromic binding site and are thus capable of binding only a single dimer of p53. Instead of being arranged like the consensus p53 sequence, as tandem palindromes, there is a spacing of 13 bp separating the −447 and −416 hMSH2 palindromic binding sites. This distance would tend to preclude the type of direct dimer/dimer interactions described for the consensus sequence (27). The additional spacing present between the −447 and −416 sites would seem to necessitate a different type of interaction. Two possibilities exist to allow dimer/dimer interaction in such a system, either folding or looping of the DNA to bring the two sites next to each other, as has been reported for the distal and proximal promoter regions of CDK2. (28)
proximal p53 elements in the MCK promoter (28) or perhaps interaction with another transcription factor, bound to an adjacent element.

Support for the second alternative that the −447 and −416 hMSH2 elements require interaction with another transcription factor comes from the observation that the −447 and −416 elements are not able to transactivate hMSH2 expression in Saos-2 cells solely in the presence of p53 alone (21). Pretreatment of the cells with ionizing radiation or co-transfection with the AP-1 binding protein c-Jun were required for p53-mediated expression through both the −447 and −416 sites. An examination of the hMSH2 promoter sequence shows the presence of AP1 binding sites flanking the −447 and −416 elements (21). Therefore, it seems plausible that in certain cells (Saos-2) and under certain conditions (ionizing radiation), AP-1 binding is required for p53 activation through these sites. One might speculate that transactivation through the −447 and −416 elements requires the formation of a complex involving heterodimer-dimer interaction between a dimer of p53 and an AP-1 binding complex. In other cell types (A2780), p53 activation of hMSH2 occurs independent of other transactivating factors through the −242 hMSH2 element. This element does not appear to require additional factors, as co-transfection of p53 into the null SK-OV-3 cell line was sufficient to produce activation of the −281 hMSH2 promoter (Fig. 6). Furthermore, the −281 hMSH2 promoter construct does not contain either of the AP-1 sites that are presumably required for interaction with the −447 and −416 p53 elements, thereby eliminating direct AP-1 involvement with the transactivation activity observed with the −242 hMSH2 element.

Recent observations concerning cell-specific interactions of the p53 protein on p53 binding sites in other genes may provide potential clues regarding the complex, sometimes divergent, tissue-specific regulation of the hMSH2 gene. Cell type-specific regulation of p53 target genes has been reported for the bax promoter (29). These studies indicate that in Saos-2 cells, both the bax and p21 promoters showed p53-dependent activation, whereas in similar experiments performed in MDA-MB-453 cells, p53 activated the p21 promoter but failed to activate the bax promoter. This difference was attributed to differences in p53 interaction with the p21 versus bax response elements because of their different conformations. The p21 element is comprised of a tandem repeat palindrome similar to the −242 hMSH2 element, whereas the bax element is arranged as three adjacent half-sites akin to the −447 and −416 sites. Thus, the inference drawn from these studies is that the interaction of p53 on the bax promoter (and by analogy the −447 and −416 hMSH2 sites) is somewhat different from the binding seen on the consensus sequence. This binding appears to involve conformationally distinct, tissue-specific forms of the protein present in Saos-2 cells that are capable of binding to a single palindromic site. This, thus, differences between the structures of the −242 hMSH2 element and the elements at −447 and −416 bp may account for the difference in activity of elements in Saos-2 cells compared with A2780. Clearly additional transfection studies combined with in vitro binding assays will be required to resolve this issue and provide greater insight into the complex cell-specific regulation of hMSH2 expression by the currently identified p53 response elements.

Finally, it must be noted that p53 is but one member of a family of proteins, the other two being p63 and p73 (30). All of these proteins share a high degree of homology, as high as 63% in their DNA binding domain (31), and each has been shown to be capable of binding to the p53 consensus binding sequence (30). The p53 family is further diversified through alternate splicing of the p73 mRNA product to generate several protein variants (32), as well as post-translational modifications of all three members, primarily via phosphorylation at multiple sites (32). The rational for the complexities inherent in the p53 family are at present not well understood. Current thinking presumes that the complexity is related to the important function these proteins play in both developmental expression and maintenance of the cellular genome.

Given the diversity of the p53 family, a potential source of tissue-specific transcriptional activation might involve competition by other family members for binding to p53 elements. Both p63 and p73 have been shown to effectively bind to p53 elements (31). When p73 was transfected into A2780 cells the degree of endogenous p53 transcriptional activity was markedly reduced (33). This reduction required an intact DNA binding domain in the transfected p73 protein and thus was presumably the result of direct competition for binding to the p53 element. Additional studies have shown that p73 overexpression in A2780 cells also leads to a decrease in the transcription of the p53 gene resulting in reduced levels of p53 protein and hence diminished promoter activity of p53 responsive genes (33). Tissue-specific regulation of various p53 responsive genes could also be accounted for by the observation that various members of the p53 family show differences in their susceptibility to inactivation. For example, p53 and p73 show striking differences to adenovirus inactivation with p53 inactivated by the E1B 55 kDa oncoprotein with no effect on p73 activity (34).

Another potential source of tissue-specific expression of p53 responsive genes is the ability of p63 and p73 to bind p53 elements and transactivate expression of p53 responsive genes. For example, p63 has a similar binding specificity to the p53 Waf1 site but differs in binding to Gadd45 and T3SF sites (35). Given the fact that p53 binds to DNA elements as a dimer it is also possible that p63 or p73 could form heterodimers with p53 and either disrupt or enhance transactivation of a target gene. Alternatively, p63 and/or p73 could disrupt p53 activity without binding DNA, simply by sequestering it as an inactive heterodimer in solution. Finally, alternate splice products of the p63 and p73 proteins lacking a transactivation domain but retaining an intact DNA binding domain could function as competitive inhibitors, as has been shown to occur in developing and adult tissues of p73 knock-out mice (36). Similar findings have been described in developing neurons where the presence of nerve growth factor (NGF) increases the expression of a truncated form of p73 that binds to p53 response elements but is unable to transactivate expression. The presence of the truncated p73 protein on the p53 element acts as an anti-apoptotic signal to counteract the pro-apoptotic activity of p53 (37). In this system, when NGF is withdrawn the levels of truncated p73 fall and p53 exerts its apoptotic role and neuronal death ensues. These studies further emphasize the importance of understanding how the presence or absence of various members of the p53 family can dramatically affect the ability of a given p53 response element to transactivate transcription in any given cell.

In conclusion, the presence of multiple cell-specific, p53 response elements within the hMSH2 proximal promoter region will require additional studies to determine their respective roles in regulating the expression of the hMSH2 gene in various tissues. Toward this goal, studies are currently underway in our laboratory to determine the relative affinities of the p53 family members for these sites and their potential interactions with one another as dimer partners. Additional studies of the proximal promoter regions of the other proteins that form the MutS complex, hMSH3 and hMSH6, as well as the MutL core protein hMLH1 are also underway.

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C. Terry Warnick, Bashar Dabbas, Clyde D. Ford and Kevin A. Strait

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