Differential Cellular Expression of the Human MSH2 Repair Enzyme in Small and Large Intestine

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Abstract

The human MSH2 (hMSH2) protein is responsible for the initial recognition of mismatched nucleotides during the postreplication mismatch repair process. Loss of hMSH2 function has been demonstrated to lead to the accumulation of replication errors, resulting in a mutator phenotype, which may be responsible for the multiple mutations required for multistage carcinogenesis. Alterations of the hMSH2 gene has been linked to approximately 60% of hereditary nonpolyposis colon cancer cases. Colon tumors in hereditary nonpolyposis colon cancer patients originate within benign neoplastic adenomas and display replication errors in the form of microsatellite instability. The aim of this study was to investigate the cellular expression of the hMSH2 protein in cells of the large and small intestines. Using antibody specific for hMSH2, we have determined that this protein is highly expressed in cells of the crypts of Lieberkühn that are undergoing rapid renewal in both the ileum and colon. Proliferative perilibroblasts in the colon also showed significant presence of the hMSH2 protein. These results confirm the hypothesis that hMSH2 is expressed in highly proliferative cells of the gut, and mutations in this gene could, therefore, be expected to expedite the progression of adenoma to carcinoma in this tissue.

Introduction

HNPCC is one of the most prevalent cancer-predisposing syndromes known, affecting as many as 1 in 200 individuals in industrialized nations (1). In the United States alone, approximately 56,000 deaths and 150,000 new cases have been estimated for this year (2). HNPCC is an autosomal dominantly inherited disease in which individuals within HNPCC families have a 50% chance of inheriting a defective HNPCC-linked gene and, if present, a lifetime risk of 90–100% of developing one or more bowel cancers (3). These cancers generally originate within benign neoplastic polyps termed adenomas (4, 5). The adenomas in HNPCC patients, although not numerous, exhibit more severe pathological features than do sporadic adenomas present in the general population. These features include development at an earlier age, a larger size, a more villous histology with high-grade dysplasia, and a higher tendency to undergo malignant conversion (4–6).

Four genes (hMSH2, hMLH1, hPMS1 and hPMS2) have been linked to HNPCC and account for better than 95% of the cancers in known HNPCC families (7–11). Within these kindreds, hMSH2 on chromosome 2p21–22 accounts for approximately 60% of the cases (7). The human genes are members of an evolutionary conserved family of MMR genes (12). In Escherichia coli, these genes, referred to as the mutLHS pathway, have been extensively characterized and were identified as mutations that increased the rate of spontaneous mutation, resulting in a mutator phenotype (13–15).

An effect of the mutator phenotype is the production of replication errors in simple repetitive DNA, resulting in microsatellite instability. This phenotype has been observed in tumors from HNPCC patients and indicates a direct role for the human MMR genes in genomic stability (16–18). The development of colorectal carcinoma has been determined to be the result of at least six independent mutations (19). The mutator phenotype has been proposed to account for the multiple mutations required for this multistage carcinogenesis (20, 21). It has been proposed that HNPCC genes do not initiate neoplastic transformation; rather, they allow the accumulation of replication errors, accelerating the progression of the adenoma to adenocarcinoma (8, 22). Recently, hMSH2 was found to be capable of binding to mismatched nucleotides, initiating the MMR process (23, 24). A loss of this function may lead to a mutator phenotype and, hence, multistage carcinogenesis within the adenoma of the large bowel (24).

The aim of this study was to investigate the cell-specific expression of the hMSH2 protein in tissues of the large and small intestines. Using antibody specific for hMSH2, we show that this protein is highly expressed in the rapidly proliferating cells of the crypts of Lieberkühn of both the ileum and colon. These cells are responsible for the continuous production of differentiated cells that will migrate over a 2–4-day period before being sloughed into the lumen. These results confirm the premise that hMSH2 is highly expressed in proliferative cells and, therefore, mutations in this gene would hasten the progression of adenoma to carcinoma in gut.

Materials and Methods

Tissue. Biopsy specimens were fixed in 4% buffered formaldehyde, embedded in paraffin, and sectioned at 6 µm. The results presented here were corroborated with tissue from several patients (data not shown).

Antibody Preparation. Polyclonal rabbit antiserum was prepared by Oncogene Sciences, Inc. (Cambridge, MA) by immunizing three rabbits (HRP, Inc., Denver, PA) with 200 µg of PrepCell (Bio-Rad, Hercules, CA)-purified hMSH2 overexpressed in bacteria. A GST fusion construct was used for immunization and antibody titer (24). After three boosts of 100 µg antigen per boost at approximately 4-week intervals, high titers of MSH2 cross-reacting sera were detected in all rabbits. One sera preparation yielded an extremely high titer, and the antibody was subsequently purified via protein-A chromatography. Western analysis revealed that the purified antibody preparation detected only one major protein that was equivalent to hMSH2, which was verified by antibody quenching with purified hMSH2 before blotting (data not shown).

Western Blot Analysis. Total cell extracts were from human liver, the glioma cell line A1235, and the myeloid leukemia cell line HL-60. Twenty µg
of liver and 10 μg of each of the cell line extracts were fractionated on a 7.5% SDS-polyacrylamide gel, and Western blotting was performed as described previously (25). Anti-hMSH2 antibody was used at a concentration of 3 μg/ml for Western blots, and cross-reacting proteins were detected using the chemiluminescence Western blotting kit from Boehringer-Mannheim (Indianapolis, IN) as directed.

**Immunohistochemistry.** The sections were baked at 65°C for 1 h, deparaffinized in xylene, and hydrated through a series of graded alcohols (100, 95, 70, and 50%) to distilled H₂O. Endogenous peroxidase activity was then quenched with a 25-min incubation in 0.3% H₂O₂ in methanol, followed by rinsing in PBS [0.1 M K₂HPO₄ and KH₂PO₄ (pH 7.4)-150 mM NaCl]. Non-specific antibody binding was blocked by incubation for 30 min with 10% goat serum in PBS. Primary anti-hMSH2 antibody (rabbit antihuman MSH2 polyclonal) was then incubated with the sections overnight at 4°C at a concentration of 25 μg/ml in 10% goat serum in PBS. The following day, the sections were washed 3 times for 5 min in PBS, followed by incubation with the secondary antibody (biotinylated goat antirabbit IgG, Vector Laboratories, Burlingame, CA) at 15 μg/ml in 10% goat serum for 1 h. After two 5-min PBS washes, the sections were incubated with avidin and biotinylated horseradish peroxidase complex (ABC elite kit, Vector Laboratories) for 45 min. The sections were then incubated with the chromogen diaminobenzidine (Vector Laboratories). After development of sufficient signal, the sections were washed briefly in distilled H₂O and dehydrated through a graded alcohol series to xylene. The sections were coverslipped using Permount (Fisher Scientific, Itasca, IL) and photographed. To control for antibody specificity, preimmune IgG was used as the primary antibody in place of the anti-hMSH2 antibody at a concentration of 50 μg/ml. For histological staining, the coverslips were removed with xylene, and the sections were hydrated and stained with either hematoxylin and eosin or toluidine blue, dehydrated, and coverslipped.

**Results and Discussion**

The expression of hMSH2 was first determined by Northern analysis of RNA prepared from various normal human tissues. The highest expression was found in tissues of the testis and thymus (Fig. 1). Lower levels of expression were observed in all other tissues shown, including colon, as well as skin, lung, heart, skeletal muscle, and liver (data not shown). These RNA samples represented all of the cell types present in a tissue type, thus excluding the possibility of determining whether hMSH2 expression was globally present or confined to specific cell types.

Fig. 1. Northern analysis of hMSH2 RNA expression in human tissues. A Northern blot of RNA extracted from human tissues (Clontech, Palo Alto, CA) was probed with a full-length hMSH2 cDNA clone (7). As an internal gauge of RNA levels, the blot was also probed with β-actin cDNA supplied by the manufacturer (Clontech). The hMSH2 message migrated at 3.5 kb, and the β-actin message migrated at 2.0 kb. Both cDNA probes hybridized to the expected size transcript when used separately. The tissue types corresponding to the RNA in each lane is shown at the top of the figure.

To determine cellular expression, we performed immunohistochemical analysis using hMSH2-specific polyclonal antibody. The specificity of the human MSH2 antibody was examined using total cellular extracts from the human cell lines A1235 (astrocytoma) and HL-60, a cell line of leukemic myeloid precursor cells (Ref. 26; Fig. 2). By Western blot analysis, the antibody only cross-reacted with a single band at Mr 105,000, the molecular weight of human MSH2. Human MSH2 immunohistochemistry of the ileum demonstrated a strong reaction in the nucleus of striated-bordered cells in the crypts.

Fig. 2. Western blot analysis using hMSH2 antibody. Total cell extracts (20 μg) from the human cell lines A1235 (astrocytoma) and HL-60 (cell line of leukemic myeloid precursor cells) were run on a 7.5% SDS-polyacrylamide gel, blotted, and reacted with purified antibody to the human MSH2 mismatch DNA repair enzyme. Only a single protein band of Mr 105,000 was observed. Numbers on right, molecular weight in thousands.
of Lieberkühn (27). The hMSH2 antibody did not recognize every cell, nor did it recognize cells bordering the outside of the crypts (Fig. 3). The goblet cells in the crypts of Lieberkühn showed no cross-reactivity with the hMSH2 antibody (Fig. 3). There was some light staining of nuclei in enteroctyes of the proximal villus, and very sporadic staining of the distal villus nuclei was occasionally observed. Incubation with preimmune IgG as a primary antibody in place of the anti-hMSH2 antibody resulted in no staining of any cells (Fig. 3), nor did staining with second antibody alone (data not shown).

In the ileum, enterocytes are generated in the crypts of Lieberkühn; over a period of approximately 2–4 days, they migrate out of the crypts and up the villi toward the tip, where they are sloughed into the intestinal lumen (28). Therefore, the predominant expression of the hMSH2 DNA repair enzyme in the immature cells of the crypts correlated with hMSH2 expression in highly proliferative, immature cells, as expected for a DNA repair enzyme involved in MMR. In contrast, previous work with the O\textsuperscript{6}-methylguanine DNA methyltransferase, a DNA repair protein that removes alkyl groups from modified bases, showed a homogeneous staining pattern from the crypts all the way to the tips of the villi in rat (29). The major APE in humans (30) has been shown to be predominantly expressed in the nuclei of the crypts of Lieberkühn and in the proximal enteroctye nuclei, but the enteroctye nuclei abruptly stopped staining with APE antibody well before they had reached the villus tip.\textsuperscript{4} Therefore, DNA repair proteins of the three different but intimately related pathways [direct reversal (O\textsuperscript{6}-methylguanine DNA methyltransferase); mismatch (MSH2); and base excision repair (APE)] all show a different and distinct pattern of expression in the small intestine.

As in the small intestine (ileum), the large intestine (colon) is composed of a serosa, muscularis externa, submucosa, and mucosa, and all are continuous with the small intestine (27). The mucosa is less complex compared to the small intestine or stomach and contains a surface epithelium composed of columnar cells with thin striated borders and some goblet cells, which continue into the glands (crypts of Lieberkühn) that are lined by a single-cell-thick layer of the epithelium cells (27). Villi are not present in the colon. The hMSH2 antibody predominantly stained the lower one- to two-thirds of the crypts, and little or no staining was observed in the outer one-third (Fig. 4). Again, preimmune IgG (Fig. 4) or second antibody alone (data not shown) did not result in any background antibody staining of any cells (Fig. 4). Goblet cells did not stain with the hMSH2 antibody (Fig. 4), as observed in the ileum (Fig. 3); however, there were a large number of fibroblast cells surrounding the crypts that stained with the

hMSH2 antibody (Fig. 4). In the colon, the columnar absorptive cells originate in the lower two-thirds of the crypts as immature cells that migrate up the crypts before being lost into the lumen of the gut (31). Accordingly, the cells in the lower two-thirds of the colon crypts are undifferentiated proliferative cells that are constantly moving through the cell cycle and, therefore, undergoing increased metabolic activity and DNA synthesis. As expected, these cells express high levels of hMSH2. Cells that have differentiated and are making the trip to an eventual sloughing in the lumen may not require the functions of the hMSH2 protein and, as such, have little or any of this enzyme present in them. As in the ileum, the trip from the lower crypts to the mucosal surface and sloughing into the lumen has been estimated to occur over a 2–4-day period (28).

The results presented here show the distribution of hMSH2 in the small and large intestines. The specificity of the immunohistochemical reactions we used was demonstrated by the cross-reactivity of our hMSH2 antibody to only one protein in a Western blot analysis and the absence of signal when preimmune antibody was used. The aim of this study was to investigate the cell-specific expression of the hMSH2 protein in cells of the large and small intestines. Using antibody specific for hMSH2, we have demonstrated that this protein was highly expressed in cells of the crypts of Lieberkühn that are undergoing rapid renewal in both the ileum and colon. These cells are responsible for the continuous production of differentiated cells that will migrate over a 2–4-day period, before being sloughed into the lumen. Proliferative perifibroblasts in the colon also showed significant presence of the hMSH2 protein. These results confirm the hypothesis that hMSH2 is expressed in highly proliferative cells of the gut, and mutations in this gene could, therefore, be expected to expedite the progression of adenoma to carcinoma in this tissue.

References


