A Truncated hMSH2 Transcript Occurs as a Common Variant in the Population: Implications for Genetic Diagnosis

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Abstract

Germline mutations of the hMSH2 gene are responsible for many cases of hereditary nonpolyposis colorectal cancer. While screening for hMSH2 gene mutations in hereditary nonpolyposis colorectal cancer kindreds, we observed that a previously reported germline mutation is in fact a common, alternatively spliced variant in the population. Using RT-PCR and the protein truncation test, the hMSH2 exon 13 deletion variant was found in more than 90% of individuals. The exon 13 deletion transcript was only present in lymphocyte RNA, no abnormalities were detected in genomic DNA flanking exon 13, and the deletion transcript is apparently not translated. These findings highlight further that caution should be exercised in providing genetic risk assessment on the basis of currently used germline mutation detection strategies.

Introduction

HNPPC is a common dominantly inherited cancer susceptibility syndrome characterized by an early-onset colorectal cancer and an increased frequency of extracolonic neoplasms, including endometrial, gastric, and urological tumors (1, 2). HNPPC is associated with an incomplete penetrance, and multigeneration extended families with clear dominant transmission of colorectal cancer are rare. Epidemiological evidence suggests that HNPPC may account for 1–5% of all colorectal cancers. By current convention, HNPPC families are classified by the following clinical criteria (Amsterdam criteria): (a) at least three individuals in two successive generations affected with colorectal cancer and one of them being a first-degree relative of the other two; (b) at least one affected individual diagnosed at <50 years of age; and (c) exclusion of the diagnosis of FAP, a distinct autosomal dominant colorectal cancer predisposition syndrome (3).

Recent studies have implicated mutations of DNA MMR genes in HNPPC (4–8). These genes represent human homologues of the prokaryotic mutS and mutL genes, whose primary function is to initiate methyl-directed repair of mismatched DNA during replication. To date, inherited mutations of four MMR genes, hMSH2, hMLH1, hPMS1, and hPMS2, have been identified in the germline of HNPPC patients, and hMSH2 mutations are predicted to account for about one-third of all HNPPC cases (9). Somatic hMSH2 mutations of the remaining wild-type allele have been demonstrated in tumors of HNPPC patients with an inherited hMSH2 mutation. The majority of germline and somatic hMSH2 mutations are inactivating in nature and result in a truncated gene product. Here we demonstrate that a frameshift deletion causing a truncated hMSH2 transcript, which was reported previously as a germline mutation (10–12), occurs as a common variant in the population. We have characterized this transcript for its expression properties and population frequency. The implications of these findings in the context of predictive genetic diagnosis of HNPPC are discussed.

Materials and Methods

All protocols involved in patient selection and accrual of blood and tissue specimens were approved by the Human Ethics Committee of the University of Toronto.

Patient Selection. Patients were selected from referrals to the Steve Atanas Stavro Familial GI Cancer Registry of Mount Sinai Hospital in Toronto. Putative HNPPC families were identified using our registry’s modified Amsterdam criteria (13), which include three individuals affected with gastrointestinal/genitourinary/gynecological cancers in two successive generations, with at least one of them affected with colorectal cancer and with no restriction on age at diagnosis.

Tissue Sampling. Matched normal and tumor specimens were obtained from affected patients at the time of tumor resection. Fresh tissues were snap frozen immediately in liquid nitrogen and stored at −70°C.

Nucleic Acids Extraction and PCR. Genomic DNA and total RNA were extracted from peripheral blood and tissue specimens using DNAzol and Trizol according to the manufacturer’s protocol (Canadian Life Technologies Inc., Burlington, Ontario, Canada). DNA was stored at 4°C and RNA at −70°C. RT-PCR was carried out as follows. cDNA (20 μl) was generated from total RNA (2–5 μg) using random hexamer (1 μg; Pharmacia Biotech, Baie d’Urfé, Quebec, Canada), 1× first-strand buffer (Bethesda Research Laboratories, Burlington, Ontario, Canada), 0.5 mM deoxynucleotide triphosphates, 10 mM DTT, and 200 units Superscript II reverse transcriptase (Bethesda Research Laboratories). cDNA (1 μl) was amplified by PCR using primers K3F (nucleotides 1911–1930) and Mh6R (nucleotides 2438–2457) in a reaction mix (20 μl) consisting of 1.0 mM magnesium chloride, 0.25 mM deoxynucleotide triphosphates, and 1.5–2.0 units AmpliTaq (Roche Molecular Systems Inc., Branchburg, NJ). Amplification conditions were initial denaturation at 95°C for 2 min; 30 cycles, each consisting of denaturation (95°C, 1 min), annealing (58°C, 1 min), and extension (72°C, 1 min); and a final extension at 72°C for 5 min. Samples were electrophoresed on either 1% agarose or 12% polyacrylamide gel matrix in 1× Tris-borate EDTA (Tris base (89 mM), boric acid (89 mM), and EDTA (pH 8.0; 2 mM)) buffer.

PTT and Sequence Analysis. An in vitro-synthesized protein truncation assay was carried out on total RNA isolated from peripheral blood lymphocytes of putative HNPPC patients as described previously (5). Briefly, hMSH2 cDNA was amplified in two overlapping segments (nucleotides 1911–1951 and Mh6R (nucleotides 2438–2457) in a reaction mix (20 μl) consisting of 1.0 mM magnesium chloride, 0.25 mM deoxynucleotide triphosphates, and 1.5–2.0 units AmpliTaq (Roche Molecular Systems Inc., Branchburg, NJ). Amplification conditions were initial denaturation at 95°C for 2 min; 30 cycles, each consisting of denaturation (95°C, 30 s), annealing (58°C, 1 min), and extension (72°C, 1 min); and a final extension at 72°C for 5 min. Samples were electrophoresed on either 1% agarose or 12% polyacrylamide gel matrix in 1× Tris-borate EDTA (Tris base (89 mM), boric acid (89 mM), and EDTA (pH 8.0; 2 mM)) buffer.

Western Blot Analysis. Total proteins were isolated from peripheral blood lymphocytes using the lysis buffer (100 ml) containing glycerol (10 ml), β-mercaptoethanol (5 ml), 10% (10 g/100 ml) SDS (30 ml), 1.25 m Tris buffer (pH 6.7–6.8; 12.5 ml), urea (6 g), and 0.1% bromphenol blue (20 ml). Monoclonal antibodies to amino (Ab-1) and carboxyl (Ab-2) terminal of...
hMSH2 protein (Oncogene Science, Cambridge, MA) were used, and Western blotting was carried out according to the manufacturer’s protocol.

Results

Putative hMSH2 Mutation Analysis

To investigate hMSH2 alterations, we analyzed total RNA from lymphocytes of putative HNPCC patients. In one patient (M. B.), PTT analysis of segment 2 (codons 816–934) demonstrated the presence of a truncated gene product in addition to a wild-type protein (Fig. 1). To determine the site of this truncating mutation, we screened the hMSH2 cDNA by RT-PCR analysis of multiple short overlapping segments. Amplification of hMSH2 cDNA spanning codons 615–796 (primers K3F and Mh6R) revealed two transcripts (Fig. 1). Of these, the larger transcript (546 bp) corresponded to the expected wild-type size, and the smaller transcript (341 bp) represented a novel isoform. Sequence analysis of the smaller transcript confirmed a deletion of 204 bp corresponding to exon 13 of hMSH2 cDNA. An examination of the hMSH2 sequence indicates that this is a frameshift deletion that causes a stop codon (TAA) at nucleotide positions 2299–2301 in exon 14 and a truncated protein of Mr 826,000. This mutation has been described previously as a germline mutation responsible for HNPCC (10–12).

hMSH2 Exon 13 Deletion Segregation Analysis in a Putative HNPCC Family

After the identification of the hMSH2 truncating mutation in the lymphocytes of patient M. B., we examined other at-risk members of her family. We used primers K3F and Mh6R to conduct RT-PCR analysis of lymphocyte RNA isolated from affected and nonaffected family members. All were found to carry the hMSH2 exon 13 deletion transcript in addition to the wild-type cDNA. We conducted genotype analysis of all available family members using an intragenic microsatellite marker, D2S123 (5), and a T→C polymorphism at the –6 position in the intronic splice acceptor site of hMSH2 exon 13 (15). Segregation analysis indicated that the deletion of hMSH2 exon 13 did not correlate with a specific chromosomal haplotype (data not shown). Taken together, these results indicate that the frameshift deletion of hMSH2 exon 13 was not a germline mutation responsible for HNPCC in this family.

Population Frequency of hMSH2 Exon 13 Deletion Transcript

We next investigated the occurrence of this transcript in individuals from other HNPCC and non-HNPCC families. Peripheral blood lymphocyte RNA was obtained from a total of 54 individuals (8 with HNPCC, 29 with FAP, 7 with sporadic colorectal cancers, and 10 normal with no colorectal cancer) and analyzed by RT-PCR of hMSH2 exons 12–14 as described earlier. Fifty individuals were found to carry the truncated transcript, and four did not. Furthermore, sequencing of the smaller transcript from three unrelated individuals from this group of samples confirmed that the putative truncated transcripts corresponded to hMSH2 exon 13 deletion. Thus, the exon 13 deletion transcript is a common variant with a population frequency of >90%.

Characterization of hMSH2 Exon 13 Deletion Transcript

Absence of a Genomic Deletion. We then investigated the molecular mechanisms underlying the hMSH2 exon 13 deletion. The hMSH2 genomic locus spans approximately 73 kb and consists of 16 exons (16). Of these, exon 13 is flanked by two introns 1.0 and 1.7 kb in size. To examine whether the exon 13 deletion occurred at the chromosomal level, we amplified genomic DNA of individuals with and without this deletion using the same primers, K3F and Mh6R. A single PCR product of approximately 3.2 kb was observed. Thus, the hMSH2 exon 13 deletion does not occur because of a genomic deletion. Additional sequence analysis of these 3.2-kb PCR products confirmed the wild-type sequence (data not shown).
of exon 13 deletion transcript by RT-PCR screening of matched M, 100,000 corresponding to normal hMSH2 protein is seen with amino terminal (Ab-1, and 2) and without (Lane 3) the IMSH2 exon 13 deletion transcript. A single product of Western blot analysis.

Tissue-Specific Expression. We examined the expression profile of exon 13 deletion transcript by RT-PCR screening of matched peripheral blood lymphocytes and biopsies of normal and tumor specimens (Fig. 2). Analysis of 13 colorectal cancer, 5 breast cancer, 1 ovarian cancer, and 4 lymphoma cases indicated that the truncated transcript is expressed exclusively in lymphocyte RNA.

Western Blot Analysis. The expression of the deleted transcript was examined by Western blot analysis of total proteins isolated from peripheral blood lymphocytes using amino and carboxyl terminal monoclonal antibodies to hMSH2 protein. A single product of M, approximately 100,000 corresponding to the size of the predicted wild-type protein was observed for specimens with and without the exon 13 deletion (Fig. 3).

Discussion

We have conducted a detailed analysis of an hMSH2 truncated transcript caused by a deletion of exon 13. Having examined transcriptional and translational properties and population frequency, we conclude that this deletion transcript occurs as a common variant in the general population. Despite this high frequency of the hMSH2 deletion transcript as detected by RT-PCR, PTT analysis of lymphocyte RNA of individuals carrying the putative deletion did not always detect a truncated product. This may be explained partially by varying levels of the deletion transcript seen among different individuals and/or the efficiency of the "in vitro" translation of these transcripts in the PTT assay. Moreover, in multiple replicate experiments, the abundance of the deletion transcript was consistently lower (2–10-fold) than the wild-type transcript. We investigated molecular mechanisms that cause this deletion. A complete sequence analysis of the flanking introns did not reveal any difference between individuals with and without the truncation transcript. Thus, the deletion of exon 13 does not appear to be due to a splice site defect nor to a genomic deletion, and the molecular mechanisms responsible for generation of this deletion likely occur at the transcriptional or posttranscriptional level. Analysis of several different tissues and tumors showed that the truncation transcript is expressed in peripheral lymphocytes but not in the other tissues studied. Furthermore, the altered transcript does not appear to generate a stable, truncated hMSH2 protein detectable by Western blot analysis.

Our results demonstrate that, in certain instances, screening of lymphocytes or lymphoblast samples at the cDNA or RNA level for hMSH2 gene defects not only may lead to a misdiagnosis of HNPCC, but will have profound implications for counseling and genetic risk assessments of other family members. In HNPCC, germline mutations of MMR genes affect specific organs including the colon and rectum, endometrium, and stomach, and other tissues. Furthermore, a significant proportion of reported hMSH2 and hMLH1 mutations are exon-spooling defects (9). Whether some of these truncated transcripts exhibit a similar tissue-specific expression is not yet known. For hMLH1, generation of multiple transcripts due to alternative splicing has been reported; however, these transcripts are expressed in several different tissues, including colorectal and stomach (17). Perhaps for MMR genes, genetic diagnosis of germline mutations should also be carried out in the susceptible tissues rather than in peripheral lymphocytes only. Alternatively, in appropriate families, additional confirmation of the carrier status should be obtained by linkage analysis. Most importantly, several academic institutions and biotechnology companies have initiated genetic screening programs for families with HNPCC, and our results suggest that caution should be exercised in genetic counseling based on the results of "simple blood tests" in HNPCC families.

Acknowledgments

We gratefully acknowledge the contributions of Drs. H. Ozcelik and M. Zielinska for breast, ovarian, and lymphoma tissue specimens and Helixx Technologies (Toronto, Ontario, Canada) for Tris-glycine gradient gels used for Western blot analysis. We acknowledge the families who participated in this study.

References


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*Cancer Res* 1996;56:2289-2292.

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