Neurofibromatosis and Early Onset of Cancers in hMLH1-deficient Children

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Abstract

Hereditary nonpolyposis colon cancer is a common hereditary disorder caused by the germ-line mutations of DNA mismatch repair (MMR) genes, especially hMLH1 and hMSH2. We report here the first identification of human compounds with a homozygous inactivation of a MMR gene. In a typical hereditary nonpolyposis colon cancer family, MMR-deficient children conceived from matings between heterozygotes for a hMLH1 deleterious mutation exhibited clinical features of de novo neurofibromatosis type 1 and early onset of extracolonic cancers. This observation demonstrates that MMR deficiency is compatible with human development but may lead to mutations during embryogenesis. On the basis of clinical symptoms observed in MMR-deficient children, we speculate that the neurofibromatosis type 1 gene is a preferential target for such alterations.

Introduction

HNPCC is a common autosomal dominant disorder characterized by early onset of colorectal cancer (mean age, approximately 40–45 years) in the absence of gastrointestinal polyposis (1). In addition to large bowel cancers, carcinomas of the ovary, small intestine, ureter, renal pelvis, and stomach are present with increased frequency in HNPCC families. Cancer predisposition is due to heterozygous germ-line mutations in one of the DNA MMR genes (2), hMSH2, hMLH1, hPMS1, hPMS2, and hMSH6/GTBP. Mutations in hMSH2 and hMLH1 account for the vast majority of the cases. The presence of a wild-type allele of these genes appears to be sufficient for normal MMR activity. Cancer progression in predisposed individuals results from the occurrence of a somatic alteration of this normal copy, leading to a mutator phenotype (3). We report here the identification of children with a constitutional homozygous inactivation of the hMLH1 cancer susceptibility gene. The deficiency of MMR activity was associated to particularly severe symptoms associating neurofibromatosis and early development of cancers.

Patients and Methods

Family Collection. Individuals were collected from genetic consultations in the Center Léon Bérard. Members of the family provided specimens for this study with informed consent. Affection status of the individual patients was based on complete clinical investigation, including histopathology of the tumors.

Analysis of MMR Gene Status. DNA was extracted from peripheral blood, buccal mucosa cells, or frozen tumor tissues by proteinase K digestion and phenol purification. Each exon and exon-intron junction region of the hMLH1 and hMSH2 genes was amplified with primers and conditions previously described (4). For the hPMS1, hPMS2, and hMSH6/GTBP genes, analysis of the entire coding region was performed by heteroduplex analysis after reverse transcription PCR. Conditions and primers are available on request. Heteroduplex analysis and in vitro synthesis of protein assay were performed as previously described (4).

Microsatellite Instability Detection. DNA was obtained from normal cells of buccal mucosa and was diluted in water to approximately 1–3 genome equivalents per PCR. Microsatellite marker D2S123 was amplified in 50 μl containing 0.2 μM primers, 1.5 mM MgCl2, 200 μM dNTP, 1 unit Taq polymerase (Perkin-Elmer), and 1 μCi of [α-32P]dATP. PCR was performed with 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 60 s. Negative controls (with no DNA) and positive controls with the equivalent of 100 genomes per PCR were used in each run.

Results

The large kindred HM-1 (Fig. 1), originated from North Africa, fulfilled all criteria of HNPCC as defined by the International Collaborative Group on HNPCC (1). Eleven family members developed colorectal cancers, 8 of them before the age of 50. However, this HNPCC family was characterized by an early onset of neoplastic and non-neoplastic disorders in young children conceived from a consanguineous mating between first cousins. Girl 92 died at age 2 from an undifferentiated non-Hodgkin malignant lymphoma, and her sister (girl 95) was diagnosed with acute myeloid leukemia at age 6, for which she was treated by allogeneic transplantation, and with medulloblastoma at age 7. Furthermore, both sisters had clinical features of NF1; girl 92 had multiple but strictly hemispheric “café au lait macules” and a pseudoarthrosis of the tibia, whereas girl 95 had nine café au lait spots, seven of them over 15 mm, and multiple dermal neurofibromas. No other family member had neurofibromatosis. hMSH2, hMLH1, hPMS1, hPMS2, and hMSH6/GTBP gene status was tested in four patients with cancer (individuals 31, 62, 67, and 91). We detected in all four patients a heterozygous missense mutation in hMLH1 (exon 2, codon 67, GGG→TGG, Gly→Trp). Several criteria suggest that the hMLH1 missense mutation is deleterious: (a) the amino acid at this position is a conserved residue among all MutL homologues; (b) this residue belongs to the consensus MutL box, which is essential for DNA MMR function (5); (c) this mutation was found in all tested affected individuals from family HM-1; (d) the wild-type allele was lost in tumor cells of individual 67 (data not shown), suggesting the involvement of hMLH1 inactivation in tumor progression process; (e) substitution of this amino acid was reported in three other HNPCC families (6–8); and (f) this mutation was not detected in 60 normal control DNA samples, confirming data reported by others (8).

Genetic testing in normal cells of buccal mucosa of child 95 revealed that she was homozygous for the hMLH1 missense mutation (Fig. 2). As expected from this observation, her parents were heterozygous. No sample was available for child 92, but the observation of clinical features associating extracolonic cancer and neurofibromatosis supports the hypothesis that both sisters had a similar defect.
Normal cells from MLH1-deficient mice exhibited a genetic instability in which repeated sequences were characteristically altered (9). The microsatellite instability was evaluated in buccal mucosa cells from girl 95 and from her parents. As expected, a strong mutator phenotype was observed in the homozygote compound (Table 1), confirming the constitutional defect in DNA MMR.

Discussion

Given the involvement of hMLH1 in the repair of mismatched DNA formed during replication and recombination, one can expect that its constitutional inactivation leads to a dramatic increase of the probability of genetic mutations during early development. Considering this hypothesis, the observation of neurofibromatosis symptoms in both sisters is of particular interest. NF1 is a dominant disease affecting approximately 1 in 3500 individuals, caused by the inheritance of a mutant allele of the NF1 gene. Clinical features of the disease include café au lait macules, neurofibromas, freckling, optic glioma, hamartomas of the iris and osseous lesions (10). The penetrance of the disease is virtually 100% by the age of 5 years. Nevertheless, no other member of the family HM-1 was known to
have clinical manifestations of neurofibromatosis. In particular, a careful examination of both parents was unable to reveal any diagnostic criteria of NF1. Although we cannot exclude the hypothesis of a germ-line mosaicism in a clinically normal parent, a mechanism of postzygotic somatic mutation resulting in mosaicism in affected children is strongly suggested by the segmental clinical manifestations in girl 92. Such a mechanism was recently reported in patients with NF1 (11). The NF1 gene is a large gene with 59 exons spanning about 350 kb of genomic DNA (12). It is characterized by a very high mutation rate, estimated at 1 in 10,000 alleles per generation, about 10-fold higher than most genes. Factors that might contribute to this high mutation rate include (a) the large size of the gene; (b) recombinations with pseudogenes that would act as a reservoir of mutations; and (c) the presence within the gene of repeated sequences that are highly susceptible to mutation and intrasstrand recombination (12–14). All of these characteristics may render NF1 highly susceptible to mutations in the absence of MMR activity. On the basis of our observation, it is thus tempting to speculate that in hMLH1-deficient individuals, NF1 constitutes a preferential target for heterozygous mutations during embryogenesis. From studies in animal models (15), we can expect that at this stage, homozygous mutation at the NF1 locus would prevent embryonic development.

In addition to neurofibromatosis, both sisters developed extracranial cancers at an early age. This exceptional susceptibility to cancer probably results from the mutator phenotype caused by the constitutional inactivation of hMLH1. Indeed, the decreased ability to repair genetic alterations increases the likelihood of mutations of oncogenes and tumor suppressor genes (3). Among those, the inactivation of the second allele of the NF1 gene may be a key step of tumor progression. The NF1 gene encodes a cytoplasmic GTPase activating protein called neurofibromin, which is involved in cell growth regulation (16). Although the search for NF1 alterations has proven difficult, data on NF1 mutations in tumors of patients with NF1 and in other types of neoplasms support the view that NF1 is a tumor suppressor gene. Interestingly, young patients with neurofibromatosis are at increased relative risk of malignant disorders, such as juvenile chronic myelogenous leukemia and acute myeloid leukemia (17), a hematopoietic cancer developed by the child 95. Consistent with Knudson's "two-hit" model for inactivation of tumor suppressor genes, malignant tumors from these patients show loss of heterozygosity (17). In hMLH1-deficient children, the risk of such a second event would be dramatically increased by the constitutional genetic instability, explaining both the type of cancers observed in these individuals and the early age of development. This mechanistic hypothesis is based on the observation of clinical features of NF1 in both sisters. Due to the lack of available samples, we were not able to search for NF1 mutations in normal and tumor cells from these individuals. However, such studies are in progress in MLH1-deficient mice.

In conclusion, we report here the first identification of human compounds with a homozygous mutation of a MMR gene. Recently, Hackman et al. (18) reported the observation of a woman heterozygous for two different hMLH1 missense mutations on both alleles. This woman developed a breast cancer at age 35, a clinical feature quite different from our cases. However, as suggested by the authors, the mild phenotype of the woman may be due to a residual hMLH1 activity from one allele. In the HM-I family, both the high predisposition of heterozygotes to colorectal cancers and the severe phenotype of the homozygotes demonstrate that this mutation is deleterious. This is further confirmed by the loss of the wild-type hMLH1 allele in the tumor of the heterozygous compound 67. Due to the HNPCC frequency, individuals with constitutional inactivation of MMR activity may not represent exceptional cases. Furthermore, we believe that the association of MMR deficiency and NF1 phenotype is not purely coincidental. Pratt et al. (19, 20) reported independent cases of patients with early development of multiple colon carcinomas, lymphomas and clinical features of de novo NF1. These individuals were conceived from consanguineous marriages, strongly suggesting that the predisposition was linked to the inheritance of a homozygous mutation. No genetic testing was performed in these families. However, in light of our results, hMLH1 (or another MMR gene) should be a good candidate to these predispositions. During the preparation of this report, we learned that Ricciardone et al. (21) identified a Turkish HNPCC family with three children, conceived from a consanguineous mating, diagnosed with leukemias and lymphomas. The colon cancer predisposition was caused by the inheritance of a hMLH1 gene alteration. All three children exhibited clinical features of neurofibromatosis. The observation of similar clinical features in an independent family strengthens our hypothesis that in the presence of a constitutional genetic instability, the NF1 gene constitutes a key target of mutation. Such a hypothesis will be tested in human MMR-deficient cell lines and in animal models. This would provide a novel link between two thus far unrelated pathways involved in cancer development.

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References

HOMOZYGOUS MLH1 MUTATION AND NEUROFIBROMATOSIS


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