Microsatellite Instability and Immunohistochemical Analysis of MLH1 and MSH2 in Normal Endometrium, Endometrial Hyperplasia and Endometrial Cancer from a Hereditary Nonpolyposis Colorectal Cancer Patient

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Received August 27, 2001; accepted December 25, 2001

Hereditary nonpolyposis colorectal cancer (HNPCC)-related endometrial cancer is associated with mutations in DNA mismatch repair genes. However, chronological changes of these genes in the endometrium have not been studied in women from HNPCC families. Tissue samples of normal endometrium, endometrial hyperplasia without atypia and endometrial cancer were collected at different times from a 41-year-old Japanese woman with a family history of HNPCC. Combined microsatellite instability (MSI) and immunohistochemical analysis of MLH1 and MSH2 predicted the presence of a mutation in MSH2 when she had endometrial hyperplasia without atypia 7 months before the diagnosis of endometrial cancer. Endometrial hyperplasia without atypia may indicate an early development of endometrial cancer in women from HNPCC families.

Key words: hereditary nonpolyposis colorectal cancer – endometrial cancer – endometrial hyperplasia – mismatch repair gene – microsatellite instability

INTRODUCTION

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant inherited disorder which is characterized by an early onset of colorectal cancer and is associated with extracolonic cancers of the endometrium, stomach, ovary, small bowel, renal pelvis and ureter (1). Endometrial cancer is the second most common malignancy in female HNPCC patients (2). The results of recent molecular biological studies have shown that HNPCC-related endometrial cancer is associated with a high incidence of mutations in DNA mismatch repair genes (3). However, as far as we know, there have been no studies that investigated mutations in DNA mismatch repair genes at different times in patients with a family history of HNPCC (i.e. normal endometrium, endometrial hyperplasia and endometrial cancer). This paper reports a woman from whom tissue samples were collected at different times. The results of a histological examination were normal at the initial examination, complex endometrial hyperplasia without atypia 4 years after the initial examination and endometrial cancer 7 months after the endometrial hyperplasia diagnosis. Microsatellite instability (MSI) analysis was conducted and MLH1 and MSH2 immunohistochemical expression was examined. The results showed chronological changes in MSI and MSH2 expression in the endometrium of this patient.

CASE REPORT

The subject was a 41-year-old Japanese woman, gravida 5, para 2, who met the new clinical criteria for HNPCC (4). Her father and an uncle on her father’s side had colon cancers and another uncle and an aunt on her father’s side had stomach cancers. Also, another aunt on her father’s side had both colon and endometrial cancers. At the age of 36 years, the patient visited Tsukuba University Hospital owing to menstrual irregularity. Her endometrial smear was normal and a histological examination of the endometrium revealed a normal endometrium. At the age of 38 years, she underwent left colectomy to remove...
descending colon cancer (well differentiated adenocarcinoma) and the result of a histological examination of the endometrium was also normal. Subsequently, at the age of 40 years, following a histological examination she was diagnosed as having complex endometrial hyperplasia without atypia. She was followed up and 7 months later she had vaginal bleeding and based on a histological examination was diagnosed as having endometrial cancer (well differentiated endometrioid adenocarcinoma). Consequently, she was admitted to undergo surgery. Preoperative magnetic resonance imaging (MRI) and hysteroscopy showed that the cancer was localized in the body of the uterus. We performed modified radical hysterectomy, bilateral salpingo-oophorectomy and pelvic lymph node dissection. Pathological examination revealed the endometrial cancer to be FIGO stage Ib, endometrioid adenocarcinoma, G1. She was discharged without further treatment. At present (December 2001, 6 years after the surgery), she is being treated on an outpatient basis, with no evidence of disease.

**MSI Analysis**

DNA was extracted from paraffin-embedded tissue samples from the initial examination (normal endometrium), from the examination 4 years after the initial one (complex endometrial hyperplasia without atypia) and the excised uterus (endometrial cancer). For corresponding normal tissue, DNA was extracted from a lymph node that was removed at surgery. DNA samples were analyzed with three microsatellite primers that amplified D2S123, D3S1067 and BAT26. PCR amplification and MSI assessment were performed as described previously (5). Abnormal bands were seen at D3S1067 and BAT26 using the endometrial hyperplasia and cancer samples, thus confirming positive MSI status (Fig. 1).

**Immunohistochemical Findings**

The paraffin-embedded tissue samples used in the above MSI analysis were subjected to MLH1 and MSH2 immunohistochemical staining as described previously (6). Although MLH1 expression was detectable in the nuclei of normal endometrium, complex endometrial hyperplasia without atypia and endometrial cancer, MSH2 expression was only detectable in the normal endometrium (Fig. 2).

**Discussion**

Endometrial cancer is the second most common malignancy after colorectal cancer in female HNPCC patients (2). Compared with sporadic endometrial cancer, HNPCC-related
Defective mismatch repair in endometrium endometrial cancer is associated with a higher incidence of mutations in DNA mismatch repair genes (3). However, it has been reported that, even in women with a family history of HNPCC caused by mutations in DNA mismatch repair genes, MSI and alteration in MLH1 and MSH2 expression are absent in histologically normal endometria (6). In the present case, we were able to investigate MSI and alteration of MLH1 and MSH2 expression level before the onset of endometrial cancer when the patient was diagnosed as having normal endometrium and complex endometrial hyperplasia without atypia. MSI and immunohistochemical changes in MSH2 expression were seen when she had the endometrial hyperplasia. Also, the same changes that were seen when she was diagnosed as having endometrial cancer, predicting the presence of a mutation in MSH2 seen during the endometrial hyperplasia, were involved in the carcinogenesis of the endometrial cancer. This case could provide valuable information about the role of mutations in DNA mismatch repair genes in HNPCC-related endometrial cancer. This case reaffirms the importance of endometrial hyperplasia in endometrial examinations for women with a family history of HNPCC. In the future, it will be important to establish methods that could identify women from HNPCC families who are likely to develop endometrial hyperplasia by analyzing genes other than DNA mismatch repair genes. This might lead to the prevention of endometrial cancer in HNPCC families.

Moreover, in this case, the time period between the diagnosis of normal endometrium and that of complex endometrial hyperplasia without atypia (4 years) was longer than the time period between the diagnosis of endometrial hyperplasia and that of endometrial cancer (7 months). Therefore, prophylactic hysterectomy should be considered after childbirth when women from HNPCC families are diagnosed as having endometrial hyperplasia with or without atypia. Surgery including lymph node dissection is needed once endometrial cancer is confirmed, but less invasive surgery may be sufficient at the stage of endometrial hyperplasia. This point needs to be examined further by studying more cases.

In conclusion, the present case reaffirms the importance of endometrial hyperplasia in endometrial examinations for women with a family history of HNPCC. In the future, it will be important to establish methods that could identify women from HNPCC families who are likely to develop endometrial hyperplasia by analyzing genes other than DNA mismatch repair genes. This might lead to the prevention of endometrial cancer in HNPCC families.

References