Clinicopathological Features in Endometrial Carcinoma with Mismatch Repair Deficiency among Chinese Women Younger Than 50 Years of Age

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Abstract
Objective: Mismatch repair (MMR) deficiency can lead to genome instability and closely correlated with carcinogenesis of endometrial carcinoma and mainly caused by MMR gene germline mutation known as Lynch syndrome (LS) or epigenetic mechanisms of MLH1 promoter methylation. The main objective of the present study is to correlate the clinicopathological features in endometrial carcinoma from 92 Chinese women younger than 50 years of age with the MMR status.

Methods: In total 92 cases of consecutive hysterectomy specimens with EC in patients younger than 50 years of age were analyzed. We performed immunohistochemistry staining for 4 MMR proteins and two groups (MMR deficiency group and MMR normal group) were subdivided according to the outcome of immunohistochemistry staining.

Results: 38 cases (42%) with the negative expression of MMR proteins are selected for analysis of their clinical and pathological features. This group included 34 cases of endometrioid adenocarcinoma, one case of clear cell adenocarcinoma, three cases of the mixed types including clear cell, serous and neuroendocrine carcinoma mixed with classic endometrioid adenocarcinoma respectively. In addition to squamous differentiation, 29% cases also exhibit other differentiations including mucinous, clear cell, a lot of papillary structures, dedifferentiated, and secretory types. There are 10 cases showing features of peritumoral and tumor infiltrating lymphocytes. Seven cases have synchronous carcinoma of other sites besides the uterus.

Conclusions: The MMR deficient group showed more morphological differentiations including mucinous differentiation, frequent tumor infiltrating lymphocytes, and more frequent synchronous carcinomas of other sites than tumors with a normal immunophenotype of the MMR genes. Features of dedifferentiated EC originated in the lower uterine segment were not identified in our study. Endometrial carcinoma in Chinese patients younger than 50 years of age is more likely to be LS associated carcinoma.

Introduction

The DNA mismatch repair (MMR) system is known for recognizing and fixing errors during replication and preserving genome stability. Defects of the MMR system can lead to genome instability and is involved in carcinogenesis with a mutator phenotype tumor[1]. It is reported to be associated with 10–15% of colorectal cancer and up to 30% of endometrial cancers [2]. The MMR deficiency, mainly caused by germline mutations in 4 MMR gene or epigenetic mechanisms of MLH1 promoter methylation, can be manifested as loss of expression of one or more MMR proteins.

Germline mutations in MMR genes can give rise to the autosomal dominant disease[3,4], Lynch Syndrome (LS), previously referred as hereditary nonpolyposis colon cancer (HNPCC). The lifetime cumulative risk of endometrial carcinoma in women with Lynch syndrome is as high as 40% to 60%[5,6], which equals to or exceed the risk of colorectal carcinoma. Endometrial carcinoma in more than half of the cases is usually the first primary tumor[7,8]. Correctly recognizing this disease is significantly important for patient and their family members to decrease the cancer risk. It is suggested that the association of tumor morphology and MMR protein status can enhance the detection of Lynch syndrome[9,10].

The objective of the present study is to correlate the clinicopathological features (including stage, tumor type, grade and morphology features) in 92 consecutive endometrial carcinoma from Chinese women younger than 50 years of age with tumor’s the MMR status (including MLH1, PMS2, MSH2 and MSH6).

Materials and Methods

Sample Selection
Resection specimens were obtained from 92 patients who underwent surgical resection of endometrial carcinoma at
the Central Hospital of Gynecology and Obstetrics (Tianjin, China) from 2011 to 2014. All patients involved have signed an informed consent, which permitted the use of their specimens for our present study.

**Histopathologic Review of Tumors**

For all tumors, all hematoxylin-stained slides were reviewed independently by two observers. The following variables were assessed: Tumor grade (low grade and high grade), histological subtype (endometrioid, nonendometrioid, mixed type), morphology features (nonsquamous differentiation, features of peritumoral or tumor infiltrating lymphocytes), the presence or absence of histo-prognostic criteria (such as perineural EP or embols or vessel invasion EV). Tumors were staged according to the tumor-node-metastasis (TNM) staging systems (AJCC 7th Ed.)

**Immunohistochemistry Procedure**

These samples were fixed in 10% formaldehyde solution, dehydrated, and placed in paraffin for histological analyzes. Four-micrometer-thick sections were prepared and mounted on poly-L-lysine-coated slides. Briefly, slides were deparaffinized, and endogenous peroxidase activity was blocked by incubation with 3% H2O2. Heat-induced antigen retrieval was performed using the Ventana CC1 mild reagent (Ventana Medical Systems) for 30 to 60 min. After treatment with 10% normal goat serum to block nonspecific protein binding, pre-diluted primary antibodies against MLH1, MSH2, MSH6, and PMS2 were applied, followed by incubation with horseradish peroxidase-conjugated multimer antibody reagent. The antigen-antibody reaction was visualized using diaminobenzidine as a chromogen.

Immunohistochemistry (IHC) analysis was performed using the following monoclonal antibodies: anti-MLH1, anti-MSH2, anti-MSH6, and anti-PMS2 (Zhongshan biotechnology limited company, Beijing, China). Normal endometrial tissue and stromal cells served as an internal positive control for staining. Completely Loss of expression was recorded when nuclear staining was absent in malignant cells, but preserved in normal epithelial and stromal cells. Two observers assessed all cases independently.

**Statistical Analysis**

We used SPSS software (Version 16) for the statistical analysis. The x square test was used to determine the statistic significance concerning the difference of MMR protein expression based on immunostaining in normal tissue and primary tumors. The correlation between the MMR protein expression and clinicopathologic variables of tumors are also determined in a similar manner.

**Results**

Summary of MMR gene expression in Chinese patients less than 50 years old with endometrial carcinoma (Table 1). Totally, 92 cases of endometrial carcinoma in patients younger than 50 years were identified within the study period. At least one MMR protein was absent in 38 (42%) of the 92 tumors investigated. The MSH2 loss was the most common abnormality, followed by the absence of MSH6 and PMS2. Combined loss of MLH1 and PMS2 occurred in 7 cases (18.4%) while isolated PMS2 loss occurred in 7 cases. Combined loss of MSH2 and MSH6 occurred in 15 cases (39.4%) while isolated loss of MSH2 or MSH6 occurred in 4 and 2 cases respectively. Additional three cases exhibited combined loss of PMS2, MSH2.

<table>
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<tr>
<th>Age</th>
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**Patient Characteristics in Endometrial Carcinoma with MMR Deficiency**

Overall patient age ranged from 24 to 50 years (median 40) in 38 cases of endometrial carcinoma with MMR deficiency. There were 15 (38%) patients who are younger than 40 years while the percent was slightly lower in the normal MMR groups (33%). The majority of patients presented with stage 1a disease (26 cases, 67%), while 6 (15.7%), 2 (5.3%), and 4 (10.5%) patients presented with stage 1b, stage 2, and stage 3 or 4 disease, respectively. Only five patients with the loss of IHC-MMR showed personal and/or family history (13%). The majority of our patients with the loss of MMR protein expression are absent of personal or family history (33/38) of LS-associated tumors.

**Morphology Features**

34 cases (92%) of Endometrioid adenocarcinoma were identified in the MMR deficiency group. Four cases of Non-endometrioid carcinoma were identified, including one case of clear cell carcinoma, three cases of mixed types of clear cell, serous and neuroendocrine carcinoma mixed with classic endometrioid adenocarcinoma respectively. The number of type II carcinoma or non-endometrioid carcinoma is statistically higher in the MMR deficient group than in the standard MMR group (four cases versus one case).

The endometrial carcinomas were subtyped into low grade (including FIGO grade 1 or 2 endometrioid adenocarcinoma) and high grade endometrial carcinoma[12] (HGEN, consisting of FIGO grade 3 endometrioid carcinoma, dedifferentiated carcinoma and type II carcinoma). Among the 38 endometrial adenocarcinoma with the MMR deficiency, 29 cases (76.3%) were low grade and nine cases (23.7%) were high grade. While in the normal MMR group, high-grade cases accounting for 28.5% and no statistical significance was found between these two groups.

There are 13 cases (34.2%) of endometrial carcinoma with MMR deficiency displaying non-squamous differentiation. There are seven cases showing mucinous differentiation, another three cases showing clear cell, serous or villoglandular differentiation respectively, one case displaying a lot of papillary structures (Figure 1), one case showing secretory adenocarcinoma (Figure 2) and one case of dedifferentiated carcinoma (Figure 3). The number of cases with various non-squamous differentiations in the MMR deficient group is statistically different than that in cases showing normal MMR expression. There are 10 cases exhibiting features of peritumoral or tumor infiltrating lymphocytes (Figure 4).
Other Histological Features

Six cases (16%) of deep myometrial invasion and seven cases (18%) of lymphovascular space invasion were identified. No statistical significance was found between the MMR deficient group and normal MMR counterparts.

Four cases (10.5%) of endometrial carcinoma appeared to be originated from the lower uterine segment and additional 13 cases (34.2%) involved the mucosal or myometrium of the lower uterine segment. Although not statistically significant, the ratio of endometrial carcinoma involving lower uterine segment is higher than that (16.7%) in the normal MMR counterpart.

Seven MMR deficient patients have synchronous tumors in other sites besides the uterus. Four cases are concurrent ovarian endometrioid carcinoma or clear cell carcinoma; two cases are with rectal or colonic cancer. The last case was occurring together with, interestingly, pelvic and abdominal multifocal gastrointestinal stromal tumor (GIST).

Discussion

We have analyzed a cohort of 92 consecutive hysterectomy specimens with endometrial carcinoma in patients younger than 50 years of age and revealed that the rate of abnormal MMR protein expression by IHC is 41.3% (38/92). The rate of loss of expression of MMR protein in unselective endometrial carcinoma is proximately 22.4-35.3% according to some literatures[7,13,14]. In women less than 50 years old, the incidence of MMR loss ranges from 16.2% to 34% [11,15,16] in western countries. However, our rate is slightly higher than that being reported, which may be ascribed to the differences in the ethnic backgrounds of patient populations or case selections[17].

In our study, only 7 cases (18.4%) showed combined loss of MLH1 and PMS2. This rate is significantly lower than that in unselected endometrial carcinoma, which is usually in the range of 58.2%-77.3[7,13]. The difference may be due to the distribution discrepancies of MMR protein expression in patients younger than 50 years of age. Due to the fact that up to 77% of endometrial carcinoma with MMR deficiency[13,18] is associated with epigenetic mechanisms of MLH1 promoter methylation silencing transcription[19,20], the low incidence rate of loss of MLH1 in our cohort signified the low methylation rate in women before 50 years. A report[21] about Microsatellite instability and DNA methylation in endometrial tumors demonstrated that the unmethylated rate in women younger than 50 years with MSI increased to 62%, significantly higher than that of 17% in older women. Our findings are consistent with that younger woman with MMR deficiency exhibited low methylation rate.

Deficient MMR activity would account for the accumulation of mutations throughout the genome[22-25]. In fact, Endometrial carcinoma is diagnosed at an overall significantly earlier
Several studies[10,30] have reported one of the constant
vestigation. -

than that in the normal MMR expression group. We observed
ma and one of dedifferentiated carcinoma. The number of cases
showed clear cell differentiation, one case with serous differenti
For these, seven cases showed mucinous differentiation, one case
inflammatory response, which can be separated into “(a) peritumoral
features seen in MMR deficiency associated tumors is the host
ative EC, which comprises FIGO grade 1 or 2 endometrioid
carcinomas and undifferentiated carcinomas. One earlier study
reported the features of tumor heterogeneity, which was not well
accepted by some authors because the actual incidence of LS
associated carcinoma having dedifferentiated component is only
5%[10,29]. Our data showed only one case (3%) of the MMR de-
icient endometrial carcinoma with dedifferentiated carcinoma
consistent with the view that tumor heterogeneity is likely not a particular feature for LS.

The correlation between the anatomic origins of endo-
trial carcinoma and Lynch syndrome is controversial. Some
vestigators identified a positive “correlation between the anato-
omic origin of the cancer and individuals with LS: Up to 29% of
patients with ECs centered in the lower uterine segment had LS”[32]. Another group reported the incidence of the lower uter-
segment involvement in the LS patients was merely 5.3%[33].
Our study focused on Chinese women with MMR deficiency
younger than 50 years of age. We have found about 10% of the
endometrial carcinoma in the MMR deficiency group are origin-
ated in the lower uterine segment, which showed no statistical
difference compared with that in the normal MMR group. How-
ever, the actual incidence of involvement of the lower uterine
segment was 34%, which is statistically significant different than
that in the normal MMR group.

In summary, the MMR deficient group showed more
non-squamous histomorphological differentiations including
cases with mucinous differentiation. The MMR deficient group
is also more likely to have tumor infiltrating lymphocytes and
more frequent synchronous carcinomas of other sites than tu-
mors with a standard MMR immunophenotype. Features of de-
differentiated EC and features of frequently originated from the
lower uterine segment were not found in our study. The majority
of our patients with the loss of MMR protein expression does
not have a personal or family history (33/38) of LS-associated

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