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Clinical characteristics of Lynch-like cases collaterally classified by Lynch syndrome identification strategy using universal screening in endometrial cancer

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HIGHLIGHTS

- Lynch syndrome (LS) screening is recently recommended for endometrial cancer (EC).
- Molecularly LS-suspected Lynch-like cases (LL) lack germline pathogenic mutations.
- In 348 ECs, clinical features of LS, LL, and sporadic cancer (SC) were compared.
- LL showed intermediate features between LS and SC, and clearly differed from SC.

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ABSTRACT

Objective. Lynch syndrome (LS), an autosomal-dominant inherited disorder, increases the risk for LS-associated cancers (LS-AC). Molecular LS assessment for all cases is referred to as universal screening (U/S) and is recommended for endometrial cancer (EC) and colorectal cancer. Lynch-like cases (LL) lack LS-pathogenic mutations despite being suspected as LS by U/S, but have been poorly investigated in EC. The aim of this study was to capture the features of LL in EC and to devise LL management in EC.

Methods. U/S, consisting of immunohistochemistry and reflex methylation analysis, was applied to 348 Asian ECs, and sporadic cancer (SC) cases were screened out. Genetic testing was offered to "suspected-LS" cases selected by U/S. The features of the LS, LL, and SC groups were recorded and compared.

Results. U/S screened 306 ECs as SC. The recurrence rates of suspected-LS and SC cases were 14.3% (6/42) and 26.5% (81/306), respectively. Of the 42 suspected-LS cases, 10 were identified as LS, 17 were classified as LL, and 15 did not undergo genetic testing. In the LS group, the frequency of personal history (50%) and family history (100%) of LS-AC were prominent. Of note, the prevalence of family history of LS-AC and gastric cancer was significantly higher in the LL group than in the SC group (76.5% vs. 38.6% and 47.1% vs. 25.2%, respectively).

Conclusions. Herein, we report the features of LL classified by LS identification via U/S in Asian EC. LL should be candidates for tailored surveillance based on regionality and family history.

1. Introduction

Lynch syndrome (LS) is an autosomal-dominant inherited disorder mainly due to a predisposing germline mutation in the DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) or the epithelial cell adhesion molecule (*EpCAM*) gene, and is associated with increased risk for various cancers, particularly colorectal cancer (CRC) and endometrial cancer (EC). The detection of LS patients with LS-associated cancers (LS-AC) is important for the prevention and prediction of their subsequent LS-AC and can provide their relatives with the opportunity for genetic evaluation and prophylactic management of LS-AC. Studies regarding family members with germline MMR gene mutation identified via probands with CRC have supported the development of more suitable identification methods and management guidelines [1]. Based

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on its cost effectiveness and strong scientific evidence, molecular LS assessment for all patients with CRC is strongly recommended by the American Gastroenterological Association Institute [2].

Women with LS account for 2–6% of patients with EC [3–8]. In women with pathogenic germline MMR gene mutation, the lifetime risk is 43–48% for CRC, 40–62% for EC, 2–13% for gastric cancer (GC), and 6–14% for ovarian cancer (OC), and the incidence of other LS-AC also increases greatly [9,10]. EC frequently precedes CRC and functions as a sentinel cancer for women with LS [11]. EC caused by LS is likely to occur at a young age and has a lower mortality rate than other LS-AC. Hence, the identification of LS women with EC would have clinical significance in the prediction of subsequent cancers and could offer profound benefits to future expansion of prophylactic and/or individualized medicine.

Microsatellite instability (MSI) testing and immunohistochemistry (IHC) of MMR proteins are valuable for molecular LS assessment in CRC. Molecular LS assessment in all patients is referred to as universal screening (U/S) and has been gaining a foothold as a primary screening method that is an alternative to conventional criteria based on clinical data [12–14]. In EC, the majority of tumors with MLH1 and/or PMS2 deficiency on IHC are sporadic cancer (SC) caused by *MLH1* promoter hypermethylation (MLH1-PHM) [5,15], and procedures that consist of IHC and reflex methylation analysis are regarded as a highly sensitive U/S strategy. On the other hand, the procedures adding molecular analyses to some types of clinical selection have been studied as a more efficient screening strategy [16].

Based on LS screening, germline genetic testing would be proposed to patients with EC as well as CRC through genetic counseling. Lynch-like cases (LL) refer to cases having neither germline pathogenic MMR mutations nor somatic MLH1-PHM in spite of MMR protein deficiency and/or MSI-High in the tumor [17]. LL accounts for 56–71% of suspected-LS patients with CRC and 16–64% of suspected-LS patients with EC [3,5,6,7,17–19]. Acquired biallelic somatic MMR gene mutations are responsible for a subset of LL and may be associated with somatic (or occasionally germline) mutations in DNA polymerase epsilon (POLE) exonuclease domain that increase spontaneous mutation rates (Ultramutated phenotype) [20]. Awareness of LL in CRC is starting to grow [21], but no definite guidelines for LL have yet been formulated [1]. In EC, even the clinical features of LL patients have not been sufficiently investigated.

In this study, U/S using immunohistochemical staining for MMR proteins (MMR-IHC) and reflex methylation analysis was retrospectively conducted in 348 unselected patients with EC. Clinical identification, including genetic testing, was performed to capture the features of LS in Asian patients with EC. The clinical features of patients with LS, LL, and SC classified on the basis of our identification strategy were recorded and the differences between the three groups were verified.

2. Materials and methods

2.1. Study population and procedures (Fig. 1)

Out of 360 newly diagnosed patients with EC at Akita University Hospital between January 2003 and December 2013, 348 cases for whom evaluable tumor specimens were available were identified retrospectively (12 cases with insufficient cancer tissue volume for reliable IHC were excluded from this study population). All patients were Asians living in Japan. The patients' clinical data were collected from medical records and clinical inquiry. The family history of LS-AC was collected from first- and second-degree relatives (not including individuals). This population was almost the same as our previous study [15,16] participants, but their information was modified by additional inspection in this study. MMR-IHC was performed on the tumors of all patients to assess MMR protein expression. MLH1 methylation assay was performed on tumors with MLH1 and/or PMS2 protein deficiency to exclude sporadic EC. Thus, we selected "suspected-LS" patients as candidates for genetic analysis. The corresponding MMR gene mutations were predicted from MMR-IHC results and germline genetic testing was carried out by using direct sequencing and/or multiplex ligation-dependent probe amplification (MLPA) techniques. Patients who underwent germline genetic testing were classified into LS or LL. All study participants who underwent genetic analysis provided written informed consent in the prescribed document. The Institutional Review Board of Akita University approved our study design.

2.2. MMR-IHC

MMR-IHC was performed on tumors from all EC patients to assess MMR protein (MLH1, MSH2, MSH6, and PMS2) expression, according to standard procedures. An appropriate paraffin-embedded tissue was cut at 4-μm thickness. The tissue sections were deparaffinized in xylene and rehydrated in graded alcohol. Subsequently, antigen retrieval was performed in 10 mmol/L Tris-EDTA buffer (pH 9.0) in a microwave oven for 20 min. These sections were allowed to cool at room temperature. Next, the primary antibodies were applied overnight at 4 °C. The primary antibodies against MLH1 (clone ES05; dilution 1:50; Dako, Glostrup, Denmark), MSH2 (clone FE11; dilution 1:50; Dako), MSH6 (clone EP49; dilution 1:50; Dako), and PMS2 (clone EP51; dilution 1:40; Dako) were used. The antigen-antibody reaction was visualized with the Envision kit (Dako). The slides were counterstained with hematoxylin. Adjacent normal endometrium and lymphocytes in the slides were used as internal positive controls. We judged the complete absence of nuclear staining in the tumor cells as loss of MMR protein expression.

2.3. MLH1 promoter methylation analysis

We previously reported that isolated loss of PMS2 immunohistochemical expression was frequently caused by heterogeneous MLH1-PHM [15]. Hence, *MLH1* promoter methylation analysis was performed on tumors with MLH1 and/or PMS2 protein deficiency to exclude sporadic EC. The tumor DNA was extracted from mapped formalin-fixed, paraffin-embedded tissue sections to provide tumor samples for the assay. The SALSA MS-MLPA kit ME011 mismatch repair genes (MMR) (MRC-Holland, Amsterdam, The Netherlands) was used to detect aberrant CpG island methylation in the promoter of MMR genes, including 5 probes for *MLH1*. The MS-MLPA assay was performed as described by the manufacturer. We focused on the promoter C region (probe 3), which provides the best correlation with MLH1 expression. Based on a previous study associated with gene silencing [22], the dichotomization threshold to distinguish hypermethylated versus non-methylated samples was set at 15%.

2.4. Prediction of MMR gene mutations

The MMR proteins function as heterodimers. MSH2 forms a heterodimer with MSH6 or MSH3 and MLH1 forms a heterodimer with PMS2 or PMS1. The expression of MSH6 depends upon that of MSH2, and the expression of PMS2 depends upon that of MLH1. Hence, the corresponding MMR gene mutations were predicted from loss pattern of MMR protein expression. Tumors retaining MMR protein expression were regarded as sporadic EC.

2.5. Germline genetic testing

Out of 42 "suspected-LS" cases, 27 patients voluntarily donated their peripheral blood for this study. Based on the prediction of MMR gene mutations, germline genetic testing was carried out by using standard techniques. Detection of point mutations was conducted by using exon-by-exon PCR and direct sequencing of the whole coding sequence at intron-exon boundaries for each gene. EpCAM and large rearrangements (e.g., deletions, insertions, and inversions) in the MMR gene

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