Possible Risk Factors of Pulmonary Metastases in Patients With International Federation of Gynecology and Obstetrics Stage I Endometrioid-Type Endometrial Cancer

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Objective: Limited data have been obtained in regard to pulmonary metastasis (PM) in patients with stage I endometrial cancer. The aims of the study were (1) to present the clinical and pathological characteristics of patients with PM in the setting of stage I endometrioid-type endometrial cancer (EEC) and (2) to define possible factors that may be used to predict PM.

Methods: Six hundred thirty patients with stage I EEC, including 12 with PM, 19 with extra-PM (EPM), and 599 with no recurrence, were observed. Paired samples of primary and metastatic tumors from a patient were used for exome sequencing to identify potential gene mutations associated with PM.

Results: There was no significant difference in the age, Ki-67, lymphatic vascular space invasion, and grade 3 among the 3 groups (P > 0.05). More squamous epithelial differentiation was observed in PM (7/12), as compared with patients with EPM (1/19) (P < 0.05) and no recurrence (20/599) (P < 0.05). The tumor size of the patients with PM was bigger than that of nonrecurrent patients (29.8 ± 16.6 vs 18.5 ± 16.3 mm, P < 0.05). More percentage of patients with deep myometrial invasion (IB) were found in PM (6/12) (P < 0.05) as compared with patients with EPM (3/19) (P < 0.05) and no recurrence (76/599). CDH10, ARID1A, and EMT-associated gene mutations were identified in metastatic tumor tissue but not in primary tumors from a patient with EEC and lung metastases.

Conclusions: Squamous epithelial differentiation, large tumor size, and deep myometrial invasion might be risk factors for PM in patients with stage I EEC. CDH10, ARID1A, and EMT-associated gene mutation may promote the initiation of lung recurrence. However, further studies are needed to determine the precise mechanisms associated with lung metastasis in these patients.

Key Words: Endometrial cancer, Pulmonary metastases, Recurrence

Received December 29, 2016, and in revised form February 12, 2017. Accepted for publication March 5, 2017.

(Int J Gynecol Cancer 2017;27: 1206–1215)
Endometrial carcinoma (EC) is the most common invasive malignancy of the female reproductive tract in developed countries.¹ The incidence of EC is increasing rapidly in China, where it is the third most common malignancy in women.² Most patients are usually given a diagnosis in the early stages, which is associated with good survival. Unfortunately, some of these patients will experience local and/or distant recurrences throughout all stages of the initial disease. Approximately 10% to 15% of patients with early-stage disease experience tumor recurrence after initial treatment.³ The lung is a less common site for EC metastasis via hematogenous routes because the incidence of lung metastasis ranges from 2.3% to 4.6% (for all stages and all pathological types). It has been reported that pulmonary metastasis (PM) was associated with stage IV disease and deep myometrial invasion.⁴ ⁵ However, a subset of patients with early-stage and low-grade disease experience PM for reasons that remain unclear. To date, no data can predict the occurrence of PM in patients with early-stage endometrioid-type EC (EEC).

To present the clinical, pathological, and biological characteristics of patients with stage I EEC and PM, as well as to define the possible factors that predict pulmonary recurrence, we retrospectively analyzed patients in our hospital with stage I EEC who developed PM and extra-PM (EPM). We also performed exome next-generation sequencing of cancer-related genes to identify potential mutations that might be associated with PM in both primary and PM tumor tissues in 1 patient.

MATERIALS AND METHODS

Patients

Data for this study were obtained from a large institutional review board–approved database of patients with EC who were treated at the Obstetrics and Gynecology Hospital of Fudan University from 2008 through 2014. This interval was chosen to minimize the impact of cohort effects on our data analysis. Figure 1 summarizes the process by which the cohort was defined and the PM cases were identified. Nine hundred six patients with EC who were managed with surgery, postoperative chemotherapy, and/or radiotherapy between January 2008 and December 2014 in our hospital were observed. After a careful retrospective review of the clinical and pathological data, 12 patients with stage I (International Federation of Gynecology and Obstetrics [FIGO] 2009, IA or IB) EEC with PM alone or with recurrence at other sites were identified. Meanwhile, 19 patients with stage I EEC with EPM and 599 patients with stage I EEC without any recurrence were analyzed as controls. All patients were followed until death or until the end of the study period (December 31, 2015). All patients underwent laparoscopic total hysterectomy and bilateral salpingo-oophorectomy, with or without pelvic and aortic lymph node dissection, depending on the stage (IA or IB) and tumor size.

Clinicopathological Characteristics

Histopathologic assessment, regarding all slides from the patients in our research, was performed by attending pathologists at our institution. The pathological features of tumor tissues, including Ki-67 expression, the presence of lymphatic vascular space invasion (LVSI), and the degree of squamous epithelial differentiation, were analyzed. A retrospective extensive review of medical records was performed of patients with EC metastatic to the lung to obtain specific clinical data and overall outcomes (Table 1).

Follow-Up

The data obtained during follow-up included the age of the patients, the date that PM was diagnosed, the treatment strategy, and the health status of the patients on the last day of follow-up. All patients were observed until December 2015 or the date that the patient died of the disease.

Hematoxylin and Eosin and Immunohistochemistry

Formalin-fixed and paraffin-embedded sections were reviewed after the selection of representative sections for immunohistochemistry. Histological (based on hematoxylin and eosin [H&E]) and immunohistochemical analyses were performed according to standard protocols, as previously described.⁸ The TTF1, Ki-67, and Napsin A antibodies that were used in these assays were purchased from Abcam (Cambridge, UK).

Genome Sequencing

Two of the patients with PM received surgery therapy of metastatic lesions in the lung. However, archived formalin-fixed paraffin-embedded (FFPE) tumor samples from both primary and metastatic tissues from 1 patient were available for genome analysis. Each tumor sample was determined as a primary EEC or PM by pathologists, with a minimum of 70% of tumor cellularity. Paired samples containing peripheral blood control and primary and metastatic tumors were applied for cancer-related gene exome next-generation sequencing to identify potential mutations associated with pulmonary recurrence.
| Patient No. | Age at Initial Diagnosis, y | Recurrence Periods After Initial Treatment, mo | Primary Tumor Size, mm | FIGO 2009 Stage/Grade | Initial Treatment* | Adjuvant Therapy | Squamous Epithelial Differentiation | LVS | Ki-67 | Metastases to Other Sites | Treatment for PM | Survival After PM,† mo |
|------------|-----------------------------|-----------------------------------------------|-----------------------|-----------------------|-------------------|-----------------|-----------------------------------|-----|-------|--------------------------|-----------------|-----------------------|
| 1          | 52                          | 14                                            | 15                    | IB/G1                 | SS                | No              | +                                 | −   | 40%   | No                       | pelvic CT + RT  | 79+                   |
| 2          | 55                          | 5                                             | 20                    | IA/G1                 | NSS               | No              | −                                 | +   | 40%   | pelvic 40%                | CT + RT         | 51                    |
| 3          | 66                          | 41                                            | 60                    | IA/G1                 | NSS               | No              | +                                 | −   | 70%   | pelvic No CT + RT 31      | CT + RT         | 31                    |
| 4          | 52                          | 60                                            | 20                    | IB/G2                 | SS                | No              | −                                 | −   | 40%   | pelvic 30%                | pelvic CT + RT  | 51                    |
| 5          | 57                          | 42                                            | 30                    | IB/G1                 | NSS               | No              | +                                 | −   | 30%   | pelvic No surgery 17      | CT + RT         | 3                     |
| 6          | 78                          | 17                                            | 50                    | IB/G1                 | NSS               | No              | −                                 | −   | 20%   | pelvic Gastric 5          | CT + RT         | 3                     |
| 7          | 54                          | 21                                            | 10                    | IA/G3                 | SS                | CT              | +                                 | −   | 20%   | pelvic No CT 15           | CT + RT         | 15                    |
| 8          | 58                          | 16                                            | 35                    | IA/G2                 | SS                | No              | −                                 | −   | 20%   | pelvic Liver 25+          | CT + RT         | 15                    |
| 9          | 57                          | 7                                             | 30                    | IB/G2                 | SS                | No              | −                                 | −   | 75%   | pelvic, liver CT + RT 3   | CT + RT         | 3                     |
| 10         | 59                          | 20                                            | 30                    | IB/G3                 | SS                | CT + RT         | +                                 | +   | 80%   | pelvic 80%                | pelvic CT + RT  | 13                    |
| 11         | 81                          | 36                                            | 50                    | IB/G3                 | NSS               | CT              | −                                 | −   | 90%   | pelvic Bone CT + RT 11    | CT + RT         | 11                    |
| 12         | 67                          | 24                                            | 8                     | IA/G1                 | SS                | No              | −                                 | −   | 70%   | pelvic surgery CT + CT 8+ | CT + RT         | 8+                    |
| Mean       | 61.3                        | 25.3                                          | 29.8                  | —                     | —                 | —               | —                                 | —   | 49.6% | —                         | —               | —                     |

*All patients received laparoscopic surgery.
†Till December 2015.
CT, chemotherapy; NSS, nonstaging surgery: hysterectomy + bilateral salpingo-oophorectomy; RT, radiotherapy; SS, staging surgery: hysterectomy + bilateral salpingo-oophorectomy + pelvic lymphadenectomy.
Briefly, total DNA was isolated from frozen peripheral blood and archived FFPE tissue samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and the GeneRead DNA FFPE Kit (Qiagen, Hilden, Germany), respectively. Deoxyribonucleic acid concentration was determined using the Qubit dsDNA HS assay kit (Life Technologies), and genomic DNA integrity was assessed by agarose gel electrophoresis. Exome sequencing libraries were prepared using the KAPA Hyper Prep Kit (KAPA Biosystem, Roche), and enrichment was performed with the SeqCapEZ Exomev3.0 Kit (Nimblegen, Roche) following the manufacturer’s instructions. Genomic DNA was sheared to an average size of 200 to 300 bp using the Covaris M200 sonicator. Approximately 1 to 1.5 μg of fragmented genomic DNA was used for end-repairing, A-tailing, and adaptor ligation. Samples were bar coded using illumina-indexed adaptors. Size selection was performed before polymerase chain reaction enrichment using Ampure XP beads (Beckman). After library construction, libraries from the same group of samples (based on DNA quality) were pooled together for capture enrichment. Captured libraries were sequenced with the Illumina Hiseq platform using 150-bp paired-end sequencing mode.

Bioinformatics Methods

A mean of 28-GB raw data (FASTQ) were generated for each sample by illumina sequencers. First, the adapter sequence in the raw data was removed, and low-quality reads that have too many Ns or low base quality were discarded. Second, Burrows-Wheeler Aligner was used to align sequencing reads to the reference genome hg19. SAM format files were generated by Burrows-Wheeler Aligner. Third, the SAM format files were further processed to BAM files using Samtools. After these processes, variant calling was performed using our in-house software. For somatic mutation filtration, we compared the data of the patients with PM, EPM, and no recurrence. Somatic SNVs are single-nucleotide variations that occur in any non-germ cell of the body after conception, such as those that initiate tumorigenesis. For somatic mutation calling, we also applied VarScan to identify paired normal (blood) sample and tumor sample-specific SNVs by simultaneously comparing read counts, base quality, and allele frequency between the blood/normal tissue and tumor tissue. After SNVs are identified, annotation was also performed using our in-house software to annotate the variants from the VCF file and integrate information from multiple databases. The final variants can feed to the downstream advanced analysis pipeline.

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Fisher exact test, P values, and false discovery rates were applied in the GO and pathway analyses, according to a previous study.

Statistical Analysis

The data of patients with PM, EPM, and no recurrence were compared using Fisher exact test, χ² test, and t test. Analyses were performed using SPSS version 13.0 software for Windows (SPSS, Inc, Chicago, IL). Differences between groups were considered statistically significant at P < 0.05.

RESULTS

Among the patients who were reviewed, 78.7% (713/906) of whom were given a diagnosis of FIGO stage I (IA or IB). A total of 6.3% (45/713) of the patients with stage I experienced recurrence after primary treatment. Among them, 68.9% (31/45) had EEC. Twelve patients experienced a primary pulmonary recurrence, 7 of which were solitary. The remaining 19 patients had EPM. In the study period, 599 patients with stage I EEC with no recurrence (nonrecurrent) were identified. The clinicopathological characteristics of the 12 patients with PM are listed in Table 1. The mean age was 61.3 years (range, 52–81 years). According to the 2009 FIGO staging criteria, 6 patients (50%) were classified as stage IA, and 6 patients (50%) were classified as stage IB. The mean primary tumor size was 29.8 (range, 8–60) mm in diameter. Eight patients underwent laparoscopic staging surgery (hysterectomy + bilateral salpingo-oophorectomy + pelvic lymphadenectomy), whereas the others underwent laparoscopic hysterectomy + bilateral salpingo-oophorectomy. Three patients received chemotherapy (TP) after surgery because of poor cancer cell differentiation (grade 3). After careful review of the slides by the attending pathologist, squamous epithelial differentiation was observed in 58.3% (7/12) of the patients with lung metastasis alone. The Ki-67 positivity rate was 49.6% (range, 20%–90%). Lymphatic vascular space invasion was observed in only 1 case. The mean recurrence period after initial treatment was 25.3 (range, 5–60) months (Table 1).

To further define the possible clinical and pathological risk factors associated with PM in patients with stage I EEC, we compared the data of the patients with PM, EPM, and no recurrence. As shown in Table 2, there was no significant difference in the age, Ki-67 positivity rate, LVS1 positivity, and grade 3 differentiation of tumor cells among the 3 groups (all P > 0.05). The tumor size of the patients with PM was bigger than that of nonrecurrent patients (29.8 ± 16.6 vs 18.5 ± 16.3 mm, P < 0.05), whereas there was no significant difference between groups with PM and EPM. The rate of squamous epithelial differentiation in patients with PM (58.3%, 7/12) was significantly higher than that of patients with EPM (5.3%, 1/19) and no recurrence (3.3%, 20/599), whereas there was no statistical difference between patients with EPM and no recurrence. More percentage of patients with deep myometrial invasion (IB) were found in the group with PM (50.0%, 6/12) as compared with the group with EPM (15.7%, 3/19) (P < 0.05) and the nonrecurrent group (12.7%, 76/599) (P < 0.05). There was no statistical difference between patients with EPM and no recurrence with deep myometrial invasion (P > 0.05).
Among 12 patients with PM, only 2 patients received surgery, and the other patients received chemotherapy and/or radiotherapy (Table 1). Representative images of H&E stains and immunohistochemical stains of primary tumors and lung metastases from patients with FIGO stage I EEC are illustrated in Figure 2. As is shown, the PM tumor tissues were

![Representative images](image)

**FIGURE 2.** Representative H&E staining and immunohistochemical results of primary tumors and lung metastases in patients with EEC. As was shown, TTF-1 was positive in PM tumor tissue and negative in primary EEC. Napsin A, a new marker for lung adenocarcinoma, was negative both in primary and metastatic tumor tissues. Ki-67 was strongly expressed in both tissues.

| Patients                  | PM (N = 12) | EPM (N = 19) | Nonrecurrent (N = 599) | P     |
|---------------------------|-------------|--------------|------------------------|-------|
| Age, y                    | 61.3 ± 9.7  | 57.4 ± 8.6   | 54.0 ± 9.0             | 0.131 |
| Tumor size, mm            | 29.8 ± 16.6 | 23.3 ± 17.4  | 18.5 ± 16.3            | 0.017 |
| Squamous epithelial differentiation | 7 (58.3%)  | 1 (5.3%)    | 20 (3.3%)             | 0.000 |
| Ki-67                     | 49.6% ± 25.8% | 40.0% ± 26.8% | 37.2% ± 33.2%        | 0.202 |
| LVSI positive             | 1 (8.3%)    | 3 (15.7%)    | 53 (8.8%)             | 0.652 |
| Stage IB                  | 6 (50.0%)   | 3 (15.7%)    | 76 (12.7%)            | 0.001 |
| Grade 3                   | 3 (33.3%)   | 3 (15.7%)    | 46 (7.7%)             | 0.099 |

(1), PM vs nonrecurrent; (2), PM vs EPM; (3), EPM vs nonrecurrent.
positive for TTF-1, whereas EEC tumor tissues were negative for TTF-1. Staining for Napsin A, a new marker for lung adenocarcinoma, was negative in both primary and metastatic tumor tissues. In contrast, Ki-67 was strongly expressed in both tissues.

To explore the genomic alterations that may attribute to PMs in patients with EC, we performed whole exome sequencing in available paired primary and metastatic tumors from 1 patient. Briefly, we obtained a mean of 28-GB raw data for frozen blood (LSL_blood) and FFPE tumor tissues (LSL_primary and LSL_metastasis). The mean depth is approximately 120× that the metastatic tumor sample is of the lowest depth (~88×) (Supplementary Table 1, http://links.lww.com/IGC/A482). Both germ line and somatic mutations were analyzed. Our data indicated that no genetic high-risk factors were detected in exome sequencing data, which is consistent with the family history screening and immunohistochemical testing results (data not shown). In total, 405 filtered somatic mutations were found in primary tumors, and 563 mutations were found in metastatic tumors. Among them, 125 mutations were shared by both tumors, suggesting that the lung metastasis origins form the endometrioid carcinoma (Fig. 3). Most primary tumor-specific mutations (280/405, 69%) were lost in its metastatic counterpart, and metastatic tumor obtained more than 78% (438/563) private somatic mutations. Even for the shared variants, allele frequency altered in most mutations. These data demonstrated a distinct mutation profiling in lung metastasis compared with its endometrial origin, highlighting the evolution of tumor genomes during disease progression. Subsequently, we applied GO and pathway analyses for genes in the shared, primary, or metastasis-specific categories. Intriguingly, we observed a significant enrichment of genes related to cellular response to estradiol stimulus in the shared category, which further supports the common endometrial origin (Fig. 4; Supplementary Table 2, http://links.lww.com/IGC/A483). Several primary tumor-specific mutated genes (DAB2, NOTCH1, TGFB1, and BCL9L) were closely associated with positive regulation of epithelial to mesenchymal transition. Notably, in all 3 categories, we observed enrichment of genes related to the GO term “homophilic cell adhesion via plasma membrane adhesion molecules.” Mutation status analysis showed that a large fraction (8/21) of these mutations belongs to high-impact mutations (frameshift, stop-gain/lost, splicing). These data indicate that defective cell-cell adhesion is a common feature in tumorigenesis, despite tumor heterogeneity and dynamic clonal expansion.

Finally, we focused on individual mutations to identify potential drivers for tumorigenesis and metastasis. We merged top mutated genes in uterine corpus endometrial carcinoma from TCGA, TumorPortal, and COSMIC databases, respectively. Mutations in our data on these 50 genes were further analyzed in addition to other mutations, which are recorded in COSMIC database (Supplementary Table 3, http://links.lww.com/IGC/A484). Interestingly, we observed dramatic changes in mutation profiling in the primary and metastatic tumors, highlighting cancer as an evolutionary process. For example, disruptive PTEN and NF1 mutations were identified with a very high allele frequency in primary tumor, but not in metastatic tumor. This suggests that the predominant subclone in the primary tumor was lost during metastasis. On the contrary, the metastatic tumor obtained ATM, ARID1A, CDH10, and SLP1 mutations with a high frequency (Table 3). We were particularly interested in which metastatic tumor-specific mutations might contribute to the lung metastasis. On the basis of the nature and allele frequency of mutations, as well as the literature, we proposed that CDH10 mutation (NM_006727, c.G2060A, p.R687Q) and ARID1A mutation (NM_006015, c.822delG, p.M274fs) might be beneficial for clonal adaptation during metastasis. Both mutations are predicted to be damaging, and previous studies have shown that these genes are frequently mutated in tumors, and loss/down-regulation of these genes promotes cell proliferation, migration, and cancer metastasis.

**DISCUSSION**

In our study, we present 12 patients with lung metastasis with or without other recurrences out of 31 patients with recurrent stage I EEC. According to the literature, the survival rate of patients with lung metastasis is generally poor, with 1-year survival rates of 20% to 30% and 5-year survival rates of less than 10%. It was reported that the resection of PMs...
FIGURE 4. Gene ontology and pathway analyses for genes in the shared, primary, or metastasis-specific categories. A, Primary tumor. B, Metastatic tumor. C, Shared.
combined with chemotherapy (paclitaxel and carboplatin) and/or hormone therapy (progestins) is an optimal treatment regimen for patients with EC with metastasis to the lung. Only 2 of our patients with PM underwent surgical treatment after recurrence was detected.

Risk factors for recurrence in stage I EC have been defined in 2 large randomized trials, one European and one American, as follows: (1) being older than 60 years in PORTEC-1 and being older than 70 years in GOG99, (2) more than 50% myometrial invasion (PORTEC-1 and GOG99) and grade 3 disease (PORTEC-1 and GOG99), and (3) presence of L VSI (GOG99). However, the significance of the different factors varies, and none of the systems are accurate in the prediction of the risk of recurrence, which means that future risk stratification systems need to be improved by the addition of new predictors. Stage IV disease and deep myometrial invasion were thought to be associated with PM in patients with EC. Our results showed a high rate of deep myometrial invasion in patients with PM as compared with patients with EPM and no recurrence, but still, half (6/19) of the patients had stage IA disease.

Old age (≥60 or 70 years) as a risk factor of recurrence of EC was widely accepted, but it was questioned by some authors. One research suggested that, when older patients with EC were matched with younger patients based on tumor stage, grade, and adjuvant management, the prognostic impact of old age disappeared. We got the same result; there was no statistical difference in the age of patients with PM, EPM, and no recurrence, suggesting that old age was not a risk factor of PM. In addition to the risk factors mentioned previously, according to our results, large tumor size was also observed in patients with PM as compared with patients with EPM and no recurrence. Growing lines of evidence suggest that L VSI is a poor prognostic indicator in EC. Through a review of the available literature on L VSI, we are able to demonstrate that it is an independent risk factor for nodal metastasis, as well as for distant recurrence. In our study, only 1 patient had positive L VSI, which suggests that there is no relationship between L VSI and PM. As was reported, the expression of Ki-67 can significantly affect therapeutic decisions in selected patients with EEC. The high Ki-67 expression in patients with EEC is related to an increased risk of relapse. In our study, although the Ki-67 rate was very high in patients with PM, there was no statistical difference when compared with that in nonrecurrent patients.

Intriguingly, according to the careful review of the slides from all tumor tissues, we found that 7 of 12 patients with PM had tumors with squamous epithelial differentiation, which was extremely low in patients with EPM or no recurrence. All 7 patients had PM alone with no recurrence at any other sites. Our findings suggested that squamous epithelial differentiation might be one of the risk factors for PM in patients with early-stage EEC. Squamous epithelial differentiation is often associated with endometrial adenocarcinoma and with benign lesions, such as endometrial hyperplasia and chronic endometritis. Although this change within ECs has long been recognized by pathologists, its biologic significance has been the subject of continued debate. Whereas some authors have found a worsened prognosis for women who have tumors with squamous features, others have

| Gene   | Blood_AF | Primary_Metastasis_AF | Mutation | RefSeq  | Coding  | Amino_Acid_Change | Cosmic | SIFT_pred | PolyPhen2_pred | MutationTaster_pred | Pathogenic | ClinVar  |
|--------|----------|-----------------------|----------|---------|---------|------------------|--------|-----------|----------------|---------------------|-------------|----------|
| ARID1A | 0.324    | 0.163                 | Stop-gain | c.1303T | NM_006015 | p.R435X          | A      | T         | D              | Pathogenic           | 0.05        |          |
| ATM    | 0.324    | 0.412                 | Missense  | c.822delG | NM_000051 | p.Q274X          | A      | T         | P              |          | 0.461      |          |
| CDH10  | 0.333    | 0.246                 | Missense  | c.826delG | NM_000051 | p.Q274X          | A      | T         | P              |          | 0.461      |          |
| KCHN4  | 0.325    | 0.333                 | Missense  | c.826delG | NM_000051 | p.Q274X          | A      | T         | P              |          | 0.325      |          |
| NFI    | 0.412    | 0.429                 | Stop-gain | c.2072dupC | NM_000051 | p.R687X          | A      | T         | P              |          | 0.429      |          |
| PIK3CA | 0.381    | 0.196                 | Missense  | c.A13140C | NM_000051 | p.R410X          | A      | T         | P              |          | 0.381      |          |
| PTEN   | 0.427    | 0.431                 | Missense  | c.306delG | NM_000051 | p.R102X          | A      | T         | P              |          | 0.421      |          |
| SLPI   | 0.421    | 0.421                 | Missense  | c.306delG | NM_000051 | p.R102X          | A      | T         | P              |          | 0.421      |          |

A, disease causing automatic; B, benign; D, deleterious, score 0.05, probably damaging; P, possibly damaging; T, tolerated, score < 0.05.
reported the prognosis to be better than that of conventional endometrial adenocarcinomas. It was also reported that HPV is one of the causative factors of squamous differentiation in the endometrium.

We applied whole exome sequencing to investigate the molecular profile of a low-grade endometrioid carcinoma, as well as of a lung metastasis, in a female patient. The primary and metastatic tumors shared some of the mutations, and GO analysis showed an enrichment on cellular response to estradiol stimulus, indicating that the lung metastasis indeed originates from the primary endometrium tumor. In our molecular analysis, we detected mutations in genes such as ARID1A, AKAP9, ATM, PIK3CA, and PTEN, which are frequently affected in these tumor entities. However, the mutation profiling was quite different in the primary and metastatic tumors. We further showed that some mutations were lost during the metastasis formation, whereas some mutations were specifically detected in metastatic tumor. Intriguingly, our data showed that EMT plays a very important role during metastasis. First, mutations on several positive regulators of EMT (DAB2, NOTCH1, TGFB1R, and BCL9L) were lost during metastasis formation. Second, disruptive mutations on cell adhesion molecules were identified in both primary and metastatic tumors. Last but not least, we identified CDH10 mutation in the metastasis sample, which we proposed to be an advantageous factor for tumor cell migration and metastasis in our case. Several lines of evidence suggest that CDH10 may play the role of tumor suppressor. CDH10 has been reported to be primarily expressed in human brain and prostate. It has also been reported that, in lung squamous cancer cells, the knockdown of CDH10 promotes cell proliferation, soft agar colony formation, cell migration, and cell invasion, whereas the overexpression of CDH10 inhibits cell proliferation. CDH10 was found to be frequently mutated in both lung squamous cell carcinoma and adenocarcinoma, which indicates the importance of CDH10 mutations in the development of non–small cell lung cancer. Taken together, we postulate that mutant CDH10 may play important roles, or at least partial roles, in the invasion and metastasis of EC. Besides the CDH10 mutation, ARID1A mutation also might contribute to tumor metastasis. ARID1A is one of the important cancer-related genes identified by large-scale cancer genome sequencing in recent years. Mutations in the chromatin remodeling gene ARID1A have recently been identified in most types of cancer, such as gastric cancer, colon cancer, bladder cancer, ovarian cancer, liver cancer, EC, and breast cancer. Loss of ARID1A in primary tumor was significantly associated with endometrioid grade 1 or 2 and clear cell histology, diploid tumor cells, younger patient age, and deeper myometrial infiltration. Mutations of ARID1A gene in primary liver cancer cause an enhanced invasiveness and metastatic ability.

In summary, squamous epithelial differentiation, large tumor size, and deep myometrial invasion might be risk factors for PM in patients with stage I EC. Old age, Ki-67 rate, and high grade of tumor (grade 3) were not correlated with PM in such patients. Our data showed a strikingly different mutation profiling in lung metastatic tumor compared with the primary endometrial tumor. CDH10, ARID1A, and EMT-associated gene mutation may promote the initiation of lung recurrence. However, the cases of such patients are extremely rare, and further studies are needed to figure out the precise mechanisms associated with lung metastasis in these patients.

ACKNOWLEDGMENTS

The authors thank American Journal Experts for their linguistic revision of the article.

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