Aberrant p53 expression in metastatic lymph nodes is a significant predictor of recurrence in stage IIIC endometrial cancer

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Abstract

In endometrial cancer (EC), lymph node (LN) metastasis significantly impacts prognosis. In this study, the clinicopathological and molecular characteristics of 33 patients with EC with regional LN metastasis (FIGO stage IIIC) were investigated. We evaluated the mutational status of p53 and DNA mismatch repair (MMR) proteins in positive LNs (102 lesions), mutational variation between primary and paired metastatic lesions, inter-lesion heterogeneity in the metastatic lesions, and their association with clinical outcomes. Immunohistochemically, 13 patients (39.4%) displayed aberrant p53 expression in metastatic lesions, and a concordant rate of 93.4% was found between the primary and metastatic lesions. Genetic diversity between primary and metastatic lesions and among metastases was validated by evaluating p53 and MMR proteins. In Kaplan–Meier analysis, patients with aberrant p53 expression in metastatic LNs exhibited worse progression-free survival (PFS) than those with wild-type expression (p=0.024). Aberrant expression of p53 in the primary lesion did not show a significant difference in PFS compared with wild-type expression. In Cox univariate analysis, only p53 expression in metastatic LNs was significantly associated with recurrence (p=0.031). In conclusion, p53 expression in metastatic LNs is an independent and superior predictor of PFS in patients with EC with regional LN metastasis.

Introduction

Endometrial cancer (EC) is the most common gynecological malignancy worldwide, and its incidence is increasing. Japan exhibits the same tendency with > 12,000 new cases annually [1–6].

Conventionally, EC is classified into two groups according to clinical and endocrine features [7–9]. Type I EC is characterized by highly and moderately differentiated low-grade tumors (G1 or G2), mostly endometrioid adenocarcinomas associated with hyperestrogenism. Moreover, type I EC often contains atypical hyperplasia considered to be precursor lesions, and its prognosis is favorable. Type II EC is characterized by high-grade tumors (G3), including serous, clear cell, endometrioid, carcinosarcoma and undifferentiated carcinomas. Type II EC is not correlated with hyperestrogenism; it arises from atrophic endometrium and carries a poor prognosis. The Cancer Genome Atlas (TCGA) classifies EC into four distinct categories based on molecular genetics, while clinical behavior varies according to the four subtypes [7]. These findings highlight the significance of molecular characteristics for a holistic understanding of malignancies and their management strategies.

Approximately 90% of cancer-related deaths are thought to be due to failure to control metastasis rather than the primary tumor [10]; thus, the molecular characteristics of metastatic lesions might have a more critical role in prognosis than those of primary tumors. Intra-tumor heterogeneity is thought to be present in the earliest days of carcinogenesis [11, 12], and metastatic lesions may have heterogeneous characteristics even at the start of treatment. Therefore, the analysis of only primary lesions might lead to a misunderstanding of metastatic tumors. Most previous studies, including those based on TCGA data, have focused on primary lesions without mentioning metastatic lesions. To the best of our knowledge, no study has previously investigated the molecular genetics of each metastatic lesion in the cohort.

Stage IIIC EC is the most common locally advanced sub-stage. The current FIGO staging classification system subdivides stage IIIC (metastases to pelvic or para-aortic LNs or both) EC into IIIC1 (positive pelvic LNs) and IIIC2 (positive para-aortic LNs with or without positive pelvic LNs) [6, 13]. LN metastasis is an important prognostic factor [14]. In stage III EC, previous studies have found that the lymph node ratio (LNR), defined as the percentage of positive LNs to total dissected LNs, is associated with prognosis [15–17]. The relative prognostic values of the LNR, molecular characteristics, and a combination of the two have yet to be elucidated.

The current study focused on the analysis of p53 and MMR protein expression in primary and metastatic LNs, clinicopathological characteristics, and their association with clinical outcomes.

Methods

Patients

This retrospective study enrolled 33 patients with stage IIIC EC who were treated at the Okayama University Hospital between January 2011 and December 2019. Patients who received preoperative chemotherapy and/or did not undergo systematic lymph node dissection were excluded from the study. We obtained institutional review board approval for the study from the ethics committee of the Okayama University (approval number: 1901-022). Informed consent was obtained from all the participants. All procedures were performed in accordance with relevant ethical standards and institutional ethics committee regulations.

According to the Japan Society of Gynecologic Oncology (JSGO) guidelines [18], stage IIIC Patients with EC with extra-uterine lesions are categorized as high-risk for relapse, and adjuvant treatment is strongly recommended. Thus, all the patients in this study underwent systemic chemotherapy with or without vaginal brachytherapy after surgery.

The following clinicopathological data were extracted from medical records: age, FIGO stage, histology, length of the short axis of enlarged LNs confirmed by imaging findings, tumor volume (calculation: height × width × length / 2), tumor size (the largest diameter of the three dimensions), location of the foci, location and number of LN metastases, LNR, and clinical outcomes.

Immunohistochemistry (IHC) of p53 and MMR proteins

We prepared formalin-fixed paraffin-embedded (FFPE) blocks of all positive metastatic LNs and representative primary tumors that contained large amounts of tumor tissue. Histological slides were reviewed by the authors (KO and KN), and no apparent specimen-specific morphological differences were confirmed. All FFPE specimens were cut into 4-μm thick slices. Then, the FFPE sections were deparaffinized with xylene and rehydrated using an ethanol gradient. Endogenous peroxidase activity was blocked with H_2O_2, followed by antigen retrieval using citrate buffer for p53 or ethylenediaminetetraacetic acid buffer for MMR proteins at 98°C for 20 min. Next, the slides were

incubated at 15–25°C with primary antibodies against p53 from Ventana Medical Systems (mouse, monoclonal, DO-7, prediluted, 60 min), MLH1 (mouse, monoclonal, M1, prediluted, 120 min), PMS2 (mouse, monoclonal, A16-4, prediluted, 60 min), MSH2 (mouse, monoclonal, G219-1129, prediluted, 60 min), and MSH6 (rabbit, monoclonal, SP93, prediluted, 30 min). The sections were then incubated with biotinylated secondary antibodies (Vectastain ABC mouse or rabbit IgG kit, PK-4001 or PK-4002; Vector Laboratories). Specific antigen-antibody reactions were visualized with diaminobenzidine tetrahydrochloride, and hematoxylin was used for nuclear counterstaining.

IHC evaluation of p53 and MMR proteins

The expression status of p53 was divided into two categories: wild-type and aberrant. Aberrant p53 expression was defined as strongly positive nuclear staining in >80% of the tumor cells, focally aberrant expression, or completely negative staining (null pattern) [19]. To identify the heterogeneous p53 expression, we divided wild-type expression into two subgroups according to the percentage of positive tumor cells: <10% and 10–80%. In involved LNs, when at least one LN displayed aberrant expression, the overall p53 expression was classified as aberrant.

Tumor cells with diffuse negative nuclear staining for at least one MMR protein (MLH1, PMS2, MSH2, and MSH6) were regarded as MMR-deficient (dMMR). The surrounding stromal cells adjacent to the tumor cells were used as a positive control. Tumors with >10% of its area displaying subclonal loss of these proteins were identified as heterogeneous and regarded as dMMR [19]. In involved LNs, a mixed case of MMR-proficient (pMMR) and dMMR was identified as inter-LN heterogeneity and regarded as dMMR.

Statistical analysis

Analyses were performed using SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P<0.05$. Comparisons between two groups were performed using the Mann–Whitney U test. Pearson's χ^2 test was used to test for independence. Survival curves were calculated using the Kaplan–Meier method with the log-rank test. A univariate Cox regression model was used to evaluate the prognostic factors for progression-free survival (PFS).

Results

Clinicopathological characteristics

The clinicopathological findings and outcomes are summarized in Fig. 1 and Table 1. The age at diagnosis ranged from 32–77 years (median, 57 years). Histologically, 14 patients (42.4%) had low-grade tumors (endometrioid G1 or G2) and 19 (57.6%) had high-grade tumors (any histology besides endometrioid G1 or G2). The number of resected and positive LNs ranged from 20–84 (median 41) and 1–20 (median 5), respectively. The LNR ranged from 1% (1/72) to 100% (20/20). The median tumor volume was 42.0 cm^3 (2.7–963.1 cm^3) and the median largest tumor size was 5.6 cm (2.0–16.3 cm). Furthermore, the median duration of observation was 62 months (10–136 months); 14 patients (42.4%)

relapsed, and four patients died, including one patient who died of aortic dissection. The median PFS was 47 months.

Expression status of p53 in primary and metastatic lesions

We performed IHC analysis of p53 expression (Fig. 2) and identified 20 (60.6%) and 13 (39.4%) cases as wild and aberrant phenotypes, respectively, in both, primary and metastatic lesions. Among the 20 cases of wild-type primary lesion, one showed aberrant p53 expression in the metastatic lesion, and among the 13 cases of aberrant p53 expression in the primary lesion, one case showed wild-type p53 expression in the metastatic lesions. Therefore, according to the definition of p53 expression status, concordance between primary and metastatic lesions was observed in 31 out of 33 cases (93.9%). Six (19.4%) out of thirty-one cases exhibited heterogeneous expression between primary and metastatic lesions, of which both were classified as wild or aberrant. Of these, three cases presented a wild phenotype with <10% positive staining in the primary lesion and a mixture of <10% and 10–80% (mixed pattern) positive staining in the metastatic LNs. Additionally, one case had a wild phenotype with 10–80% positive staining in the primary lesion and a mixed pattern in the metastatic LNs. Another case presented as focally aberrant due to the >80% positive staining in the primary lesion and a null pattern in the metastatic lesion. The last case was an aberrant phenotype with >80% positive staining and a mixed pattern in the primary lesion, and >80% positive staining in the metastatic lesion (Supplementary Table S1 and Supplementary Fig. S1).

Profiles of MMR-related proteins

Representative examples of IHC staining for MMR proteins are shown in Fig. 3. As confirmed by IHC analysis, 16 cases (48.5%) were classified as dMMR and 17 (51.5%) as pMMR. No cases of discordant MMR status between primary and metastatic lesions were observed. Among the 16 dMMR cases, 14 (87.5%) showed diffuse loss of both, MLH1 and PMS2, one (6.3%) showed subclonal loss of MSH6 alone, and the last case showed subclonal loss of all four MMR proteins. Of the 14 dMMR cases with diffuse loss of MLH1 and PMS2, eight (57.1%) presented heterogeneous staining of MSH2 and/or MSH6. Finally, inter-LN heterogeneity was observed in six cases (Supplementary Fig. S1).

According to the heterodimer formation pattern of the MMR proteins, dMMR was divided into two main classifications: MLH1-PMS2 deficiency and MSH2-MSH6 deficiency. The dMMR cases were further divided into two groups: the first (1) consisted of cases with MLH1-PMS2 *or* MSH2-MSH6 deficiency and the second (2) consisted of cases with MLH1-PMS2 *and* MSH2-MSH6 deficiency. Among the 16 dMMR cases, seven cases fell into the first classification and nine cases fell into the second classification.

Survival analysis with respect to molecular stratification

Survival analysis revealed that patients with aberrant p53 expression in primary lesions and metastatic LNs had shorter PFS than those with wild type p53 expression (p=0.137 and p=0.024, respectively);

however, the difference in primary lesions was not significant (Fig. 4a and 4b). MMR expression was not significantly associated with PFS (p=0.135).

Of the dMMR cases, one of the seven cases (5.9%) falling into classification (1) and four of the nine cases (44.4%) falling into classification (2) experienced recurrence, although these findings were not statistically significant (p=0.197).

Receiver operating characteristic curve of LNR

Receiver operating characteristic curve analysis was used to determine the optimal cutoff values of LNR for predicting recurrence. The analysis identified an LNR ≥0.11 (area under the curve =0.691, sensitivity: 71.4%, specificity: 63.2%) as the most accurate cutoff value for predicting recurrence in this cohort (Fig. 5).

Correlation between p53 expression in metastatic lesions, LNR, and patient characteristics

Aberrant p53 expression was significantly associated with older age and MMR proficiency. Moreover, marginal differences in histology, enlarged LNs, tumor volume, and recurrence were observed between aberrant and wild type p53 expression (Table 2a). An LNR ≥0.11 was significantly correlated with older age, existence of bilateral LN metastases, and recurrence (Table 2b). Finally, dMMR status was only significantly associated with younger age (p=0.016) (Supplementary Table S2).

Cox univariate analysis of the association between patient characteristics and PFS

The correlation between patient characteristics and PFS was assessed by Cox univariate analyses. Bilateral LN metastases and an LNR ≥0.11 were marginally associated with PFS (p=0.052 and p=0.062, respectively). Finally, aberrant p53 status in metastatic lesions was significantly associated with PFS (p=0.031) (Table 3).

Discussion

Recently, owing to next-generation sequencing technology, the molecular characteristics and mutational profiles of EC have been identified. Specifically, TCGA has classified EC into four categories: POLE (ultramutated), MSI (hypermutated), copy-number low (endometrioid), and copy-number high (serous-like) and found these classifications to be associated with prognosis [13].

During initial management of EC, surgery is often the standard of care, and identifying the risk of relapse based on the pathological findings of excised specimens has a strong influence on decision-making during the course of postoperative treatment [5, 18]. The pathological findings useful for determining risk include histology, the degree of muscle invasion, lymph vascular space invasion, and the presence or absence of extrauterine lesions [18]. However, these factors do not account for the importance of the molecular and mutational signatures of EC in prognosis. Molecular characteristics of endometrioid

carcinoma were first mentioned in the revised 5th edition of the WHO classification of female genital tumors [20].

Although many studies have investigated molecular genetics using next-generation DNA sequencing, this technique is expensive and impractical to apply in routine clinical settings. Intra-tumor heterogeneity is thought to be present in the earliest days of carcinogenesis [11, 12], and metastatic lesions may have heterogeneous characteristics even at the start of treatment. Therefore, analysis of only primary lesions might lead to a misunderstanding of metastatic tumors.

TP53 mutation occurs early in tumorigenesis and is one of the most important molecular factors associated with unfavorable prognosis [2, 12, 21]. According to TCGA, analysis of dMMR is also crucial for prognosis and MMR deficiency arises in the early stages of tumorigenesis. Moreover, the inherited pathogenic germline variants in MMR genes cause Lynch syndrome [5, 7, 19, 22, 23]. Most previous studies, including those using TCGA data, were based on data from primary lesions only; no study has yet investigated the molecular features of each metastatic lesion. In contrast to next-generation sequencing, IHC is easy to perform and apply in routine clinical practice. Moreover, IHC can reveal the status of *TP53* mutation and MMR deficiency [21, 23]. To the best of our knowledge, this is the first study to analyze the association of clinical outcomes with p53 and MMR protein status of both, primary lesions and all metastatic LNs using IHC and clinicopathological data.

TP53 mutation is the single most influential factor affecting prognosis [21] and aberrant p53 expression was observed in 39.4% of the patients in our cohort. Previously reported frequencies of p53 overexpression vary widely for a range of reasons, including the study cohort. For example, Haraga et al. reported a p53 overexpression rate of 14.7% in a cohort of patients with all stages of EC, and Saijo et al. reported aberrant p53 status in 63% of patients with endometrial carcinosarcoma [19, 24].

William et al. reported a variation in the number of mutations in primary lesions and their paired metastases; even among common driver mutations, the concordance rate of primary and metastatic lesions was only 83% [2]. The concordance rate of truncal mutations, such as those in *TP53* was also less than 100% [2]. For example, in the current study, the concordance rate of p53 status between primary and metastatic lesions was 93.9%; and even within the same classification (both primary and metastatic lesions as wild or aberrant), six cases (19.4%) showed heterogeneous expression. Three out of six (50%) cases exhibited low-grade endometrioid histology, and two out of the three cases exhibited dMMR. Köbel et al. reported the possibility of later acquisition of *TP53* mutation in endometrioid carcinoma, especially in mutator phenotype like dMMR [21]. The heterogeneous expression revealed in our study could be attributable to the subsequent occurrence of *TP53* mutations, especially in low-grade endometrioid and dMMR cases.

Many studies have evaluated MMR status in EC. Approximately 20–40% of patients with EC demonstrate dMMR, largely resulting from promoter hypermethylation and silencing of MLH1 [3, 23, 25, 26]. Ida et al. reported a dMMR rate of 60% in mixed endometrial carcinomas, while Saijo et al. reported a dMMR rate of only 10.5% in only endometrial carcinosarcoma [3, 19, 24–26]. Our cohort displayed a relatively high

dMMR rate of 48.5%, which is likely due to the inclusion of different cohorts. The variation in tumor volume among the cohorts is another possible cause of this variation. For example, Casey et al. reported that epigenetic silencing of MLH1 is significantly associated with large tumor volume [27]. Because our study evaluated patients with EC at an advanced stage, we observed considerably large tumor volumes with a median tumor size of 42.0 cm^3. Tumor size is a significant prognostic factor, and the cutoff value between large and small tumors is a diameter of 2 cm [14]. Our study observed diameters much longer than the cutoff value, and in all cases, the tumor size was ≥2 cm.

We speculate that the large tumor volume is also related to the heterogeneous staining pattern of MMR proteins. Of the 16 cases of dMMR in this study, eight presented homogenous loss of MLH1/PMS2 and heterogeneous expression of MSH2/MSH6. This same expression pattern is often observed in colorectal cancer, and the possibility of secondary MSH2/MSH6 inactivation has been proposed [28]. Because stage IIIC EC is advanced by definition, the primary lesion volume tends to be large owing to repeated cell division. Additionally, because patients with EC with dMMR have a faulty DNA mismatch repair system, more cell division occurs, resulting in increased DNA replication errors and mutations, ultimately resulting in heterogeneous MMR protein expression.

Johannes et al. reported higher levels of heterogeneity in LN metastases than in distant organ metastases [29]. Our findings of inter-LN heterogeneity corresponded to previous findings [29], and in the metastatic LNs, we could validate the polyphyletic evolution of different mutational signatures from primary lesions occurring even in fundamental molecules, such as p53 and MMR proteins.

Regarding the prognostic value of p53, Kaplan–Meier and Cox univariate analyses showed that p53 expression in metastatic lesions was significantly associated with PFS in our study. Because of its advanced nature, stage IIIC EC has relatively poor prognosis, and a significant difference according to p53 expression could be difficult to observe. However, even under these circumstances, aberrant p53 status in metastatic lesions could be a superior prognostic predictor to that in primary lesions.

The association between MMR deficiency and survival outcomes remains controversial [3]. Because EC with dMMR is characteristically hypermutated according to a TCGA study [7], it displays large amounts of neoantigens and perhaps high immunogenicity. These signatures could result in prognostic differences between patients with MMR proficient and deficient EC. Among the dMMR cases, although we failed to demonstrate significant differences between the observed classifications ((1) MLH1-PMS2 *or* MSH2-MSH6, (2) MLH1-PMS2 *and* MSH2-MSH6) (p=0.197), the recurrence rate in cases from classification two (44.4%) was higher than in cases from classification one (5.9%). We believe that the observed classifications could explain the prognostic differences.

For clinical data, LNR has been proposed as a meaningful prognostic factor in patients with stage IIIC EC [15–17]. Ali et al. found an LNR >0.15 to be an independent prognostic factor for PFS and OS [15]. Moreover, Fleming et al. reported that patients with an LNR >0.5 had significantly worse PFS than those with an LNR ≤0.5 [16]. Additionally, Polterauer et al. reported that only LNR was significantly associated

with PFS and OS in multivariate analysis [17]. Under our cutoff value by ROC curve analysis, an LNR ≥0.11 showed a marginal association with PFS, though the correlation was not significant in Kaplan–Meier or Cox univariate analyses (p=0.052 and p=0.062, respectively). Thus, based on our findings, molecular characteristics have a superior prognostic impact compared with clinical factors.

In conclusion, our study suggests that aberrant p53 expression in metastatic lesions could provide superior prognostic information than that in primary lesions in patients with stage IIIC EC. Polyclonal development from the primary lesion to individual LNs is possible, even in fundamental molecular genetics, such as p53 and MMR proteins. Further study is required to verify the association between MMR protein expression and prognosis. Although our study cohort was relatively small and confined to a single institution, this study provides a thorough evaluation and interpretation of p53 and MMR proteins in primary and all involved LNs in patients with stage IIIC EC. The technical use of this study result was for the IHC only; therefore, we believe these findings can be applied to daily medical practice easily.

Declarations

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Author Contributions

K.O., K.N. conceived this study. K.O. conducted the experiment. J.H. assisted the experiment. K.N. double-checking the experimental results. K.O., K.N. analyzed all the results. K.O., K.N. wrote the main manuscript and prepared the tables and Figures. H.M. supervised the whole study. All authors reviewed and approved the manuscript.

Data Availability

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare no competing interest.

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Tables

Table 1
Patients' clinicopathological characteristics

Characteristics	Values
Age (yr)	57 (32-77)
FIGO stage	
IIIC1	17 (51.5)
IIIC2	16 (48.5)
Histology	
Endometrioid G1, G2	14 (42.3)
Endometrioid G3	5 (15.2)
Serous	3 (9.1)
Mixed	5 (15.2)
Carcinosarcoma	3 (9.1)
Others	3 (9.1)
Number of resected LNs	
20-39	16 (48.5)
40-59	9 (27.3)
>60	8 (24.2)
Number of positive LNs	
1-3	13 (39.3)
4-6	9 (27.3)
7-9	3 (9.1)
10-14	5 (15.2)
≥15	3 (9.1)
Short axis of the largest enlarged LN	
<10mm	21 (63.6)
≥10mm	12 (36.4)
Laterality of positive LNs	
Unilateral	14 (42.3)
Bilateral	19 (57.7)

Characteristics	Values
Tumor size (largest diameter, cm)	5.6 (2.0-16.3)
Tumor volume (cm^2)	42 (2.7-963)
LNR	
<0.11	16 (48.5)
≥0.11	17 (51.5)
Reccurence	
Present	14 (42.3)
Absent	19 (57.7)
Values: median (range) or number (%)	
Abbreviation G: Grade, LN: lymph node, LNR: lymph node ratio	

Table 2
a p53 expression status of metastatic lesion and patients' characteristics

p53 phenotype	**wild type**	**aberrant type**	
	n=20	n=13	p-value
Age (median, range)	56 (32-62)	65 (37-77)	0.031*
FIGO stage			0.353
IIIC1	9	8	
IIIC2	11	5	
Histology			0.07
Low grade type	11	3	
High grade type	9	10	
LN size (≥10mm)	5	7	0.093
Bilateral LN metastases	10	9	0.275
Tumor volume (cm^2)			
median	41.5	38.2	
average±SD	58.8±61.3	190.3±287.6	0.071
LNR (≥0.11)	6	7	0.171
MMR deficient	15	1	<0.001***
Recurrence			0.073
Present	6	8	
Absent	14	5	
Abbreviation LN: lymph node, LNR: lymph node ratio, MMR: mismatch repair			

Table 2b LNR and patients' characteristics

LNR	<0.11	≥0.11	
	n=16	n=17	p-value
Age (median, range)	55 (32-65)	58.5 (47-77)	0.01*
FIGO stage			0.22
IIIC1	10	7	
IIIC2	6	10	
Histology			0.579
Low grade type	8	6	
High grade type	9	10	
LN size (≥10mm)	6	6	0.895
Bilateral LN metastases	5	14	0.003**
Tumor volume (cm^2)			
median	37.7	45.9	
average±SD	76.9±118.1	142.3±246.0	0.175
Aberrant p53 status in positive LNs	6	7	0.829
MMR deficient	7	8	0.848
Recurrence			0.049*
Present	4	10	
Absent	12	7	
Abbreviation LN: lymph node, LNR: lymph node ratio, MMR: mismatch repair			

* p <0.05, ** p <0.01

Table 3 Prognostic factors for progression-free survival by Cox univariate analysis

Prognostic factors	Hazard ratio	95% CI	p-value
PAN metastasis (stage IIIC2)	2.128	0.708-6.397	0.179
Histology (high grade type)	1.148	0.397-3.316	0.799
LN size (≥10mm)	1.702	0.588-4.932	0.327
Bilateral LN metastasis	3.553	0.988-12.771	0.052
LNR ≥0.11	3.02	0.945-9.653	0.062
Aberrant p53 status in metastatic lesion	3.234	1.111-9.410	0.031*
MMR deficiency	0.505	0.169-1.512	0.222
Abbreviation PAN: para-aorta lymph node, LN: lymph node, LNR: lymph node ratio			
MMR: mismatch repair			

Figures

Figure 1

Patients' clinicopathological and molecular characteristics, as well as prognostic outcomes.

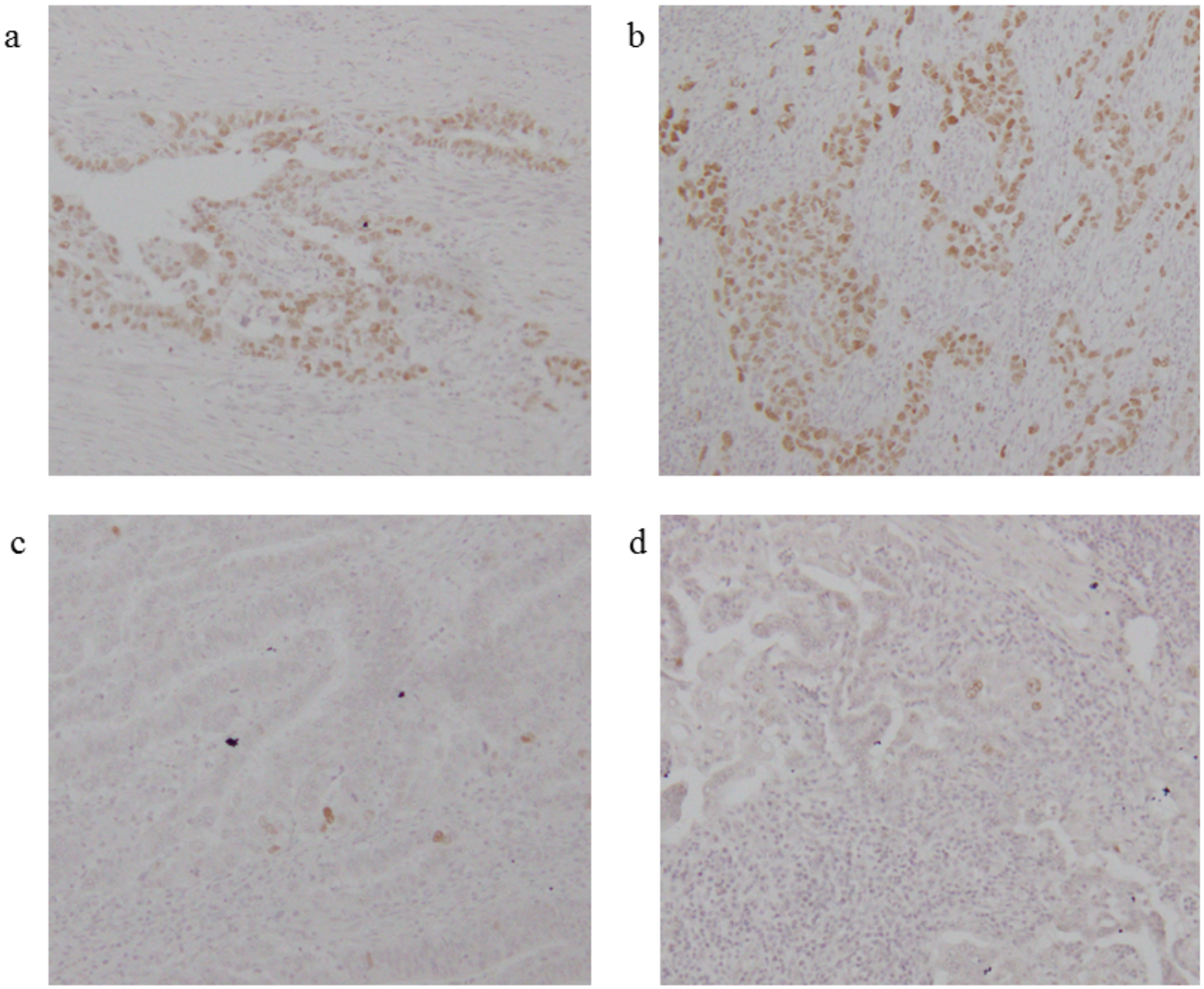

Figure 2

Representative images of p53 expression status by IHC. (a-b) Aberrant p53 phenotype in both (a) primary and (b) metastatic LNs (case 35, para-aortic LN). (c-d) Wild p53 phenotype in both (c) primary and (d) metastatic LNs (case 27, right common iliac LN).

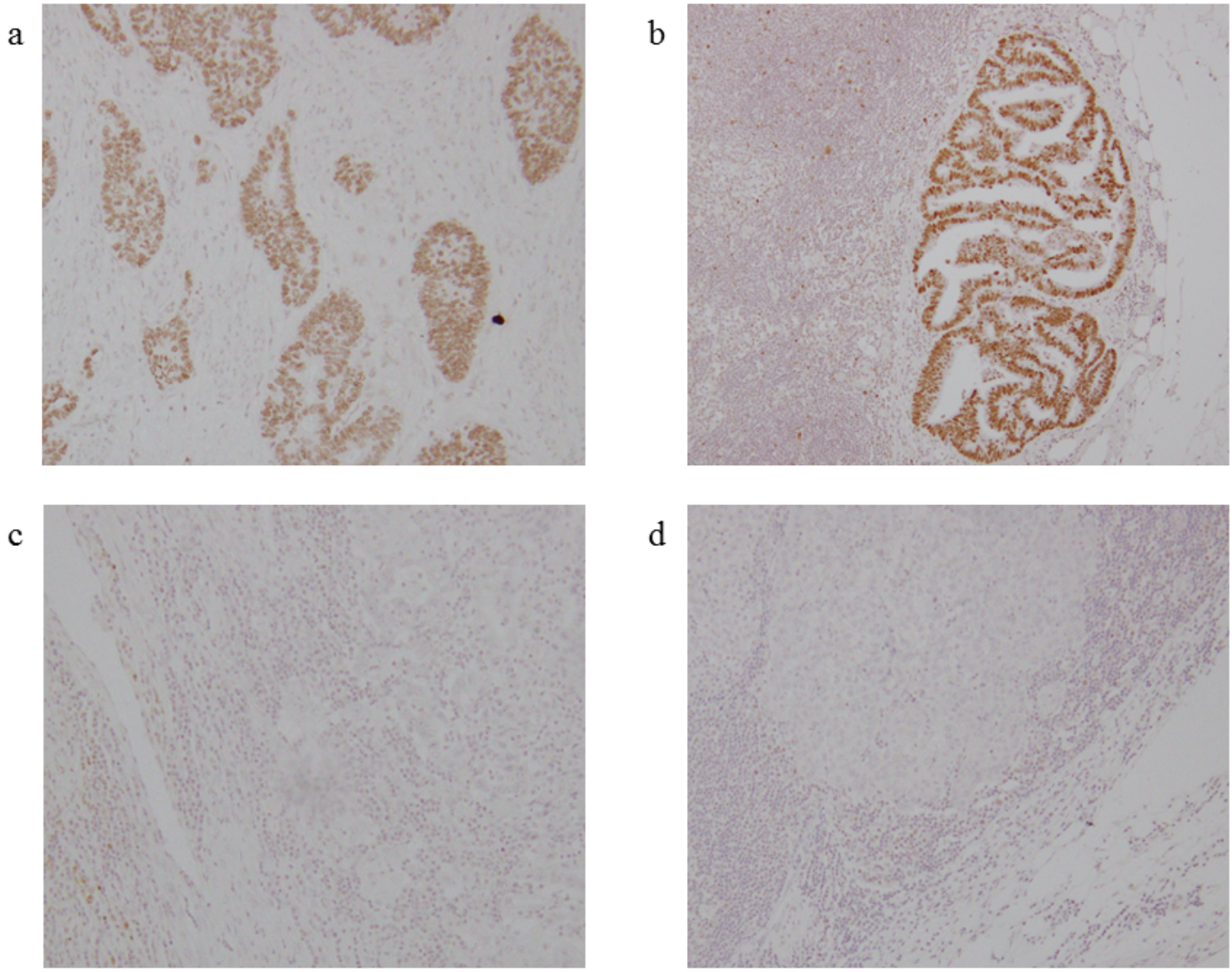

Figure 3

Representative examples of IHC staining of MMR proteins. (a-b) Diffuse positive nuclear staining of the tumor cells regarded as pMMR in (a) primary and (b) metastatic LNs (MSH6, case 24, para-aortic LN). (c-d) Diffuse negative staining of the tumor cells while surrounding positive stromal cells (positive control) were scattered regarded as dMMR (c) primary and (d) metastatic LN (PMS2, case 16, left obturator LN).

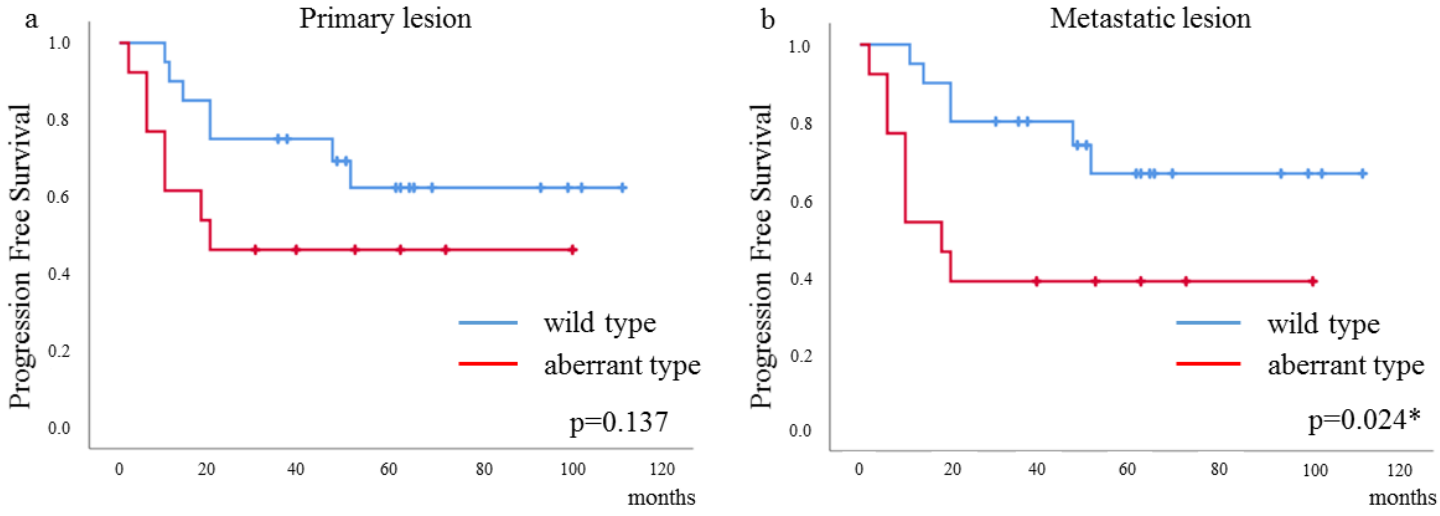

Figure 4

Kaplan–Meier curves of progression free survival of 33 ECs stratified by p53 expression status. (a) Evaluation based on p53 phenotype in primary lesions. (b) Evaluation based on p53 phenotype in metastatic lesions.

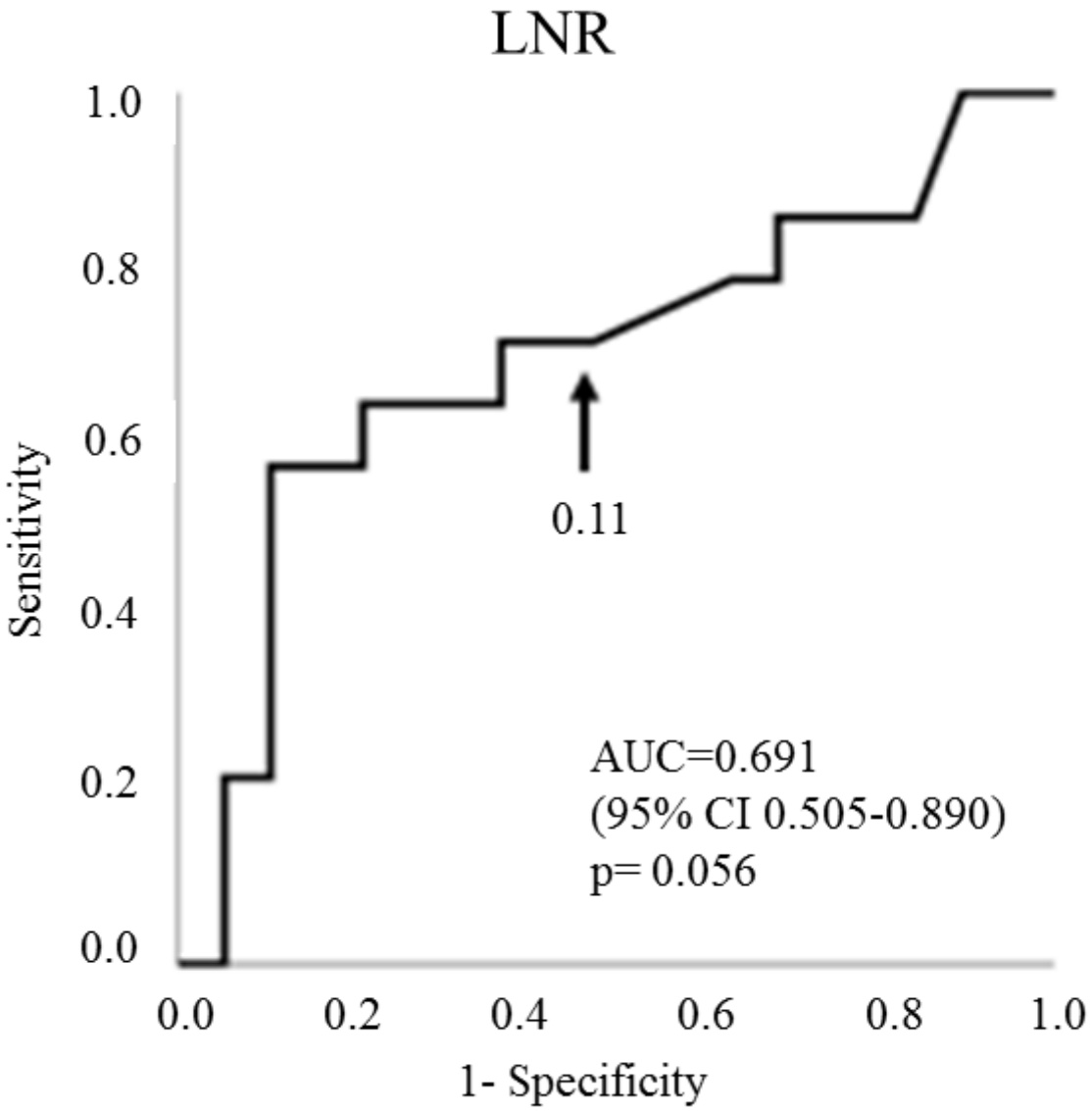

Figure 5

Receiver-operating characteristic curve analysis to determine the cutoff value of LNR for the prediction of recurrence

Supplementary Files

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- SupplementaryFigurefile.tif
- SupplementaryTablesfile.docx
- Supplementaryfigurelegends.docx