梨花醫大誌 : 第 32 卷 第 1 號 2009
Ewha Med J Vol. 32, No. 1, 2009

Utility of Immunohistochemical Markers in the Differential Diagnosis of Uterine Papillary Serous Carcinomas and Uterine Endometrioid Carcinomas

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= 국문초록 =

자궁 내막양 선암종과 자궁 장액성 선암종의 감별 진단을 위한 면역조직화학 표지자의 유용성

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목 적 : 자궁 장액성 선암종은 자궁 내막양 선암종보다 나쁜 임상적 예후를 보이기 때문에 수술 전 정확한 감별진단이 중요하다. 저자들은 파라핀 절편을 이용하여 이 두가지 종양의 감별에 유용한 면역조직화학 표지자를 찾아보고자 한다.

방 법 : 저자들은 각각 낮은 등급의 자궁내막양 선암종 19증례, 높은 등급의 자궁내막양 선암종 19증례, 자궁 장액성 선암종 13증례를 포함하는 3개의 조직 칩들을 대상으로 MLH1, MSH2, p53, PTEN, c-erb B2, estrogen receptor(ER), progesterone receptor(PR), E-cadherin, and β-catenin에 대한 면역조직화학 검사를 실시한 후 염색 패턴을 비교 분석하였다.

결 과 : 자궁 장액성 선암종이 높은 등급의 자궁 내막양 선암종보다 p53의 유의한 발현 증가(p=0.003)를 보였다. 낮은 등급의 자궁 내막양 선암종이 자궁 장액성 선암종보다 유의한 p53의 발현 감소(p<0.001), ER의 발현 증가(p<0.001), 그리고 PR의 발현 증가(p=0.038)를 보였다.

결 론 : p53 면역 염색이 자궁 장액성 선암종을 FIGO 등급에 관계없이 자궁 내막양 선암종으로부터 감별하는데 유용하였다. 하지만 p53(+), ER(-), and PR(-)의 면역 염색 조합 결과가 자궁 장액성 선암을 특히 낮은 등급의 자궁 내막양 선암종으로부터 감별하는데 보다 더 유용할 것으로 사료된다.

중심 단어 : 자궁 장액성 선암종 · 자궁 내막양 선암종 · 면역조직화학.

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Introduction

Uterine papillary serous carcinomas (UPSCs) demonstrate distinct histological features such as a high degree of cytological atypia and a papillary growth pattern[1]. UPSCs follow the similar mode of spread to that of ovarian surface epithelial carcinomas[1]. The histological distinction between UPSCs and endometrioid adenocarcinomas (EMCs) is of paramount importance because UPSCs follow a more aggressive clinical course and higher relapse rate than EMCs[1-3]. Tumor recurrence was reported to be more frequent in UPSCs than EMCs with the 1988 International Federation of Gynecology and Obstetrics (FIGO) grade 3[4]. Even a small percentage of UPSCs (even 10%) in a mixed form of carcinomas is correlated with a poor prognosis[5]. Thus, definitive preoperative or intraoperative diagnosis for UPSCs in patients with low FIGO stage ensures optimal surgical treatment and adjuvant radio-chemotherapy.

UPSCs and EMCs are well known to have different genetic pathways suggesting the dualistic model of endometrial tumorigenesis[6-8]. In UPSCs, p53 mutation, inactivation of E-cadherin and ampilification of c-erb B2 are well known[6)8)]. p53 mutation was reported even in the endometrial intraepithelial carcinoma, a putative precursor of UPSCs[9]. In EMCs, inactivation of PTEN, microsatellite instability (MSI) and mutations of K-ras and β-catenin are well known[6)8)]. Although the expression pattern of immunohistochemical markers associated with molecular pathways is considered to depend upon the histological subtypes of endometrial carcinomas, useful biomarkers to differentiate UPSCs from the EMCs, particularly FIGO grade 3 EMCs, are not well established.

We aimed to determine whether there are useful immunohistochemical markers applicable to the differential diagnosis between UPSCs and EMCs using paraffin sections.

Materials and Methods

1. Case selection and tissue microarray

Among two hundreds ten cases of EMCs and 15 cases of UPSCs from the surgical pathology files at Asan Medical Center (Seoul, South Korea) between 2001 and 2006, the selected cases included 19 low-grade EMCs (FIGO grade 1 or 2), 19 high-grade EMCs (FIGO grade 3), and 13 UPSCs.

All tissues had been routinely fixed in 4% buffered formaldehyde and processed into paraffin blocks. Two pathologists reviewed the slides to select appropriate blocks for tissue microarray.

We created one tissue microarray slide for each group, each containing three 1-mm-diameter cores per case to decrease the risk of aberrant results due to tumor heterogeneity. Briefly, representative areas of each tumor were selected and marked on the H&E slide. After extracting cores of wax (0.8mm diameter) from three prepared empty paraffin blocks, each core was replaced with the core (1mm diameter) obtained from its corresponding tissue block. Once the punches were complete, all blocks were sealed in a 60℃ oven for 10min.

2. Immunohistochemistry

A panel of immunohistochemical stainings included MLH1 (1 : 50 ; Pharmingen, San Diego, Calif), MSH2 (1 : 250 ; Pharmingen, San Diego, Calif), p53 (1 : 1,600 ; DAKO, Carpinteria, U.S.A.), PTEN (1 : 100, Novocastra, England), c-erb B2 (1 : 500 ; Dako, Carpinteria, U.S.A.), estrogen receptor (ER, 1 : 50 ; Dinona, Seoul, Korea), progesterone receptor (PR, 1 : 100 ; Dinona, Seoul, Korea), E-cadherin (1 : 100 ; ZYMED, San Francisco, U.S.A.), and β-catenin (1 : 500 ; ZYMED, San Francisco, U.S.A.). All immunostainings were performed in the Benchmark automatic immunostaining device (Ventana Medical System, Tucson, Ariz) using formalin-fixed, paraffin-embedded tissue sections. Five-micrometer-thick sections were obtained by microtome, transferred onto adhesive slides, and dried at 62℃ for 30minutes. After dewaxing and rehydrating procedures, antigen retrieval was carried out in all cases. After incubation with primary antibodies, the sections were incubated with biotinylated antimouse immunoglobulins, followed by peroxidase-labeled streptavidin in the LSAB kit (DAKO) and 3,3'-diaminobenzidine chromogen as substrate. Negative controls were obtained by omitting the primary antibodies for all slides. Slides were counterstained with Harris hematoxylin.

3. Evaluation of immunostaining

Two pathologists analyzed slides to examine any differences in intensity, percent positivity, and staining patterns among 3 groups. The results were considered significant for MLH1 or MSH2 if 100% of tumor cells lost nuclear staining. p53 immunostaining was interpreted as significant when at least two thirds of tumor cells showed nuclear staining. PTEN immunostaining was interpreted as significant when at least 90% of tumor cells lost nuclear staining. ER or PR immunostaining was interpreted as significant when at least 10% of tumor cells demonstrated nuclear staining of moderate to strong intensity. β-catenin immunostaining was considered significant when tumor cells showed any nuclear or cytoplasmic staining. E-cadherin immunostaining was interpreted as significant when more than 25% of tumor cells lost membranous staining. C-erb-B2 immunostaining was considered significant when at least 10% of tumor cells showed complete menbranous staining of moderate to strong intensity.

4. Statistical analysis

Statistical analysis between groups was performed using SPSS software (version 10 ; SPSS Inc, Chicago, III). The χ^2 test and Fisher's exact probability test were used to see any differences in the significant immunohistochemical results. P-values of less than 0.05 were considered to be significant.

Results

The results of immunohistochemical findings among 3 groups are summarized in Table 1. Cases of EMCs, low grade (Fig. 1) demonstrated significant immunohistochemical results for MLH1 (10.5%), MSH2 (15.8%), p53 (5.3%), PTEN (42.3%), ER (89.5%), PR (89.5%), β-catenin (31.6%), E-cadherin (10.5%), and c-erb B2

Table 1. Significant results of immunohistochemical stainings in EMCs and UPSCs

No. (%)	EMCs, low grade	EMCs, high grade	UPSCs
MLH1	2/19 (10.5%)	6/19 (31.6%)	2/13 (15.4%)
MSH2	3/19 (15.8%)	7/19 (36.8%)	2/13 (15.4%)
p53	1/19 (5.3%)	4/19 (21.1%)	10/13 (76.9%)
PTEN	8/19 (42.3%)	2/19 (10.5%)	2/13 (15.4%)
ER	17/19 (89.5%)	3/19 (15.8%)	1/13 (7.7%)
PR	17/19 (89.5%)	7/19 (36.8%)	7/13 (53.8%)
ß-catenin	6/19 (31.6%)	4/19 (21.1%)	2/13 (15.4%)
E-cadherin	2/19 (10.5%)	7/19 (36.8%)	5/13 (38.5%)
c-erb-B2	2/19 (10.5%)	4/19 (21.1%)	2/13 (15.4%)

EMCs : endometrioid carcinomas, UPSCs : uterine papillary serous carcinomas

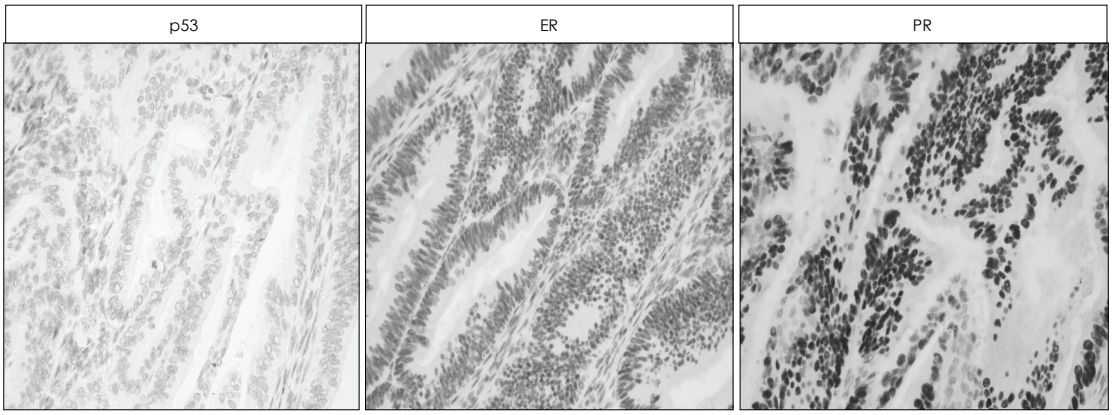

Fig. 1. Low-grade endometrial carcinomas (EMCs, LG) show an immunophenotype of p53 (−, left), ER (+, middle) and PR (+, right) in nuclei of tumor cells (×200).

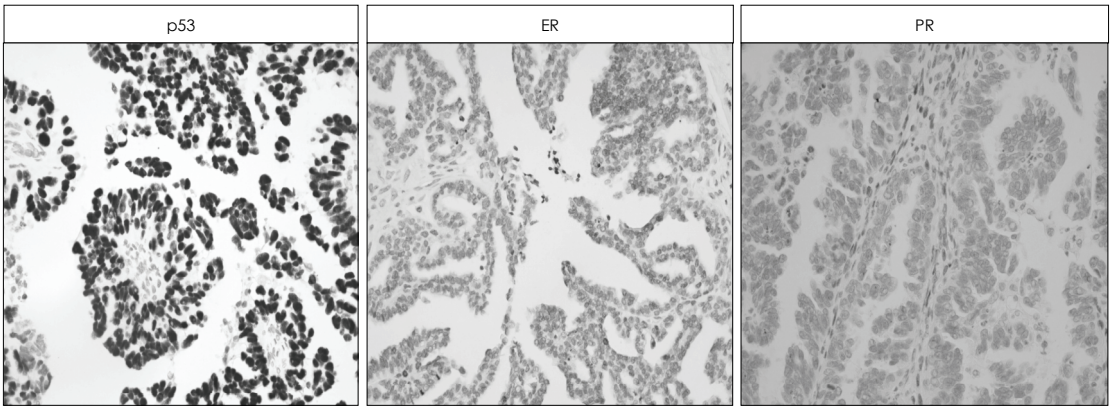

Fig. 2. Uterine papillary serous carcinomas show an immunophenotype of p53(+, left), ER(−, middle) and PR(−, right) in nuclei of tumor cells(×200) in contrast to EMCs, LG(Fig. 1).

Table 2. Utility of immunohistochemical stainings in the differential diagnosis of the UPSCs from the EMCs

Diagnosis	EMCs, LG versus UPSCs		EMCs, HG versus UPSCs	
Immunophenotype	p53(−) ER(+)	p53(+) ER(-)	p53(−) ER(−)	p53(+) ER(−)
Sensitivity	84%	69%	74%	69%
Specificity	100%	100%	77%	89%
Immunophenotype	p53(−)	p53(+)	p53(−)	p53(+)
Sensitivity	95%	77%	79%	77%
Specificity	77%	95%	77%	79%

EMCs, LG : endometrioid carcinomas, low grade, EMCs, HG : endometrioid carcinomas, high grade, UPSCs : uterine papillary serous carcinomas

(10.5%), respectively. Cases of EMCs, high grade demonstrated significant immunohistochemical results for MLH1(31.6%), MSH2(36.8%), p53(21.1%), PTEN (10.5%), ER(15.8%), PR(36.8%), β-catenin(21.1%), E-cadherin(36.8%), and c-erb B2(21.1%), respectively. Cases of UPSCs(Fig. 2) demonstrated significant immunohistochemical results for MLH1(15.4%), MSH2 (15.4%), p53(76.9%), PTEN(15.4%), ER(7.7%), PR (53.8%), β-catenin(15.4%), E-cadherin(38.5%), and c-erb B2 (15.4%), respectively.

Cases of the UPSCs showed significantly frequent immunopositivity for p53 immunohistochemical staining than those of the EMCs regardless of FIGO grades(EMCs, low grade versus UPSCs ; $p<0.0001$ and EMCs, high grade versus UPSCs ; $p=0.003$) by the χ^2 test. However, cases of the UPSCs showed significantly less frequent immunopositivity for both ER($p<0.0001$) and PR ($p=0.038$) immunohistochemical stainings than those of the EMCs, low grade(Fig. 1 and 2) by the χ^2 test.

Cases of the EMCs, low grade showed significantly frequent immunopositivity for ER and PR immunohistochemical stainings than those of the EMCs, high grade (ER ; $p<0.0001$ and PR ; $p=0.002$) by the χ^2 test. However, immunopositivity for both ER($p=0.629$) and PR($p=0.473$) immunohistochemical stainings demonstrated no significant difference in the differential diagnosis of the UPSCs from the EMCs, high grade by the χ^2 test. The remaining immunohistochemical markers showed no significant difference among 3 groups.

The utility of immunohistochemical stainings in the differential diagnosis of the UPSCs from the EMCs is summarized in Table 2. The combined use of both immunonegativity for ER and immunopositivity for p53 increased the specificity in the differential diagnosis of the UPSCs from the EMCs regardless of FIGO grades (Table 2).

Discussion

The tumorigenesis of sporadic endometrial cancer has

been explained by a dualistic model from both biological and clinical parameters[10]. Type-I EMCs, about 70−80% of cases of endometrioid carcinomas, follow an estrogen related pathway, develop in pre- and peri-menopausal women, frequently preceded by endometrial hyperplasia, express ER and PR receptors and are characterized by a favorable prognosis[10]. Type-II UPSCs, about 10−20% of cases of endometrioid carcinomas, follow an estrogen unrelated pathway, develop in post-menopausal women, frequently preceded by atrophic endometrium or endometrial intraepithelial carcinoma, demonstrate p53 mutation and are characterized by a aggressive clinical course[10]. Molecular genetics have recently provided us with more data supporting the dualistic model of type-I and type-II endometrial carcinomas[6-8].

In EMCs, 25−30% of MSI was previously reported in the literature[10)11)], and lower frequency (10−15%) of MSI was observed in our cases of EMCs, low-grade. 5−37% of p53 mutation or overexpression was previously reported in the literature[10-12)] similar to our study (5−21%). Cases of EMCs, high-grade demonstrated significantly increased p53 overexpression (21%) than EMCs, low-grade (5%) in our study although as much as 66% of p53 overexpression was reported in cases of EMCs, FIGO grade 3[13)]. Similarly, p53 overexpression was reported to be associated with high grade, advanced stage tumors and worse outcome[12)14)]. Loss of PTEN expression, the most frequently altered gene in EMCs, was observed in 42% of EMCs, low-grade in our study similar to 35-50% of the previous report[6)]. ER and PR expression was higher in EMCs, low-grade (89%) as much as the previous reports (78−85%)[15)16)]. ER and PR expression was significantly decreased in cases of EMCs, high-grade in our study. Similarly, ER and PR expression was reported to be associated with low-grade and early-stage tumors[12)]. β-catenin expression was the highest in cases of EMCs, low-grade (31%) same to that of the previous report[17)]. Although β-catenin expression in EMCs, FIGO grade 3 was reported to be as much as 47% in the previous report[18)], β-catenin expression in EMCs, high-grade was less frequent (21%) in our study. Loss of E-cadherin expression in EMCs was reported to be 5% in the previous report[19)] which is lower than that (10%) of our study. Loss of E-cadherin expression increased (36%) in EMCs, high-grade of our study. Similarly, E-cadherin expression in EMCs was reported to be negatively correlated with tumor grade and may be an independent prognostic factor for disease progression[20)]. C-erb B2 expression was higher in EMCs, high-grade than EMCs, low-grade in our study similar to the previous report[21)].

In UPSCs, MSI was uncommon and showed no significant difference between UPSCs and EMCs in our study unlikely to the previous report[22)]. 80% to more than 90% of p53 mutation or overexpression was previously reported in the literature for UPSCs[9-11)15)23)], which is higher than that (76%) of our study. It is remarkable p53 immunohistochemical staining is the most sensitive single marker in the differential diagnosis of the UPSCs from the EMCs regardless of FIGO grades (Table 2). ER and PR expression was significantly decreased in UPSCs similar to the previous reports[12)15)]. Although no expression of β-catenin was reported in the previous report for UPSCs[18)], our study demonstrated 15% of β-catenin expression. As much as 62% of loss of E-cadherin expression was reported in the previous report for UPSCs[19)] which is much higher than that (38%) of our study. Although significant difference in the E-cadherin expression was reported between endometrioid cell type and papillary serous or clear cell types[19)20)], our study showed no difference between two cell types. C-erb B2 expression in UPSCs (15%) was lower than those of previous reports (43−53%)[13)21)]. Although significant difference in the expression of c-erb B2 was reported between UPSCs and EMCs, any FIGO grade in the previous report[21)], our study revealed no difference among three groups. Among variable markers, only p53, ER and PR showed significant difference between UPSCs and EMCs, low-grade in our study.

This study is unique in that it is the most extensive comparative immunohistochemical study of uterine carcinomas comparing the similar previous studies[13)15)24)]. Similar to our study, only p53 was useful to differentiate UPSCs from EMCs, FIGO grade 3 among p53, PTEN, and c-erb B2 by N. Macwhinnie and H. Monaghan[13)]. F. Darvishian et al[24)] concluded only p53, PR, and PTEN best distinguished between EMCs and UPSCs mimicking EMCs with well-differentiated tubuloglandular morphology but high nuclear grade among p53, ER, PR,

PTEN, cyclin D1, and β-catenin. For ER, PR, and p53, the same trend was previously reported among three groups to our study[15]. The present study also adds evidence that the combined immunohistochemical analysis of endometrial carcinomas including p53, ER, or PR can more effectively differentiate between different grades and histological types (Table 2) than a use of single immunohistochemical marker. Although no mixed endometrial carcinoma is included in this study, these combined immunohistochemical analysis may be helpful in the distinction of mixed UPSCs and high-grade EMCs from pure high-grade EMCs as well as the distinction of EMCs of villoglandular features from UPSCs.

Conclusion

Immunohistochemical expression of p53 is a useful tool for differentiating UPSCs from EMC regardless of FIGO grade. Combined immunohistochemical results of p53(+), ER(−), and PR(−) would best characterize the UPSCs, and the low-grade EMCs would be best charaterized by the opposite results.

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