

International Journal of Surgical Pathology

<http://ijs.sagepub.com>

Endometrial Glandular Dysplasia: A Putative Precursor Lesion of Uterine Papillary Serous Carcinoma. Part II: Molecular Features

Sharon X. Liang, Setsuko K. Chambers, Liang Cheng, Shaopo Zhang, Yi Zhou and Wenxin Zheng
Int J Surg Pathol 2004; 12; 319
DOI: 10.1177/106689690401200405

The online version of this article can be found at:
<http://ijs.sagepub.com/cgi/content/abstract/12/4/319>

Published by:

 SAGE Publications

<http://www.sagepublications.com>

Additional services and information for *International Journal of Surgical Pathology* can be found at:

Email Alerts: <http://ijs.sagepub.com/cgi/alerts>

Subscriptions: <http://ijs.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.com/journalsPermissions.nav>

Citations (this article cites 54 articles hosted on the SAGE Journals Online and HighWire Press platforms):
<http://ijs.sagepub.com/cgi/content/refs/12/4/319>

Endometrial Glandular Dysplasia: A Putative Precursor Lesion of Uterine Papillary Serous Carcinoma. Part II: Molecular Features¹

Sharon X. Liang, MD, PhD,* Setsuko K. Chambers, MD,†
Liang Cheng, MD,‡ Shaopo Zhang, MD,‡ Yi Zhou, MD, PhD,†
and Wenxin Zheng, MD*†

Endometrial glandular dysplasia (EmGD) may be a newly defined precursor lesion of uterine papillary serous carcinoma (UPSC) by morphology. In this report, we studied molecular changes present in EmGD by the loss of heterozygosity (LOH) approach using laser capture microdissected tissue samples. Nineteen uteri showing at least 1 focus of EmGD by morphology were selected. These cases were 12 UPSC, 2 clear cell carcinomas, 1 mixed uterine papillary serous and endometrioid carcinoma, 1 uterine carcinosarcoma, 1 serous endometrial intraepithelial carcinoma (EIC), and 2 EmGD involving endometrial polyps. Seven microsatellite polymorphic DNA markers (TP53 at 17p, D1S211, and D1S162 at 1p32, D17S1323 at 17q21, D17S1330 at 17q25, D5S346 at 5q, and D2S123 at 2p) were utilized. A total of 123 laser-captured microdissection samples from 19 cases was studied with LOH method. The frequencies and patterns of LOH were analyzed and compared among benign resting endometrium (RE), EmGD, serous EIC, and UPSC. LOH was observed for at least 1 of the 7 markers in all categories of lesions, EmGD, serous EIC, and UPSC. The frequency of LOH for EmGD ranged from 4.2% to 31.3%; the range for serous EIC was 5.9% to 78.6%; and that for UPSC was 7.7% to 62.5%. The most frequent LOH in the 3 above-cited categories of lesions was identified at 17p (TP53) and 1p (D1S162). The frequency of LOH in EmGD with markers of TP53 and D1S162 was significantly higher than in RE ($p < 0.05$). With markers of D1S211 and D2S123, LOH in EmGD was higher than RE, approaching to a statistically significant level. Compared with foci of serous EIC and UPSC, however, the rate of LOH in EmGD was significantly lower only with TP53 locus (31.3% vs more than 60%, $p < 0.05$). The difference of LOH frequency with other chromosomal markers between EmGD and serous EIC/UPSC did not reach a statistically significant level. A significantly high concordant LOH pattern was found between foci of EmGD and serous EIC/UPSC ($p = 0.05$). We conclude that EmGD frequently shows LOH at multiple chromosomal loci, particularly at 17p and 1p. Significantly high concordant LOH frequency between EmGD and paired serous EIC or UPSC strongly suggests that EmGD is a noncancerous precursor lesion of UPSC, probably also of serous EIC. The clinical significance of EmGD needs further studies. *Int J Surg Pathol* 12(4):319–331, 2004

Key words: endometrial glandular dysplasia (EmGD), endometrial carcinoma, uterine papillary serous carcinoma, serous EIC.

¹Part I of this study (Morphologic Features) was published in the July 2004 issue of this journal.

From the Departments of *Pathology and †Obstetrics and Gynecology; Yale University School of Medicine, New Haven, CT; and the ‡Department of Pathology, Indiana University School of Medicine, Indianapolis, IN.

Reprint requests: Wenxin Zheng, MD, Department of Pathology, Yale University School of Medicine, 20 York Street, EP 2-608, New Haven, CT 06520-8070.

Acronyms

EIC = endometrial intraepithelial carcinoma
 EmGD = endometrial glandular dysplasia
 LCM = laser capture microdissection
 LOH = loss of heterozygosity
 RE = resting endothelium
 VEC = uterine endometrial carcinoma
 UPSC = uterine papillary serous carcinoma
 IHC = immunohistochemistry

Uterine papillary serous carcinoma (UPSC) is a distinct and aggressive subtype of endometrial carcinoma [1,2]. It is recognized as such because of its unique pathologic features, aggressive behavior, and a strong association with p53 mutations, as compared with uterine endometrioid carcinoma (UEC). UPSC comprises approximately 10–15% of all endometrial carcinoma but causes a disproportionate number of endometrial cancer deaths [3]. The factors predisposing to the development of UPSC are not clear. However, several groups, including ours, have found that early alterations of p53 may play a major role in the carcinogenesis [4–8].

Alteration of the tumor suppressor gene p53 has been described in approximately 10% to 15% of early and 40% to 50% of advanced endometrial carcinomas [4,5]. A particularly high-frequency p53 alteration is present in UPSC and other high-nuclear-grade endometrial cancers [5,7–11]. It has been found that approximately 90% of UPSC and serous EIC have p53 gene mutation by direct sequencing or single strand-conformational polymorphism (SSCP), as well as subsequent accumulation of aberrant p53 protein by immunohistochemical studies [5,11–14]. Poor prognosis of UPSC has also been attributed to p53 mutation [15,16]. Therefore p53 mutation may be a major participant in the development and progression of UPSC.

Loss of heterozygosity (LOH) on the short arm of chromosome 1 has been found in solid tumors of many organ systems, including breast, thyroid, central nervous system, gastrointestinal, and gynecologic tract [17]. Arlt et al [18] studied LOH in UPSC by using multiple microsatellite markers and found that chromosome 1p LOH was a common and specific feature for UPSC, with more than 63% of tumors revealing loss of sequences from the 1p32-p33 region. Although UPSC shares clinical features of ovarian papillary serous carcinoma (OPSC), the LOH percentage is much higher in UPSC than in OPSC (63.2% vs 26.6%) in this region [19]. These results have led to speculation that a putative tumor

suppressor gene of UPSC might be located in this region [18].

Since there is striking similarity in histopathologic appearance and poor clinical prognosis among UPSC and serous carcinomas of the ovary and peritoneum, the latter 2 are highly associated with BRCA-1 mutations, it is logical to wonder whether UPSC is also a BRCA-1-related cancer. Caduff et al [20] reported a comparative study of UPSC (n=8) and OPSC (n=26), and their results showed that both UPSC and OPSC have a significant allelic loss on 17q at BRCA-1 locus (43% vs 64%). Subsequently, BRCA-1 gene mutations were reported in women with peritoneal papillary serous carcinoma [21]. More recently, cases with a germ line mutation of the BRCA-1 gene were reported in a family of 2 affected sisters who had advanced stage of UPSC and OPSC [22]. These results suggest that BRCA-1 mutation may play a role in UPSC development, at least in a subset of patients.

In addition to the above-cited molecular events occurring in UPSC, other molecules have also been studied. These include LOH analysis of PTEN, APC, hMSH-1 and hMSH-2, microsatellite instability, and others [23,24]. Although abnormalities related to APC or hMSHs are more commonly seen in UEC, similar changes do occur in UPSC as well [13,25,26]. However, PTEN alteration seems never been reported in UPSC [27].

To study the histopathogenesis of a tumor, measurement of clonality is a well-accepted approach. Currently, analyses of chromosomal abnormality (microsatellite instability analysis), specific gene rearrangement, and patterns of oncogene activation in tumor DNA by polymerase chain reaction (PCR)-based techniques have been widely used for this purpose [28]. Of those, microsatellite instability analysis increasingly plays an important role, especially with use of paraffin-embedded tissues, because it is relatively easy to perform and abundant DNA polymorphisms are often present in tumor tissues. Microsatellite instability analysis with LOH assay has been used in discriminating multifocal from metastatic disease. This is a clinical dilemma encountered quite often in gynecologic malignancies [29–32], as well as malignancies in other organ systems [33–37].

Identification of precursor lesions of endometrial carcinoma has been a major interest of pathologists as well as of gynecologic oncologists because it has significant clinical impact. Atypical endometrial hyperplasia and endometrial intraepithelial neoplasia undoubtedly represent the precursor lesions of UEC [27,38]. However, the precursor lesion of UPSC is much less well characterized. To be considered a

precursor of UPSC, it should have evidence not only of coexistence and sequential morphologic progression from precursor to UPSC but also of similar changes at a molecular level. Therefore, it is plausible to apply a multidisciplinary approach including morphologic, clinical, and molecular studies to address the issue. In the immediate previous issue (Zheng et al, *IJSP*, 12:207–223), we defined endometrial glandular dysplasia (EmGD), a putative precursor of UPSC, by using a morphologic pathologic approach. In this study we intend to investigate the molecular changes in the lesions of EmGD by LOH assays at multiple microsatellite loci in order to determine whether EmGD is a neoplastic process and whether EmGD fits as a precursor lesion of UPSC.

Materials and Methods

Case Selection and Sample Targets

Nineteen uteri were used for this study. Among the 32 classic UPSC and 16 serous EIC uteri as described in our accompanying paper on the morphologic study [39], 8 UPSC and 1 serous EIC uteri had adequate material for this molecular study. We recruited an additional 4 UPSC, 2 clear cell carcinomas, and 1 mixed UPSC and UEC, 1 carcinosarcoma, and 2 EmGD involving endometrial polyps for this study. All 19 cases were from the Department of Pathology at Yale University School of Med-

icine. The ages of these patients ranged from 50 to 83 years with an average of 68.3. The patients were all postmenopausal. The 12 UPSC cases included 5 Stage 1A, 6 1B, and 1 stage 3C. Two clear cell carcinomas were 1 stage 1B and 1 stage 1C. One mixed carcinoma contained 75% UPSC and 25% UEC and was stage 1B. One carcinosarcoma had 30% serous carcinoma component and was stage 3C. Two EmGDs were found in endometrial polyps with no subsequent hysterectomy obtained yet. These cases are selected based on availability of tissue blocks and presence of appropriate areas for laser capture microdissection (LCM). Tumors were graded and staged by using International Federation of Obstetricians and Gynecologists (FIGO) system [40]. The patients' characteristics are summarized in Table 1. We also included 5 benign uteri from postmenopausal women who underwent hysterectomy because of nonendometrial cancer. Their ages ranged from 59 to 81 years with an average of 63.

The targeted areas included classic UPSC from the main tumor mass, serous EIC, EmGD, and adjacent resting endometrium (RE) within the uteri. If the serous EIC appeared to be closely associated with UPSC cases, we selected the lesions of serous EIC, which were at least 2 mm away from the main UPSC lesion within the same uterus. EmGD was selected based on the morphologic criteria described in the accompanying paper [39]. Multiple foci of EmGD, serous EIC, and UPSC were microdissected

Table 1. Clinical and Histologic Data of 19 Selected Cases

Case No.	Age (yr)	Histologic Dx	Nuclear Grade	Stage	Clinical Presentation and Other Findings
1	80	UPSC	3	IB	PMB
2	74	UPSC	3	IIIC	Paraneoplastic syndrome
3	70	UPSC	3	IA	Abnormal pap, breast ca, on tamoxifen
4	74	UPSC	3	IB	PMB
5	50	CCC	3	IB	PMB
6	67	UPSC	3	IB	PMB
7	69	EmGD	N/A	N/A	Stage 3 Ser OvCa
8	83	Mixed UPSC & UEC	3	IB	PMB
9	60	UPSC	3	IB	PMB
10	80	UPSC	3	IA	PMB
11	60	CCC	3	IC	PMB, abnormal pap
12	61	EmGD in EMP	N/A	N/A	Stage 3 Ser OvCa
13	68	UPSC	3	IB	PMB, breast ca & OvCa
14	60	UPSC	3	IA	Abnormal pap
15	74	MMMT	3	IIIC	PMB, breast ca
16	57	EmGD in EMP	N/A	N/A	Abn pap, EMP
17	61	UPSC in EMP	3	IA	PMB
18	73	UPSC	3	IA	PMB
19	76	UPSC	3	IB	PMB

EmGD: endometrial glandular dysplasia; UPSC: uterine papillary serous carcinoma; CCC: clear cell carcinoma; UEC: endometrioid adenocarcinoma; MMT: malignant mixed Müllerian tumor; EMP: endometrial polyp; PMB: postmenopausal bleeding; Ser OvCa: papillary serous ovarian carcinoma; Abnormal (Abn) pap: abnormal pap smears of either histocytes or atypical glandular cells or adenocarcinoma cells in the preoperative pap smears.

with laser capture method. Six foci of EmGDs and 7 foci of serous EICs were derived from 2 clear cell carcinomas, 1 mixed serous and endometrioid carcinoma, and 1 carcinosarcoma. We did not include areas of clear cell carcinoma and UEC for this study. One sample of endometrial stroma from a non-cancerous area from each uterus was included. For 2 cases of EmGD involving endometrial polyps, the stromal cells were microdissected from non-EmGD areas within the polyp. In total, 24 RE including 9 atrophic endometria, 10 weakly proliferative endometria, and 5 proliferative endometria were studied. Nineteen were derived from endometrial cancer and EmGD cases, while the remaining 5 were derived from noncancerous control uteri. The RE from cancer cases were located at least 1 cm away from either UPSC or serous EIC or other malignant component. No samples of endometrial metaplasia were examined.

All specimens were obtained under the approval of the Human Investigation Committee.

Laser-Capture Microdissection

Histologic sections were prepared from routine formalin-fixed, paraffin-embedded blocks and stained with hematoxylin-eosin for histopathologic review. Subsequent histologic sections containing targeted area(s) from each embedded tissue block were obtained and placed onto glass slides at 5-micron intervals and stained with methyl green fol-

lowing the standard protocol (Arcturus Engineering Inc., Mountain View, California). Targeted endometrial epithelial cells, including EmGD, serous EIC, invasive UPSC, and benign RE, were microdissected from methyl-green-stained slides under direct light microscopic visualization (BH2 Olympus, Tokyo, Japan), by using PixCell Iie Laser Capture Microdissection System (Arcturus Engineering Inc., Mountain View, California) as described elsewhere [41]. Approximately 300 to 500 cells were microdissected from each interested area. One control sample (not involved with any lesions) was obtained from the normal stromal tissue from each uterus. Examples of targeted areas before and after microdissection are illustrated in Fig. 1.

DNA Extraction and Detection of LOH

The microdissected tissues were placed in a 20- μ L aqueous solution containing 10 mM Tris-HCl, 1mM EDTA, 1% Tween 20, and 0.1 mg/mL proteinase K (pH 8.3) and incubated overnight at 37°C for DNA extraction [42]. The solution was boiled for 10 minutes to inactivate the proteinase K and used directly for subsequent LOH analysis as described elsewhere [34,43]. Seven oligonucleotide primer pairs for the microsatellite DNA markers selected in this study were synthesized in Research Genetics (Huntsville, AL). These included TP53, D1S211, D1S162, D17S1323, D17S1330, D5S346, and D2S123. The DNA sequence of each primer with corresponding

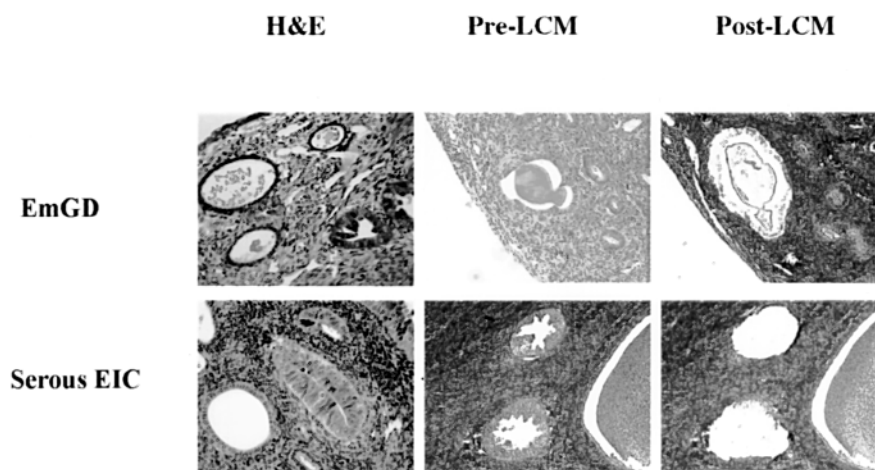


Fig. 1. Examples of precise microdissection of endometrial glands from EmGD (top) and serous EIC (bottom). The left panel is the image of H & E staining, the image in the middle is derived from pre-laser-captured microdissection (LCM), and the right is post-LCM.

known gene and chromosomal location is listed in Table 2. The TP53 and D17S1323 loci are intragenic to p53 and BRCA-1 genes, respectively [7,20]. The D1S211 and D1S162 loci were present in the short arm of chromosome 1, which are highly associated with UPSC although specific genes in these loci remain unknown [18]. The other loci (D17S1330, D5S346, and D2S123) were also related to endometrial cancers, although high specificity with UPSC has not been reported [20,44,45]. For each marker analysis, polymerase chain reactions (PCR) were performed in 10 μ L of total volume. Each reaction contained 0.2 mM deoxyribonucleoside triphosphates, 1 μ M forward and reverse primers, 0.2 μ L 32 P-labeled deoxycytidine triphosphate (6,000 Ci/mM), 0.1 unit of *Taq* polymerase, and 1 μ L DNA extraction mixture. PCR amplification was carried out for 35 cycles, with each cycle performed for 30 seconds at 95°C, 1 minute at 55°C, 1 minute at 72°C, and 10 minutes at 72°C at the final extension step (Perkin-Elmer Cetus Thermal Cycler, The Perkin-Elmer Corp., Foster City, CA). The amplified PCR products were diluted 1:1 in denaturing loading buffer containing 95% deionized formamide, 20 mM EDTA, 10 mM NaOH, 0.05% xylene cyanol FF, and 0.05% bromophenol blue (Sigma Chemical Co., St. Louis, MO), and were heated to 95°C for 5 minutes before application to 6.5% polyacrylamide gels. Electrophoresis was performed at 1,600 V for 4–7 hours, followed by autoradiography with Kodak X-OMAT-AR film (Eastman Kodak Co., Rochester, NY) for 8–16 hours. The PCR reactions for each polymorphic DNA marker were repeated at least twice, and the same results were obtained.

Allelic Loss Patterns and Statistical Analyses

The LOH analysis for each matched case was scored based on the presence or absence (complete or nearly complete absence) of an allele in the study

group as compared with normal endometrial stromal cell DNA control from the same individual [34,43]. A more than 2-fold change in the relative intensity of alleles in tumor as compared with normal DNA was considered as positive for LOH. When the genetic material from a control focus was homozygous (showing a single band) for a polymorphic marker, the sample was considered noninformative (Ni). In contrast, samples were informative if a sample contained heterozygous genetic material for the defined polymorphic marker, and in such circumstances, its DNA showed either no allelic deletion (normal retention of heterozygosity, [NR]) or showed absence of 1 allele (LOH) [46]. The concordant allelic loss pattern in EmGD, serous EIC, and/or UPSC in a single case was considered to be compatible with a monoclonal origin of the lesions. In contrast, different patterns of allelic loss are compatible with independent origins of these lesions. Nonevaluable samples (NE) were defined as samples with inadequate DNA for LOH analysis. Statistical analysis was performed with use of the two-tailed Fisher's Exact Test and/or Chi-square test. A *p* value < 0.05 was considered statistically significant.

Results

We analyzed the frequency and the pattern of allelic loss of multiple matched lesions from 19 cases with serous EIC/UPSC or its putative precursor lesions with 7 polymorphic microsatellite markers on chromosomes 1p32.1-32.2, 2p16, 5q22-23, 17p, and 17q (Table 2). From the 19 cases, a total of 123 microdissected foci was analyzed. Of those, there were 25 samples of endometrial stroma from benign areas, 18 RE, 35 EmGD, 18 serous EIC, and 19 UPSC. In addition, 5 RE from benign uteri were also dissected and included in the RE group for analysis. Representative examples of LOH with multiple markers from studied cases are illustrated in Figs. 2 and 3.

Table 2. Chromosomal Location, Probable Corresponding Genes, and Sequences of 7 Microsatellite Repeat Polymorphism Markers Used in LOH Analysis

Marker	Location	Gene	Primer Sequence	Reference
TP53	17p	p53	TACAGGGATAGGTAGCCCGAG GGATTGGGCTCTTTGTAA	Niederacher [7] 1998
D1S211	1p32.2	Unknown	AGC TAC ATG GCA GGA TCA GA GGA TTC CTT GCT CTG GAA AG	GDB*
D1S162	1p32.1	Unknown	ATA AGG GGA ACA GGT CTG GG GGG GGA AGA AGT CCG AGT AG	Arlt [18] 1996
D17S1323	17q21	BRCA-1	TAGGAGATGGATTATTGGTG AAGCAACTTTGCAATGAGTG	Caduff [20] 1998
D17S1330	17q25	CTT-16	TGGCTAGTGGACAAAAGTGGT GGGAACAAATAATGAACAAAA	Caduff [20] 1998
D5S346	5q22-23	APC	TACTCACTCTAGTGATAAATCGG TTCAGGGAATTGAGAGTTACAG	Jass [44] 2003
D2S123	2p16	hMSH-2	AAACAGGATGCCTGCCTTA GGACTTCCACCTATGGGAC	Peiro [45] 2002

GDB* website <http://www-genome.wi.mit.edu>.

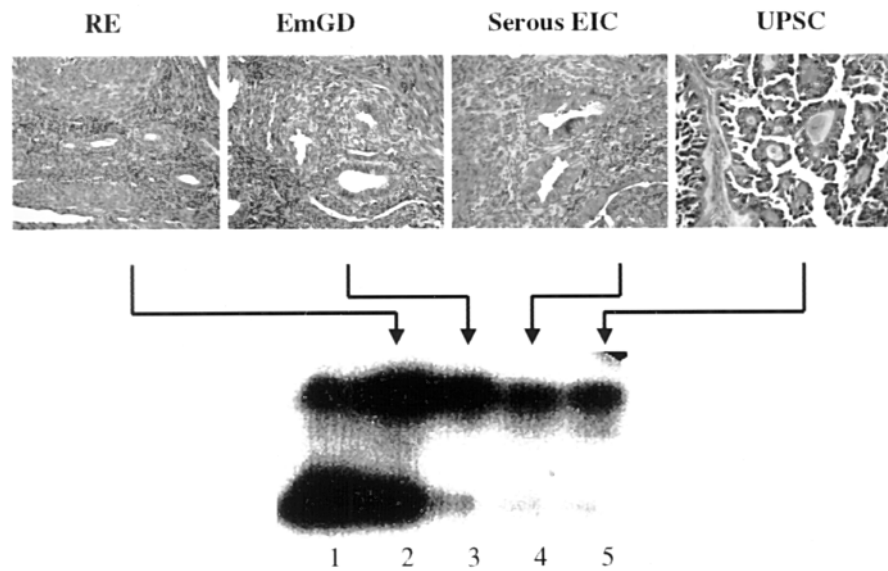


Fig. 2. Concordant LOH at TP53 in EmGD, serous EIC, and UPSC from case 4. Upper panel shows histologic examples of resting endometrium (RE), EmGD, serous EIC, and UPSC. Lower panel shows loss of lower allele in EmGD (lane 3), serous EIC (lane 4), and UPSC (lane 5). Lane 1 and 2 represent endometrial stroma and RE, respectively.

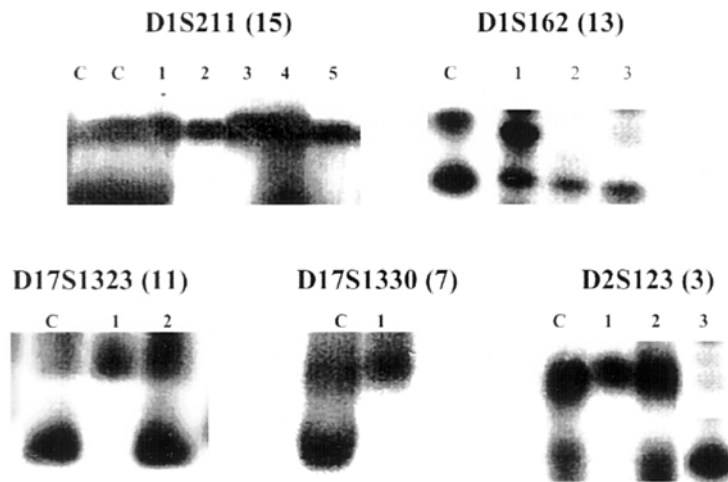


Fig. 3. Representative gels showing LOH from selected cases (numbers in parentheses) with different microsatellite markers (headings of each gel). C means resting endometrium control. Top left gel shows a concordant pattern of LOH from case 15 with marker D1S211: Lane 1 = EmGD1, 2 = EmGD2, 3 = Serous EIC1, 4 = Serous EIC2, and 5 = UPSC1. Top right gel shows loss of an upper allele from case 13 with marker D1S162: Lane 1 = EmGD1, 2 = EmGD2, 3 = Serous EIC2 (Serous EIC1 and UPSC samples were run in a separate gel, not shown here). Lower left gel shows loss of an lower allele at lane 1 (EmGD2) and normal retention at lane 2 (Serous EIC1) from case 11 with marker D17S1323. Lower middle gel shows that EmGD1 from case 7 lost 1 lower allele with marker D17S1330. Lower right gel shows an example of discordant LOH pattern from case 3 with marker D2S123: Lane 1 = EmGD2, 2 = EmGD3, and 3 = UPSC1.

Samples of case 12 were either noninformative or nonevaluable. No noninformative and nonevaluable samples were included in the statistical analysis.

Comparison of LOH Frequencies in Resting Endometrium, EmGD, Serous EIC, and UPSC

Tables 3 and 4 summarize the LOH identified in all foci of RE, EmGD, serous EIC, and UPSC among the 18 informative cases. Overall, the lesions of EmGD, serous EIC, and UPSC had at least 1 LOH in all the markers analyzed. This was in contrast to the RE, which showed only 1 LOH in 1 (TP53) of the 7 microsatellite markers ($p < 0.05$). Endometrial stromal cell controls of informative cases did not show any LOH in the 7 markers (data not shown). In general, within each case, the lesion foci of EmGD, serous EIC, and UPSC contained more LOH than the corresponding RE did. When we arranged the frequency of LOH from top to bottom among the 7 markers, the top 4 markers (LOH frequency $> 16\%$) were almost always TP53 (17p), D1S162 (1p), D1S211 (1p), and D2S123 (2p) in all endometrial lesions analyzed (Table 4). The lowest LOH frequency ($< 10\%$) was found with markers of D5S346 (5q) and D17S1330 (17q). Individually, LOH frequency in EmGD was 31.3% with TP53, 26.7% with D1S162, 18.5% with D1S211, 9.5% with D17S1323, 7.1% with D17S1330, 4.2% with D5S346, and 16.7% with D2S123. The frequency of LOH in matched serous EIC was 78.6% with TP53, 50% with D1S162, 33.3% with D1S211, 27.3% with D17S1323, 12.5% with D17S1330, 5.9% with D5S346, and 30.8% with D2S123. The LOH in corresponding UPSC areas was 62.5% with TP53, 47.4% with D1S162, 46.7% with D1S211, 28.6% with D17S1323, 8.3% with D17S1330, 7.7% with D5S346, and 23.1% with D2S123. In comparison with serous EIC and/or UPSC, a significantly lower LOH frequency was seen in EmGDs at locus of TP53 ($p < 0.05$) (Table 4). No significant statistical difference of LOH frequencies was observed among the 3 categories of lesions ($p > 0.05$) at the remaining marker loci analyzed in this study (Table 4).

LOH was identified in EmGD but not in either serous EIC or UPSC foci in 2 cases (case 11 at D17S1323 and case 10 at D5S346). In contrast, 6 cases (cases 3, 11, 14, and 17–19) showed LOH in at least 1 genetic marker in either serous EIC or UPSC, but not in its matched EmGD lesions. For example, case 19 showed LOH in UPSC foci at both chromosome 1p loci (D1S211 and D1S162) that was not recognized in corresponding EmGD lesions from the same uterus.

LOH Pattern Analysis in EmGD, Serous EIC, and UPSC

LOH with marker TP53 was found in 10 of the 18 informative cases. Among the 10 cases, 8 showed that LOH was present in at least 2 of the 3 lesions (EmGD, serous EIC, and UPSC) in the same uterus. An identical pattern of the LOH with TP53 was observed at least in 1 set of the paired lesions (compared EmGD with either serous EIC or with UPSC) in 5 (62.5%) of the 8 cases (Cases 4, 10, 11, 14, and 18). In case 4, loss of lower allele of TP53 was found in 1 of the 4 EmGDs, 2 of the 3 serous EICs, and all 2 UPSC foci (Table 3, Fig. 2). Discordant allelic loss at TP53 locus was found in 3 (37.5%) cases (cases 13, 17, and 19). In case 13, EmGD showed loss of upper allele, while 1 serous EIC and 1 UPSC focus showed loss of upper allele (Table 3). The opposite patterns of LOH were seen in cases 17 and 19 (Table 3).

In addition to marker TP53, multiple samples of concordant LOH were seen in cases 3, 4, and 10 with marker D1S162; cases 11, 14, 15, and 17 with marker D1S211; and case 13 with markers D1S211, D1S162 and D17S1323 (Table 3). In contrast, discordant allelic loss at D2S123 locus was present in only 1 additional case (case 5), which showed loss of lower allele in EmGD, but upper allele in matched serous EIC foci. Therefore, 4 additional cases (cases 3, 13, 15, and 17) with concordant LOH and 1 case (case 5) with discordant LOH were found in the markers other than TP53 when we compared the patterns of LOH between EmGD and serous EIC or UPSC (Table 3). Overall, the number of concordant LOH (9 of 13, 69.2%) was significantly higher than the number of discordant LOH (4 of 13, 30.8%) ($p = 0.05$).

No LOH pattern analysis was performed with markers D17S1330, D5S346, and D2S123 because only a few sporadic LOH were detected.

LOH Without Specific Patterns

Among the 18 informative cases, 12 had more than 1 focus of EmGD in the same uterus. Of the 12 cases, 3 showed more than 1 LOH in a single marker locus analyzed (cases 3, 4, and 14). The cases 3 and 4 showed that 2 EmGDs had identical loss of lower allele pattern at marker D1S162, while the 2 EmGDs in case 14 showed 1 loss of lower allele and the other loss of upper allele at marker TP53. Of interest, 1 atrophic endometrium showed a single sporadic LOH at TP53 locus (case 13).

Table 3. Summary of Allelic Loss Patterns in All LCM-Dissected Areas from 19 Selected Cases

Case No.	LCM Areas	TP53	D1S211	D1S162	D17S1323	D17S1330	D5S346	D2S123
1	AE	NR	NR	NR	NE	NR	NR	NR
	EmGD	NR	1	NR	NE	NR	NR	1
2	WPE	Ni	Ni	NR	NE	NR	NR	Ni
	Ser EIC	Ni	Ni	NR	NE	NR	NR	Ni
3	WPE	NR	NR	NR	NE	NR	NR	NR
	EmGD1	NR	NR	NR	NE	NR	NR	NR
	EmGD2	NR	NR	1	NE	NR	NR	2
	EmGD3	NR	NR	1	NE	NR	NR	NR
	UPSC1	NR	NR	NR	NE	NR	NR	1
	UPSC2	NR	1	1	NE	NR	NR	1
4	AE	NR	NR	NR	NR	NR	NR	NR
	EmGD1	NR	Ni	1	NE	NR	NR	NR
	EmGD2	NR	Ni	NR	NE	NR	NR	NR
	EmGD3	NR	NR	NR	NR	NR	NR	NR
	EmGD4	2	NR	1	1	NR	NR	NR
	Ser EIC1	1	Ni	1	NE	NR	NR	NR
	Ser EIC2	2	1	NR	NR	NR	NR	1
	Ser EIC3	2	NR	2	1	NR	NR	NR
	UPSC1	2	1	1	NR	NR	NR	1
	UPSC2	2	NR	2	1	1	NR	NR
5	PE	NR	NR	Ni	NE	NR	NR	NR
	EmGD	NR	NR	Ni	NE	NR	NR	2
	Ser EIC1	1	NR	Ni	NE	NR	NR	2
	Ser EIC2	1	NR	Ni	NE	NR	2	1
	Ser EIC3	1	NR	Ni	NE	NR	NR	1
6	WPE	NR	Ni	NR	NE	Ni	NR	NR
	UPSC	NR	Ni	NR	NE	Ni	NR	NR
7	AE	NR	NR	NR	NE	NR	Ni	NR
	EmGD1	1	NR	NR	NE	1	Ni	1
	EmGD2	NR	NR	1	NE	NR	Ni	NR
8	PE	NR	NR	NR	NE	NR	NR	NR
	EmGD	NR	NR	NR	NE	NR	NR	NR
	UPSC	NR	NR	NR	NE	NR	NR	NR
9	WPE	Ni	Ni	NR	NE	NR	NR	NR
	EmGD	Ni	Ni	NR	NE	NR	NR	NR
	Ser EIC	Ni	Ni	NR	NE	NR	NR	NR
	UPSC	Ni	Ni	NR	NE	NR	NR	NR
10	WPE	NR	Ni	NR	Ni	Ni	NR	NR
	EmGD1	NR	Ni	NR	Ni	Ni	NR	NR
	EmGD2	NR	Ni	NR	Ni	Ni	NR	NR
	EmGD3	2	Ni	1	Ni	Ni	2	NR
	UPSC1	2	Ni	1	Ni	Ni	NR	NR
	UPSC2	2	Ni	NR	Ni	Ni	NR	NR
11	PE	NR	NR	NR	NR	NR	NR	Ni
	EmGD1	1	2	NR	NR	NR	NR	Ni
	EmGD2	NR	NR	NR	2	NR	NR	Ni
	Ser EIC1	1	2	NR	NR	1	NR	Ni
	Ser EIC2	NR	NR	1	NR	NR	NR	Ni
12	WPE	Ni	NR	Ni	NE	Ni	NR	Ni
	EmGD1	Ni	NR	Ni	NE	Ni	NR	Ni
	EmGD2	Ni	NR	Ni	NE	Ni	NR	Ni
13	AE	1	NR	NR	NR	NR	NR	NR
	EmGD1	NR	1	NR	NR	1	NR	NR
	EmGD2	1	NR	1	NR	NR	NR	NR
	Ser EIC1	NR	1	NR	1	NR	NR	NR
	Ser EIC2	2	NR	1	NR	NR	NR	NR
	UPSC1	NR	NR	1	NR	NR	NR	NR
	UPSC2	2	1	NR	1	NR	1	2
14	WPE	NR	NR	NR	NR	NR	Ni	NR
	EmGD1	NR	NR	NR	NR	NR	Ni	NR
	EmGD2	1	1	NR	NR	NR	Ni	NR
	EmGD3	NR	NR	NR	NR	NR	Ni	NR
	EmGD4	NR	NR	NR	NR	NR	Ni	NR
	EmGD5	2	NR	NR	NR	NR	Ni	NR
	UPSC1	1	1	2	NR	NR	Ni	NR
	UPSC2	NR	NR	NR	NR	NR	Ni	NR
15	AE	Ni	NR	NR	NR	Ni	NR	NR
	EmGD1	Ni	NR	NR	NR	Ni	NR	NR
	EmGD2	Ni	2	NR	NR	Ni	NR	NR
	Ser EIC1	Ni	2	1	2	Ni	NR	NR
	Ser EIC2	Ni	NR	NR	NR	Ni	NR	NR
	UPSC1	Ni	2	1	NR	Ni	NR	NR
	UPSC2	Ni	2	NR	1	Ni	NR	NR

(continued)

Table 3. Summary of Allelic Loss Patterns in All LCM-Dissected Areas from 19 Selected Cases (*continued*)

Case No.	LCM Areas	TP53	D1S211	D1S162	D17S1323	D17S1330	D5S346	D2S123
16	PE	NR	Ni	Ni	NR	NR	NR	NR
	EmGD1	NR	Ni	Ni	NR	NR	NR	NR
	EmGD2	NR	Ni	Ni	NR	NR	NR	NR
17	WPE	NR	NR	NR	NR	NR	Ni	NR
	EmGD1	2	NR	1	NR	NR	Ni	NR
	EmGD2	NR	NR	NR	NR	NR	Ni	NR
	Ser EIC	1	NR	1	NR	NR	Ni	NR
	UPSC1	1	NR	NR	2	NR	Ni	NR
	UPSC2	1	NR	1	2	NR	Ni	NR
18	AE	NR	NR	Ni	NR	NR	NR	NR
	EmGD1	NR	NR	Ni	NR	NR	NR	NR
	EmGD2	2	NR	Ni	NR	NR	NR	NR
	Ser EIC1	NR	NR	Ni	NR	NR	NR	NR
	Ser EIC2	1	NR	Ni	NR	1	NR	NR
	Ser EIC3	2	NR	Ni	NR	NR	NR	NR
19	WPE1	NR	NR	NR	NR	Ni	NR	Ni
	EmGD1	2	NR	NR	NR	Ni	NR	Ni
	EmGD2	NR	NR	NR	NR	Ni	NR	Ni
	UPSC1	1	NR	2	NR	Ni	NR	Ni
	UPSC2	1	1	NR	NR	Ni	NR	Ni

AE: atrophic endometrium; WPE: weakly proliferative endometrium; PE: proliferative endometrium; EmGD: endometrial glandular dysplasia; Ser EIC: serous endometrial intraepithelial carcinoma; UPSC: uterine papillary serous carcinoma. 1: loss of upper allele; 2: loss of lower allele; NR: normal retention of heterozygosity; Ni: noninformative; NE: not evaluable.

Table 4. Frequencies of LOH in Foci of Resting Endometrium, EmGD, Serous EIC, and UPSC in 18 Informative Cases

Endometrium		TP53	D1S211	D1S162	D17S1323 [†]	D17S1330	D5S346	D2S123
RE (n=23*)	# IC	20	18	20	21	18	19	18
	# LOH	1	0	0	0	0	0	0
	% LOH	5.0	0	0	0	0	0	0
EmGD (n=35)	# IC	32	27	30	30	28	24	24
	# LOH	10	5	8	2	2	1	4
	% LOH	31.3	18.5	26.7	9.5	7.1	4.2	16.7
	p ¹ value	0.024	0.053	0.012	0.366	0.246	0.142	0.069
Ser EIC (n=18)	# IC	14	15	12	17	16	17	13
	# LOH	11	5	6	3	2	1	4
	% LOH	78.6	33.3	50	27.3	12.5	5.9	30.8
	p ² value	0.003	0.280	0.147	0.189	0.552	0.802	0.302
UPSC (n=19)	# IC	16	15	19	15	12	13	13
	# LOH	10	7	9	2	1	1	3
	% LOH	62.5	46.7	47.4	28.6	8.3	7.7	23.1
	p ² value	0.038	0.053	0.138	0.416	0.896	0.651	0.603

RE: resting endometrium; EmGD: endometrial glandular dysplasia; Ser EIC: serous endometrial intraepithelial carcinoma; UPSC: uterine papillary serous carcinoma.

*The RE included 18 informative cases and 5 benign uteri without dysplasia or cancer. [†]The total areas available for analysis with marker D17S1323 is less than the other markers because many of the DNA samples were not sufficient for analysis. We obtained a total of 10 RE, 26 EmGDs, 12 serous EICs, and 14 UPSCs for D17S1323 analysis.

Number of informative cases (IC) or positive LOH areas in targeted endometrium for each marker are listed as # IC and # LOH respectively. Percentage of positive LOH (% LOH) is determined by number of positive LOH areas out of total number of informative areas. The p value is determined by Fisher's and Chi-squared test. The p¹ value was derived from the comparison between EmGD and RE, while p² was from the comparisons of either serous EIC or UPSC with EmGD.

Summary of LOH in Foci of EmGD, Serous EIC, and UPSC

LOH was observed for at least 1 of the 7 polymorphic DNA markers in all categories of lesions: EmGD, serous EIC, and UPSC, in this study (Table 4). The frequency of LOH for EmGD for the 7 markers ranged from 4.2% to 31.3%; the range for

serous EIC was 5.9% to 78.6%; and that for UPSC was 7.7% to 62.5%. The most frequent LOH in the 3 above-cited categories of lesions was identified at 17p (TP53) and 1p (D1S162). The frequency of LOH in EmGD with markers of TP53 and D1S162 was significantly higher than in RE ($p < 0.05$). With markers of D1S211 and D2S123, LOH in EmGD was higher than in RE, approaching a statistically signif-

icant level ($p = 0.053$ and 0.069 , respectively). Compared with foci of serous EIC and UPSC, however, the rate of LOH in EmGD was significantly lower only with TP53 locus (31.3% vs more than 60%, $p < 0.05$). The difference of LOH frequency with other chromosomal markers between EmGD and serous EIC/UPSC did not reach a statistically significant level. A significantly high concordant LOH pattern was found between foci of EmGD and serous EIC/UPSC ($p = 0.05$).

Discussion

The dualistic model of endometrial carcinogenesis proposes 2 pathways that differ in regard to epidemiologic risk factors: histopathologic features and molecular alterations [27]. The "classic" pathway, typically for UEC, is proposed for those indolent tumors developed in an estrogen-rich environment, while the alternative pathway, typically for UPSC, is designed to interpret those aggressive tumors that are not particularly associated with estrogen stimulation. The recent morphologic recognition of EmGD in postmenopausal women has further supported the current concept of dualistic pathways in endometrial carcinogenesis.

In this study, we attempted to identify molecular changes of EmGD as part of our efforts to examine whether EmGD shows features compatible with a precursor of UPSC. We studied the level of LOH in matched tissues samples of benign RE, EmGD, serous EIC, and UPSC. We selected 7 polymorphic markers in chromosome regions of 1p, 2p, 5q, 17p, and 17q. These chromosomal regions are frequently altered in endometrial cancers, particularly in UPSC according to previous studies [13,14,17,18,20,23–26]. LCM was employed to ensure tissue purity in excess of 95%, optimizing the likelihood of successful analysis in routinely formalin-fixed, paraffin embedded tissues. All EmGD foci showed at least 1 LOH in all 7 markers, and approximately one third of EmGD showed LOH at TP53 locus, which was significantly higher than matched benign RE samples. This observation suggests that morphologically identified EmGD is not just simply a morphologic entity but represents a lesion that has undergone neoplastic transformation.

The frequency of LOH in EmGD tended to be high in chromosomal regions of 17p and 1p, but the rate of LOH in other chromosomal regions (2p, 5q, and 17q) was apparently low. As shown previously, alterations of chromosomal regions of 17p and 1p are more commonly associated with UPSC, while alterations of 2p and 5q are more commonly associated with UEC. The findings suggest that molecular

changes of EmGD are more compatible to UPSC tumorigenesis. The most frequent regions of LOH in EmGD was identified at 17p (31.3%) and 1p (22.8% for combined D1S211 and D1S162), which was also the most frequent regions of LOH identified in serous EIC (78.6% and 40.7%) and UPSC (62.5% and 47.1%, respectively). This is consistent with previous findings of frequent LOH on chromosome 17p and 1p in serous EIC and UPSC [13,17,18,20,47]. The findings suggest that alterations of these chromosomal arms may be important for the initiation and progression of UPSC. The tumor suppressor gene p53 is known to be located in chromosome 17p [48]. It is also well demonstrated that p53 gene alteration is commonly seen in serous EIC and UPSC [12–14]. LOH at TP53 is conceptually different from p53 gene mutation. However, the high TP53 LOH rate in combination with high rate of p53 overexpression by immunohistochemistry (IHC) [39] further suggests that alteration of p53 gene is likely present in EmGD, although a definitive gene mutation analysis is needed to confirm the statement.

The hypothetical multistep model of carcinogenesis as applied to many organ systems has led to explosive studies of precursor lesions. Morphologic recognition of precursors as well as their molecular changes by LOH studies at multiple chromosomal loci have been reported in many organ systems. These include prostate intraepithelial neoplasia [34], testicular intraepithelial neoplasia [49], breast duct carcinoma *in situ* [36], pancreatic intraepithelial neoplasia [50], and cervical intraepithelial neoplasia [51], and others. A significant number of LOH at multiple chromosomal loci in lesions of EmGD has also been demonstrated in this study. Strikingly similar, but significantly less in frequency, LOH patterns at multiple chromosomal loci were present in EmGD lesions compared with serous EIC and/or UPSC, strongly suggesting that a monoclonal expansion of a neoplastic cell in EmGD may be at least partially responsible for subsequent development of UPSC or possibly through serous EIC. If the multistep model of carcinogenesis is applied to UPSC development, EmGD may most likely be an earliest morphologically identifiable entity representing a putative precursor lesion of UPSC.

The extent of genetic alteration and heterogeneity was remarkably similar in serous EIC and UPSC, which is consistent with previous observations using these and other methods [13,14,17,18]. We observed very similar LOH in all the chromosomal loci in the matched cases with foci of serous EIC and UPSC (Table 3), suggesting that they are closely

linked genetically. The overall frequency of numeric chromosomal anomalies in serous EIC and UPSC was also strikingly similar (more than 60% at TP53 and 50% vs 47.4% at DIS162, etc., Table 4), suggesting a similar underlying pathogenesis for these 2 lesions. In agreement with our morphologic study in our accompanying paper [39], the molecular findings further support that serous EIC may represent an early form of UPSC instead of a true noninvasive precursor lesion of UPSC [12,52].

Recently a few cases of UPSC with alteration of BRCA-1 gene, particularly with germline mutations, have been reported [22,53]. Up to 18% LOH for BRCA-1 at 17q21 regions was seen in sporadic endometrial cancer [7]. LOH at 17q21 region (D17S1323) was detected in 9.5% EmGD, in 27% serous EIC, and in 29% UPSC foci in this study. It is known that many endometrial cancers, mainly UECs, have defective DNA mismatch repair genes [54], namely, hMSH-2. In the current study, we unexpectedly found that LOH at 2p16 region (D2S123, including hMSH-2 gene) was 17% in EmGD, 31% in serous EIC, and 23% in UPSC. However, the significance of these chromosomal regions in the development of UPSC remains to be clarified. Larger studies with BRCA-1 mutation analysis and studies particularly designed for multiple DNA mismatch repair genes in UPSC and its precursor lesions may provide better clues in terms of the roles of these genes in the development of UPSC.

In summary, it seems likely, from our morphologic [55] and molecular studies, that EmGD represents a precursor of UPSC, although the clinical significance of EmGD remains unclear. It is hoped that further systematic retrospective as well as prospective studies with associated clinical follow-up information may permit the extension and modification of the concept we proposed in these EmGD studies and that the future advances may lead to improved pathologic diagnosis and clinical management.

Acknowledgment

The authors sincerely thank Dr. Deborah Dillon (Department of Pathology, Yale University School of Medicine, Connecticut) for her assistance in laser-capture microdissection, Drs. Michael Pearl and Jay Bock (SUNY at Stony Brook, New York) for their critical review and insightful discussions.

References

1. Lauchlan SC. Tubal (serous) carcinoma of the endometrium. *Arch Pathol Lab Med* 105:615–618, 1981
2. Hendrickson M, Ross J, Eifel P, Martinez A, Kempson R. Uterine papillary serous carcinoma: A highly malignant form of endometrial adenocarcinoma. *Am J Surg Pathol* 6:93–108, 1982
3. Carcangiu ML, Chambers JT. Uterine papillary serous carcinoma: A study of 108 cases with emphasis on the prognostic significance of associated endometrioid carcinoma, absence of invasion, and concomitant ovarian carcinoma. *Gynecol Oncol* 47:298–305, 1992
4. Berchuck A, Kohler MF, Marks JR, Wiseman R, Boyd J, Bast RCJ. The p53 tumor suppressor gene frequently is altered in gynecologic cancers. *Am J Obstet Gynecol* 170:246–252, 1994
5. Zheng W, Cao P, Zheng M, Kramer EE, Godwin T. p53 and bcl-2 expression in endometrial adenocarcinoma. Comparison of serous and endometrioid subtypes (see comments). *Gynecol Oncol* 61:167–174, 1996
6. Kovalev S, Marchenko ND, Gugliotta BG, Chalas E, Chumas J, Moll UM. Loss of p53 function in uterine papillary serous carcinoma. *Hum Pathol* 29:613–619, 1998
7. Niederacher D, An HX, Camrath S, Dominik SI, Gohring UJ, Oertel A, Grass M, Hantschmann P, Lordnejad MR, Beckmann MW. Loss of heterozygosity of BRCA1, TP53 and TCRD markers analysed in sporadic endometrial cancer. *Eur J Cancer* 34:1770–1776, 1998
8. Sirchia SM, Sironi E, Grati FR, Serafini P, Garagiola I, Rossella F, Dulcetti F, Pardi G, Garsia S, Simoni G. Losses of heterozygosity in endometrial adenocarcinomas: Positive correlations with histopathological parameters. *Cancer Genet Cytogenet* 121:156–162, 2000
9. Bur ME, Perlman C, Edelmann L, Fey E, Rose PG. p53 expression in neoplasms of the uterine corpus. *Am J Clin Pathol* 98:81–87, 1992
10. Kohler MF, Berchuck A, Davidoff AM, Humphrey PA, Dodge RK, Iglehart JD, Clarke-Pearson DL, Bast RC, Marks JR. Overexpression and mutation of p53 in endometrial carcinoma. *Cancer Res* 52:1622–1627, 1992
11. King GI, Adas AA, Livolsi VA, Takahashi H, Behbaht K, McGovern P, Benjamin I, Rubin SC, Boyd J. Expression and mutational analysis of the p53 gene in uterine papillary serous carcinoma. *Cancer* 75:2700–2705, 1995
12. Sherman M, Bur ME, Kurman RJ. p53 in endometrial cancers and its putative precursors: Evidence for diverse pathways of tumorigenesis. *Hum Pathol* 26:1268–1274, 1995
13. Tashiro H, Isacson C, Levine R, Kurman RJ, Cho KR, Hedrick L. p53 gene mutations are common in uterine serous carcinoma and occur early in their pathogenesis. *Am J Pathol* 150:177–185, 1997

14. Zheng W, Khurana R, Farahmand S, Wang Y, Zhang ZF, Felix JC. p53 immunostaining as a significant diagnostic marker for uterine surface carcinoma—precursor lesion of uterine papillary serous carcinoma. *Am J Surg Pathol* 22:1463–1473, 1998
15. Todesca-Bancher D, Gitsch G, Williams KE, Kohlberger P, Neunteufel W, Obermair A, Heinze G, Breitenecker G, Hacker NF. p53 Protein overexpression: A strong prognostic factor in uterine papillary serous carcinoma. *Gynecologic Oncology* 71:59–63, 1998
16. Sung J, Quddus M, Zheng Y, Zhang ZF, Lauchlan SC, Zheng W. p53 serves as a significant prognostic marker in endometrial carcinomas. *Int J Gynecol Cancer* 10:119–127, 2000
17. Ragnarsson G, Eiriksdottir G, Johannsdottir J, Jonasson J, Egilsson V, Ingvarsson S. Loss of heterozygosity at chromosome 1p in different solid human tumours: Association with survival. *Br J Cancer* 79:1468–1474, 1999
18. Arlt MF, Herzog TJ, Mutch DG, Gersell DJ, Liu H, Goodfellow PL. Frequent deletion of chromosome 1p sequences in an aggressive histologic subtype of endometrial cancer. *Hum Mol Genet* 5:1017–1021, 1996
19. Herzog TJ, Kowalski LD, Liu H, Arlt M, Swisher E. Evaluation of a region on chromosome 1p in ovarian serous carcinoma that is frequently deleted in uterine papillary serous carcinoma. *Gynecol Oncol* 82:139–142, 2001
20. Caduff RE, Newman-Svoboda S, Bartos R, Ferguson A, Frank T. Comparative analysis of histologic homologues of endometrial and ovarian carcinoma. *Am J Surg Pathol* 22:319–326, 1998
21. Bandera C, Muto M, Schorge J, Berkowitz R, Rubin S, Mok S. BRCA1 gene mutations in women with papillary serous carcinoma of the peritoneum. *Obstet Gynecol* 92:596–600, 1998
22. Hornreich G, Beller U, Lavie O, Renbaum P, Cohen Y, Levy-Lahad E. Is uterine serous papillary carcinoma a BRCA1-related disease? Case report and review of the literature. *Gynecol Oncol* 75:300–304, 1999
23. Tritz D, Pieretti M, Turner S, Powell D. Loss of heterozygosity in usual and special variant carcinomas of the endometrium. *Hum Pathol* 28:607–612, 1997
24. Matias-Guiu X, Catusus L, Bussaglia E, Lagarda H, Garcia A, Pons C, Munoz J, Arguelles R, Machin P, Prat J. Molecular pathology of endometrial hyperplasia and carcinoma. *Hum Pathol* 32:569–577, 2001
25. Lax SF, Kendall B, Tashiro H, Slebos RJ, Hedrick L. The frequency of p53, K-ras mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: Evidence of distinct molecular genetic pathways. *Cancer* 88:814–824, 2000
26. An HJ, Lee YH, Cho NH, Shim JY, Kim JY, Lee C, Kim SJ. Alteration of PTEN expression in endometrial carcinoma is associated with down-regulation of cyclin-dependent kinase inhibitor. *p27. Histopathology* 41:437–445, 2002
27. Sherman ME. Theories of endometrial carcinogenesis: A multidisciplinary approach. *Mod Pathol* 13:295–308, 2000
28. Diaz-Cano SJ, Blanes A, Wolfe HJ. PCR techniques for clonality assays. *Diagn Mol Pathol* 10:24–33, 2001
29. Muto MG, Welch WR, Mok SC, Bandera CA, Fishbaugh PM, Tsao SW, Lau CC, Goodman HM, Knapp RC, Berkowitz RS. Evidence for a multifocal origin of papillary serous carcinoma of the peritoneum. *Cancer Res* 55:490–492, 1995
30. Shenson DL, Gallion HH, Powell DE, Pieretti M. Loss of heterozygosity and genomic instability in synchronous endometrioid tumors of the ovary and endometrium. *Cancer* 76:650–657, 1995
31. Fujita M, Enomoto T, Wada H, Inoue M, Okudaira Y, Shroyer KR. Application of clonal analysis. Differential diagnosis for synchronous primary ovarian and endometrial cancers and metastatic cancer. *Am J Clin Pathol* 105:350–359, 1996
32. Fujii H, Matsumoto T, Yoshida M, Furugen Y, Takagaki T, Iwabuchi K, Nakata Y, Takagi Y, Moriya T, Ohtsuji N, Ohtsuji M, Hirose S, Shirai T. Genetics of synchronous uterine and ovarian endometrioid carcinoma: Combined analyses of loss of heterozygosity, PTEN mutation, and microsatellite instability. *Hum Pathol* 33:421–428, 2002
33. Deng G, Lu Y, Zlotnikov G, Thor AD, Smith HS. Loss of heterozygosity in normal tissue adjacent to breast carcinomas. *Science* 274:2057–2059, 1996
34. Cheng L, Bostwick DG, Li G, Wang Q, Hu N, Vortmeyer AO, Zhuang Z. Allelic imbalance in the clonal evolution of prostate carcinoma. *Cancer* 85:2017–2022, 1999
35. Takamochi K, Ogura T, Suzuki K, Kawasaki H, Kurashima Y, Yokose T, Ochiai A, Nagai K, Nishiwaki Y, Esumi H. Loss of heterozygosity on chromosomes 9q and 16p in atypical adenomatous hyperplasia concomitant with adenocarcinoma of the lung. *Am J Pathol* 159:1941–1948, 2001
36. Maitra A, Wistuba I, Washington C, Virmani AK, Ashfaq R, Milchgrub S, Gazdar AF, Minna JD. High-resolution chromosome 3p allelotyping of breast carcinomas and precursor lesions demonstrates frequent loss of heterozygosity and a discontinuous pattern of allele loss. *Am J Pathol* 159:119–130, 2001
37. Maitra A, Gazdar AF, Moore TO, Moore AY. Loss of heterozygosity analysis of cutaneous melanoma and benign melanocytic nevi: Laser capture microdissection demonstrates clonal genetic changes in acquired nevocellular nevi. *Hum Pathol* 33:191–197, 2002
38. Mutter GL, Baak JP, Crum CP, Richart RM, Ferenczy A, Faquin WC. Endometrial precancer diagnosis by histopathology, clonal analysis, and computerized morphometry. *J Pathol* 190:462–469, 2000

39. Zheng W, Liang SX, Yu H, Rutherford T, Chambers SK, Schwartz PE. Endometrial glandular dysplasia, a putative precursor lesion of uterine papillary serous carcinoma: A morphological study. *Intl J Surg Pathol* 12:207–223, 2004
40. Kurman RJ, Zaino RJ, Norris HJ. Endometrial carcinoma. In Blaustein's Pathology of the female genital tract, Kurman RJ (ed), 4th ed. Springer-Verlag, New York, pp 439–486, 1994
41. Dillon DA, Zheng K, Costa J. Rapid, efficient genotyping of clinical tumor samples by laser-capture microdissection/PCR/SSCP. *Exp Mol Pathol* 70:195–200, 2001
42. Gu J, Roth LM, Younger C, Michael H, Abdul-Karim FW, Zhang S, Ulbright TM, Eble JN, Cheng L. Molecular evidence for the independent origin of extra-ovarian papillary serous tumors of low malignant potential. [see comments]. *J Natl Cancer Inst* 93:1147–1152, 2001
43. Cheng L, Song S-Y, Pretlow TG, Abdul-Karim F, Kung H-J, Dawson DV, Park W-S, Moon Y-W, Tsai M-L, Linehan M, Emmert-Buck MR, Liotta LA, Zhuang Z. Evidence of independent origin of multiple tumors from patients with prostate cancer. *J Natl Cancer Inst* 90:233–242, 1998
44. Jass JR, Barker M, Fraser L, Walsh MD, Whitehall VL, Gabrielli B, Young J, Leggett BA. APC mutation and tumour budding in colorectal cancer. *J Clin Pathol* 56:69–73, 2003
45. Peiro G, Diebold J, Lohse P, Ruebsamen H, Lohse P, Baretton GB, Lohrs U. Microsatellite instability, loss of heterozygosity, and loss of hMLH1 and hMSH2 protein expression in endometrial carcinoma. *Hum Pathol* 33:347–354, 2002
46. Zhuang Z, Merino MJ, Chuaqui R, Liotta LA, Emmert-Buck MR. Identical allelic loss on chromosome 11q13 in microdissected *in situ* and invasive human breast cancer. *Cancer Res* 55:467–471, 1995
47. Caduff RE, Svoboda-Neumann SM, Johnston CM, Bartos RE, Frank TS. Molecular analysis in endometrial cancer. *Verh Dtsch Ges Pathol* 81:219–227, 1997
48. Weinberg RA. Tumor suppressor genes. *Science* 254:1138–1146, 1991
49. Faulkner SW, Leigh DA, Oosterhuis JW, Roelofs H, Looijenga LH, Friedlander ML. Allelic losses in carcinoma *in situ* and testicular germ cell tumours of adolescents and adults: Evidence suggestive of the linear progression model. *Br J Cancer* 83:729–736, 2000
50. Luttges J, Galehdari H, Brocker V, Schwarte-Waldhoff I, Henne-Bruns D, Kloppel G, Schmiegel W, Hahn SA. Allelic loss is often the first hit in the biallelic inactivation of the p53 and DPC4 genes during pancreatic carcinogenesis. *Am J Pathol* 158:1677–1683, 2001
51. Guo Z, Hu X, Afink G, Ponten F, Wilander E, Ponten J. Comparison of chromosome 3p deletions between cervical precancers synchronous with and without invasive cancer. *Intl J Cancer* 86:518–523, 2000
52. Ambros RA, Sherman ME, Zahn CM, Bitterman P, Kurman RJ. Endometrial intraepithelial carcinoma: A distinctive lesion specifically associated with tumors displaying serous differentiation. *Hum Pathol* 26:1260–1267, 1995
53. Lavie O, Hornreich G, Arie AB, Renbaum P, Levy-Lahad E, Beller U. BRCA-1 germline mutations in women with uterine serous papillary carcinoma. *Obstet Gynecol* 96:28–32, 2000
54. Miturski R, Bogusiewicz M, Ciotta C, Bignami M, Gogacz M, Burnour D. Mismatched repair genes and microsatellite instability as molecular markers for gynecological cancer detection. *Exp Biol Med (Maywood)* 227:579–586, 2002
55. Zheng W, Liang SX, Yu H, Rutherford T, Chambers SK, Schwartz PE. Endometrial glandular dysplasia: A newly defined precursor lesion of uterine papillary serous carcinoma. Part I: morphologic features. *IJSP* 12:207–223, 2004