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Allelic Loss of Chromosome 16q in Endometrial Cancer: Correlation with Poor Prognosis of Patients and Less Differentiated Histology

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Deletion of certain chromosomal regions can be demonstrated in malignant cells. Chromosome 16q is one of the regions where allelic loss is frequently detected in carcinoma of the breast and many other tumors, suggesting that gene(s) which retard tumor growth may exist here. To elucidate the clinicopathological significance of chromosome 16q, loss of heterozygosity (LOH) was investigated using microsatellite polymorphism analysis in 58 patients with endometrial lesions (50 with endometrial carcinoma and 8 who had hyperplasia with or without atypia). When 11 regions of chromosome 16q were examined, LOH was found in 20 patients with carcinoma (40%) and none of the patients with hyperplasia. The tumors of 9 of the 20 patients (45%) showed total loss of 16q, while the others (55%) showed partial deletion. Tumors with LOH were histologically less differentiated than those without LOH ($P=0.038$, χ^2 test). Patients with tumors showing LOH of 16q had a worse prognosis than those without LOH according to Kaplan-Meier survival analysis ($P=0.0158$, log-rank test). In addition, LOH of 16q showed a significant relationship to prognosis by Cox regression analysis. Deletion mapping of 16q demonstrated that two regions (16q22.1 and 16q22.2–23.1) were frequently involved. Patients with 16q22.1 LOH had a poorer prognosis than those with intact 16q22.1 ($P=0.0003$, log-rank test). These findings suggest that gene(s) of which defect is possibly related to the aggressiveness of endometrial cancer are localized on a limited region of 16q that includes 16q22.1.

Key words: Endometrial cancer — Loss of heterozygosity — Chromosome 16q — Prognostic factor

Endometrial cancer is the most common neoplasm of the female reproductive tract in the United States, and its incidence is currently increasing in Europe and Japan.[1] Although the basis of endometrial carcinogenesis is largely unknown, uncontrolled growth of transformed cells is generally understood to result from the accumulation of certain genetic changes, including mutational activation of proto-oncogenes and/or lack of tumor suppressor genes.[2,3] Based on clinicopathological studies, endometrial adenocarcinoma, the most common histological phenotype of endometrial cancer, often appears to be preceded by a precancerous lesion termed atypical hyperplasia.[1] However, the actual risk of adenocarcinoma developing from atypical hyperplasia is still controversial. After adenocarcinoma develops, it may progress to invasion and subsequent metastasis through a series of changes in histological stage and/or grade. Substantial evidence supports the multistep carcinogenesis hypothesis with respect to endometrial tumorigenesis. Several different groups have suggested that K-*ras* mutation occurs at a very early stage in endometrial tumorigenesis,[4,5] while *p53* mutation, allelic loss of chromosome 17p, and amplification/overexpression of c-*erb*B-2/HER-2/*neu* are thought to be involved in the progression of endometrial carcinoma.[6–8]

Loss of heterozygosity (LOH), which may be the most common genetic alteration in human epithelial tumors, does not always indicate a specific change, since it may occur at several random chromosome regions during any stage of tumorigenesis. However, LOH indicating certain allelic losses on chromosomes is a non-random change in tumor cells. Among a number of genes localized in regions of LOH, some have been identified and characterized as tumor suppressor genes, and it seems possible that many more genes of the same class may exist at these loci.[2] In the case of endometrial cancer, significant allelic loss has been described at chromosomes 3p, 8p, 9p, 10q, 14q, 16q, 17p, and 18q. These LOHs were surveyed using conventional restriction fragment length polymorphism analysis.[6,9] In recent years, the more convenient method of polymerase chain reaction (PCR)-based microsatellite polymorphism analysis has allowed LOH to be examined precisely and the deleted regions can be localized easily.[10–12] In the present study, we focused on allelic loss of chromosome 16q, because LOH of this chromosome is frequently involved in certain neoplasms, including carcinoma of the breast, prostate, ovary, and liver.[13–16] We analyzed the allelic loss of 16q in patients with various grades of endometrial carcinoma and in patients with

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endometrial hyperplasia using PCR-based microsatellite polymorphism analysis. Employing 11 different markers for chromosome 16q, we found two commonly deleted regions that were correlated with clinicopathological features such as tumor histology and prognosis.

MATERIALS AND METHODS

Tissue samples and DNA extraction Tumor and nontumor tissue samples were obtained from the formalin-fixed and paraffin-embedded surgical materials of 58 patients who underwent operation at Ehime University Hospital (Ehime) between January 1984 and December 1991. Fifty patients had endometrial cancer (43 with adenocarcinoma, 3 with adenosquamous carcinoma, 3 with serous papillary carcinoma, and 1 with clear cell adenocarcinoma) and the other 8 patients had endometrial hyperplasia (4 with and 4 without atypia). Prior to surgical resection, neither radiation therapy nor chemotherapy was performed. All carcinomas were staged and graded according to the surgical staging method of the International Federation of Gynecology and Obstetrics and the World Health Organization histological classification, respectively,[17, 18] except for serous papillary carcinoma.[19] DNA was extracted as described elsewhere.[20] In brief, tissue which microscopically contained more than 60% tumor cells was trimmed from formalin-fixed, paraffin-embedded blocks using a razor blade and was cut into 50 μm sections. The sections were suspended in TE9 (500 μM Tris, 20 mM EDTA, 10 mM NaCl, pH 9.0) buffer with 1% sodium dodecyl sulfate (SDS) and 500 μg/ml proteinase K (Sigma, St. Louis, MO), and were incubated for 24 h at 48°C. Then the mixture was incubated for another 48 h after adding 500 μg/ml proteinase K and 1% SDS. Subsequently, DNA was extracted four times using one volume of phenol-chloroform solution, and then precipitated by ethanol and sodium chloride.

Microsatellite analysis Paired normal and tumor DNA specimens were subjected to the PCR using primers for the following 11 microsatellite loci: D16S285 (16q12), D16S390 (16q12.1–13), D16S389 (16q21), D16S301 (16q22.1), D16S347 (16q22.1), D16S318 (16q22.1), D16S450 (16q22.2–23.1), D16S395 (16q23.2–24.2), D16S289 (16q23.1–24.2), D16S393 (16q23.2–24.3), and D16S305 (16q24.3) (Table I).[21] For each PCR primer pair, two DNAs showing different levels of homozygosity were mixed and incubated in a dilution assay (data not shown). The number of PCR cycles for each primer pair was determined so as to allow detection of the allelic loss of each locus. Template genomic DNA (50 ng) was amplified for 27 cycles (exceptionally, for 35 cycles in D16S347) at the recommended annealing temperature for each primer and radiolabelled during PCR cycles in a 20 μl reaction mixture using an Astec 3000 Thermal Cycler (Fukuoka). The PCR product was diluted threefold with loading buffer and denatured at 90°C for 5 min. Then, the product was separated by electrophoresis and visualized by autoradiography. For informative cases, the intensity of the radiographic signal of heterozygous alleles in both tumor and normal DNA was measured by densitometry. Allelic loss was considered to have occurred when the ratio of these two alleles in the tumor DNA was less than 50% of that of the corresponding normal DNA.

Table I. Incidence of LOH on Chromosome 16q in Endometrial Carcinoma Detected by 11 Microsatellite Markers

Locus	Location	Incidence of LOH (%) Loss in tumor/ Informative cases
D16S285	16q12	3/20 (15)
D16S390	16q12.1–13	12/35 (34)
D16S389	16q21	4/26 (15)
D16S347	16q22.1	12/34 (35)
D16S318	16q22.1	5/12 (42)
D16S301	16q22.1	12/37 (32)
D16S450	16q22.2–23.1	17/36 (47)
D16S395	16q23.2–24.2	9/36 (25)
D16S289	16q23.1–24.2	12/39 (31)
D16S393	16q23.2–24.3	10/33 (30)
D16S305	16q24.3	7/22 (32)
Total		20/50 (40)

Statistical analysis The χ^2 test was used for comparison of LOH of chromosome 16q and several clinicopathological parameters, with one-sided P values being calculated. Kaplan-Meier survival curves were drawn to estimate the prognostic value of 16q LOH,[22] and differences in survival were compared by the means of the log-rank test and the generalized Wilcoxon test. The mean follow-up period was 76.9 months (range: 2–213 months). LOH of 16q was compared with various clinicopathologic factors related to prognosis (tumor stage, histologic differentiation, and uterine muscular invasion) by Cox proportional hazards analysis.[23] Lymph node metastasis was excluded from this analysis because of its close association with tumor stage.

RESULTS

Incidence of LOH and localization on 16q DNA from both abnormal and normal tissue of the 58 patients was examined using 11 CA repeat microsatellite markers for chromosome 16q, and allelic loss was found in 20 (40%) out of 50 patients with endometrial cancer (Fig. 1) (Table I). The region 16q22.2–23.1 was most frequently involved, being deleted in 17 (47%) of 36 informative cases (Table I). The results of chromosomal mapping of

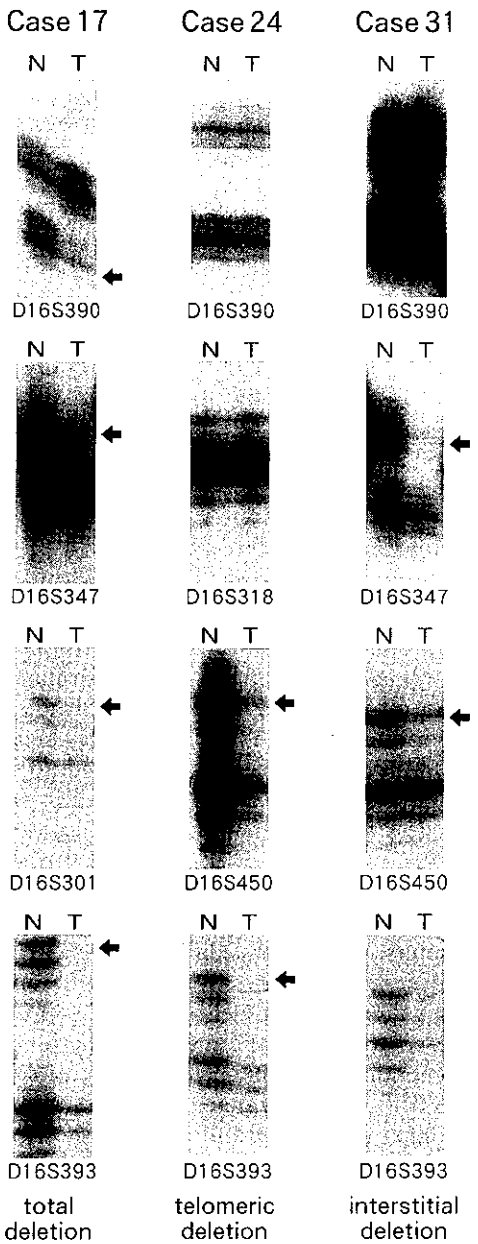

Fig. 1. Microsatellite analysis of LOH of chromosome 16q in endometrial cancer. Representative examples of total, centromeric, and interstitial deletion are presented (cases 17, 24, and 31, respectively). Marker loci are shown under each panel. N, DNA from normal tissue; T, DNA from tumor tissue; arrow, allele showing LOH. See also Fig. 2.

the deleted regions in these 20 patients are shown in Fig. 2. Nine of the patients (45%) had tumors with loss of the entire arm of 16q, and the other 11 (55%) had tumors that showed partial deletion (6 internal deletions and 5 telomeric deletions). To determine the extent of the commonly deleted regions of 16q, all deleted regions of each case were mapped (Fig. 2). It was found that the 16q22.2–23.1 region (D16S450) was most commonly deleted. The second most commonly deleted region was 16q22.1, which was detected using three microsatellite markers, D16S347, D16S318 and D16S301. The positional order of these three markers in 16q22.1 has not yet been precisely determined. Therefore, it remains uncertain whether the two frequently involved regions of 16q22.1 and 16q22.2–23.1 are close enough to be involved together or whether they were affected independently of each other.

Clinicopathological significance of allelic loss on chromosome 16q The relationship between allelic loss of 16q and various clinicopathological findings (tumor stage, histological grade, myometrial invasion, and lymph node metastasis) or the duration of survival is summarized in Table II and Fig. 3, respectively. Patients without 16q deletion had better differentiated tumors ($P=0.038$, χ^2 test) (Table II) and a better prognosis ($P=0.0158$, log-rank test; $P=0.0038$, generalized Wilcoxon test) (Fig. 3A). Among the 44 tumors informative at region 16q22.1, those with 16q22.1 deletion were associated with a worse prognosis than tumors with intact 16q22.1 ($P=0.0003$, log-rank test; $P=0.0005$, generalized Wilcoxon test) (Fig. 3B). Among the 36 tumors informative at D16S450 (16q22.2–23.1), those with 16q22.2–23.1 deletion were associated with a worse prognosis than tumors with intact 16q22.2–23.1 ($P=0.0382$, log-rank test; $P=0.0376$, generalized Wilcoxon test) (data not shown). Multivariate analysis using the Cox proportional hazards model revealed that 16q LOH showed a stronger correlation with poor prognosis than any other clinicopathological factor, including tumor stage, histological grade, and myometrial invasion (Table III). Exceptionally, the *P*-value of tumor stage was significant when either histological grade or myometrial invasion was excluded from the analysis (data not shown).

DISCUSSION

We performed microsatellite analysis of chromosome 16q in patients with endometrial lesions, and found LOH in 40% of the patients with endometrial carcinoma but in none of those with endometrial hyperplasia. We found a higher incidence of LOH than that reported previously, which has generally ranged between 18 and 23%,[10,12] except for a report of 0% by Jones *et al.*[11] This difference may be partly because fewer microsatellite markers were used in most other studies compared with our 11 markers covering almost the entire arm of chromosome 16q (Table I). We used a lower number of PCR cycles in microsatellite analysis and a lower intensity ratio be-

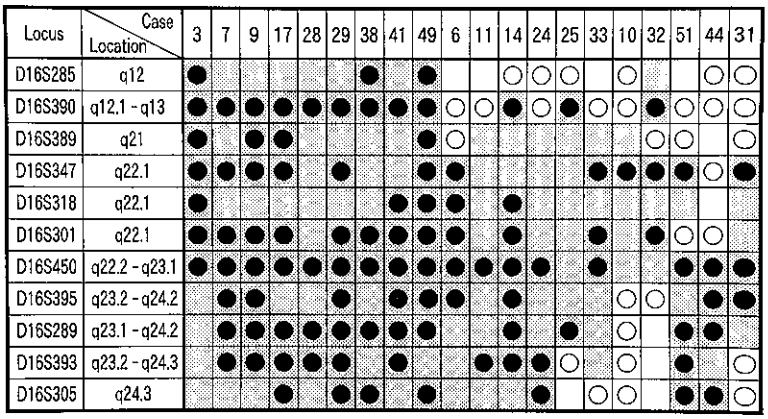

Fig. 2. Deletion map of chromosome 16q in endometrial cancer. Markers and map positions are illustrated on the left and case numbers are given at the top. Closed circles: loss of heterozygosity; open circles: retention of heterozygosity; no symbol: not informative for heterozygosity. Shaded regions show the maximum possible extent of the deletion.

Table II. Association of LOH of Chromosome 16q with Various Clinicopathological Features of Endometrial Carcinoma

Variable	16q LOH present (%)	16q LOH absent	$P^{a)}$
Stage			
I, II	13 (34)	25	NS[b]
III	5 (50)	5	
IV	2 (100)	0	
Histology			
adenocarcinoma	15 (35)	28	
grade 1	7 (24)	22	
grade 2	4 (44)	5	0.038
grade 3	4 (80)	1	
grade 2+grade 3	8 (57)	6	
adenosquamous	2 (67)	1	
clear cell	0	1	
serous papillary	3 (100)	0	
Myometrial invasion			
limited to endometrium	5 (56)	4	NS
<1/2	3 (23)	10	
>1/2	9 (39)	14	
serosal invasion	3 (60)	2	
Lymph nodal metastasis			
positive	4 (67)	2	NS
negative	16 (36)	28	

a) Statistical significance was calculated with the χ^2 test.
b) NS: not significant.

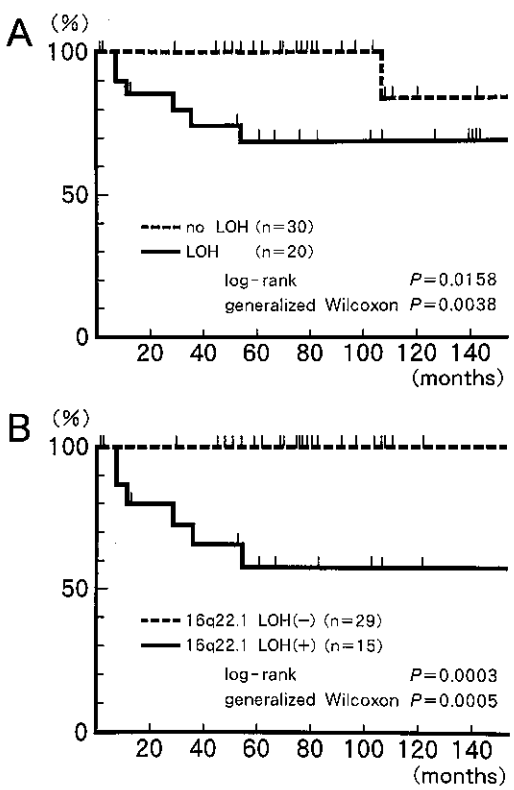

Fig. 3. A, Kaplan-Meier survival curves for patients with and without LOH of chromosome 16q. LOH: loss of heterozygosity of chromosome 16q; no LOH: retention of both alleles of chromosome 16q. Follow-up periods for the patients are indicated by vertical bars above the survival curves. LOH of chromosome 16q was associated with a worse prognosis than no LOH ($P=0.0158$, log-rank test; $P=0.0038$, generalized Wilcoxon test). B, Kaplan-Meier survival curves for 44 patients informative at the 16q22.1 region. LOH of 16q22.1 was associated with a worse prognosis than no LOH ($P=0.0003$, log-rank test; $P=0.0005$, generalized Wilcoxon test).

tween tumor and normal DNA bands for determination of positive LOH than those used in other studies, and these are also possible reasons for our higher incidence of 16q LOH.[24] The incidence of 40% was the sum of complete 16q deletion (18%) and partial deletion (22%), and the former incidence is close to that reported by others.[10, 12]

By mapping the deletions of chromosome 16q, three limited regions (16q22.1, 16q22.2–q24.2, and 16q24.3)

Table III. Cox Proportional Hazards Analysis Comparing Prognostic Factors Including 16q LOH with Survival in 50 Patients with Endometrial Carcinoma

Variable	No. of variables	*P* value	Odds ratio
16q LOH	2	0.041	10.50
Stage[a)]	6	0.057	16.60
Histology[b)]	3	0.385	2.31
Myometrial invasion[c)]	4	0.739	1.58

a) Stage was classified into Ia, Ib, Ic, II, III and IV.
b) Histology was classified into three groups: grade 1 endometrioid adenocarcinoma; grade 2; grade 3 and non-adenocarcinoma histology combined.
c) Muscular invasion was classified as follows: tumor limited to the endometrium, invasion of the inner or outer half of the myometrium, and invasion to the serosa.

have been shown to be commonly deleted in human breast cancer.[25-27] These regions were also affected in the endometrial carcinomas we examined using 8 markers: D16S347 (region 16q22.1), D16S318 (16q22.1), D16S301 (16q22.1), D16S450 (16q22.2–q23.1), D16S395 (16q23.2–q24.2), D16S289 (16q23.1–q24.2), D16S393 (16q23.2–q24.3), and D16S305 (16q24.3) (Fig. 2). The most commonly affected region lay between 16q22.1 (D16S318) and 16q22.2–q23.1 (D16S450). However, it is presently unclear whether 16q22.1 and 16q22.2–23.1 are discrete or not, and whether both regions have equal clinical importance. Our study did not provide sufficient evidence to answer these questions, although two patients showed intact alleles at the D16S301 locus (cases 51 and 44 in Fig. 2). However, both regions are often targeted in several other cancers, including hepatocellular carcinoma and adenocarcinoma of the prostate and ovary.[14-16]

Deletion of 16q may not be responsible for endometrial tumorigenesis, because LOH was detected in only 40% of cancer patients, and in none of those with hyperplasia (Table I). However, it is conceivable that 16q LOH is associated with tumor progression, because its incidence was increased in patients with less differentiated tumors and with a poorer prognosis (Table II) (Fig. 3A). To determine the surgical stage and pathological grade of endometrial carcinoma, several clinicopathological findings are carefully examined. Unfortunately, even when an early stage tumor is curatively resected, distant metastasis is often found later. Thus, it is clinically essential to establish an accurate marker for the metastatic potential of this carcinoma. At present, genetic alterations, which are supposed to be correlated with tumor aggressiveness, are not generally utilized as a prognostic marker in patients with endometrial carcinoma. Our present study indicates that among clinicopathological factors, tumor stage and 16q LOH independently correlated with patients' prognosis. However, 16q LOH was the most accurate and significant predictor of poor prognosis among all the factors we assessed (Table III).

Among the several deletions of chromosome 16q, loss of 16q22.1 was significantly correlated with survival (Fig. 3B). Although the gene(s) involved have not yet been identified, several candidates exist, including *E-cadherin*. The cadherin protein is considered to strengthen cell-to-cell adhesiveness, so its loss might weaken intercellular contact and promote the migration of tumor cells away from the primary site. Thus, lack of E-cadherin expression is thought to increase metastatic potential.[28,29] However, the *E-cadherin* gene is largely intact in endometrial or breast carcinoma, even in patients with absent or decreased E-cadherin expression.[30,31] While another regulatory mechanism for the inactivation of *E-cadherin* is still being sought, hypermethylation of the CpG island in the promotor region has been demonstrated in carcinoma of the breast and prostate.[32,33] Therefore, *E-cadherin* is a plausible candidate among the genes localized on 16q22.1 and adjacent regions. In addition, three other genes are candidates, including the breast basic conserved (*BBC1*) gene,[34] the cell adhesion regulator (*CAR*) gene,[35] and *M-cadherin*.[36] However, LOH between 16q23.2 and 16q24.3 regions might be less specific than LOH of 16q22.1 and 16q22.2–q23.1 in endometrial cancer, since the frequency was lower and corresponded less well with the clinical outcome.

In conclusion, LOH of 16q appears to occur during the progression of endometrial carcinoma. Further studies need to be performed to characterize the genes localized at 16q22.1 and adjacent regions. Such studies may help to identify gene(s) that modulate the growth of human endometrial cancer, and that have specific prognostic implications.

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REFERENCES

1) Kurman, R. J., Zaino, R. J. and Norris, H. J. Endometrial hyperplasia and related cellular changes; endometrial carcinoma. *In* "Blaustein's Pathology of the Female Genital Tract," ed. R. J. Kurman, pp. 411–486 (1994). Springer-

Verlag, New York.
2) Bishop, J. M. Molecular themes in oncogenesis. *Cell,* **64,** 235–248 (1991).
3) Fearon, E. R. and Vogelstein, B. A genetic model for colorectal carcinogenesis. *Cell,* **61,** 759–767 (1990).
4) Enomoto, T., Inoue, M., Perantoni, A. O., Buzard, G. S., Miki, H., Tanizawa, O. and Rice, J. M. K-*ras* activation in premalignant and malignant epithelial lesions of the human uterus. *Cancer Res.,* **51,** 5308–5314 (1991).
5) Sasaki, H., Nishii, H., Takahashi, H., Tada, A., Furusato, M., Terashima, Y., Siegal, G. P., Parker, S. L., Kohler, M. F., Berchuck, A. and Boyd, J. Mutation of the Ki-*ras* protooncogene in human endometrial hyperplasia and carcinoma. *Cancer Res.,* **53,** 1906–1910 (1993).
6) Okamoto, A., Sameshima, Y., Yamada, Y., Teshima, S., Terashima, Y., Terada, M. and Yokota, J. Allelic loss on chromosome 17p and *p53* mutations in human endometrial carcinoma of the uterus. *Cancer Res.,* **51,** 5632–5636 (1991).
7) Kihana, T., Hamada, K., Inoue, Y., Yano, N., Iketani, H., Murao, S., Ukita, M. and Shumpei, M. Mutation and allelic loss of the *p53* gene in endometrial carcinoma: incidence and outcome in 92 surgical patients. *Cancer,* **76,** 72–78 (1995).
8) Berchuck, A., Rodriguez, G., Kinney, R. B., Soper, J. T., Dodge, R. K., Clarke-Pearson, D. L. and Bast, R. C. Overexpression of HER-2/*neu* in endometrial cancer is associated with advanced stage disease. *Am. J. Obstet. Gynecol.,* **164,** 15–21 (1991).
9) Imamura, T., Arima, T., Kato, H., Miyamoto, S., Sasazuki, T. and Wake, N. Chromosomal deletions and K-*ras* gene mutations in human endometrial carcinomas. *Int. J. Cancer,* **51,** 47–52 (1992).
10) Fujino, T., Risinger, J. I., Collins, N. K., Liu, F., Nishii, H., Takahashi, H., Westphal, E.-M., Barrett, J. C., Sasaki, H., Kohler, M. F., Berchuck, A. and Boyd, J. Allelotype of endometrial carcinoma. *Cancer Res.,* **54,** 4294–4298 (1994).
11) Jones, M. H., Koi, S., Fujimoto, I., Hasumi, K., Kato, K. and Nakamura, Y. Allelotype of uterine cancer by analysis of RFLP and microsatellite polymorphisms: frequent loss of heterozygosity on chromosome 3p, 9q, 10q, and 17p. *Genes Chromosomes Cancer,* **9,** 119–123 (1994).
12) Peiffer, S., Herzog, T. J., Tribune, D. J., Mutch, D. G., Gersell, D. J. and Goodfellow, P. J. Allelic loss of sequences from the long arm of chromosome 10 and replication errors in endometrial cancers. *Cancer Res.,* **55,** 1922–1926 (1995).
13) Sato, T., Tanigami, A., Yamakawa, K., Akiyama, F., Kasumi, F., Sakamoto, G. and Nakamura, Y. Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. *Cancer Res.,* **50,** 7184–7189 (1990).
14) Carter, B. S., Ewing, C. M., Ward, W. S., Treiger, B. F., Aalders, T. W., Schalken, J. A., Epstein, J. I. and Issaacs, W. B. Allelic loss of chromosome 16q and 10q in human prostate cancer. *Proc. Natl. Acad. Sci. USA,* **87,** 8751–8755 (1990).
15) Sato, T., Saito, H., Morita, R., Koi, S., Lee, J. H. and Nakamura, Y. Allelotype of human ovarian cancer. *Cancer Res.,* **51,** 5118–5122 (1991).
16) Tsuda, H., Zhang, W., Shimosato, Y., Yokota, J., Terada, M., Sugimura, T., Miyamura, T. and Hirohashi, S. Allele loss on chromosome 16 associated with progression of human hepatocellular carcinoma. *Proc. Natl. Acad. Sci. USA,* **87,** 6791–6794 (1990).
17) Creasman, W. T. Announcement, FIGO stages: 1988 Revisions. *Gynecol. Oncol.,* **35,** 125–127 (1989).
18) Poulsen, C. E., Taylor, C. W. and Sobin, L. H. "Histological Typing of Female Genital Tract Tumours," International Histological Classification of Tumors; No.13 (1975). World Health Organization, Geneva.
19) Hendrickson, M., Ross, J., Eifel, P., Martinez, A. and Kempson, R. Uterine papillary serous carcinoma: a highly malignant form of endometrial carcinoma. *Am. J. Surg. Pathol.,* **6,** 93–108 (1982).
20) Kihana, T., Tsuda, H., Teshima, S., Nomoto, K., Tsugane, S., Sonoda, T., Matsuura, S. and Hirohashi, S. Prognostic significance of the overexpression of c-*erb*B-2 protein in adenocarcinoma of the uterine cervix. *Cancer,* **73,** 148–153 (1994).
21) Kozman, H. M., Keith, T. P., Donis-Keller, H., White, R. L., Weissenbach, J., Dean, M., Vergnaud, G., Kidd, K., Gusella, J., Royle, N. J., Sutherland, G. R. and Mulley, J. C. The CEPH consortium linkage map of human chromosome 16. *Genomics,* **25,** 44–58 (1995).
22) Kaplan, E. I. and Meier, P. Nonparametric estimation from incomplete observation. *J. Am. Stat. Assoc.,* **53,** 457–481 (1958).
23) Cox, D. R. Regression models and life-tables. *J. R. Stat. Soc.,* **34,** 187–220 (1972).
24) Bianchi, A. B., Navone, N. M. and Conti, C. J. Detection of loss of heterozygosity in formalin-fixed paraffin-embedded tumor specimens by the polymerase chain reaction. *Am. J. Pathol.,* **138,** 279–284 (1991).
25) Tsuda, H., Callen, D. F., Fukutomi, T., Nakamura, Y. and Hirohashi, S. Allele loss on chromosome 16q24.1-qter occurs frequently in breast cancers irrespectively of differences in phenotype and extent of spread. *Cancer Res.,* **54,** 513–517 (1994).
26) Cleton-Jansen, A.-M., Moerland, E. W., Kuipers-Dijkshoorn, N. J., Callen, D. F., Sutherland, G. R., Hansen, B., Devilee, P. and Cornelisse, C. J. At least two different regions are involved in allelic imbalance on chromosome arm 16q in breast cancer. *Genes Chromosomes Cancer,* **9,** 101–107 (1994).
27) Skirnisdottir, S., Eiriksdottir, G., Baldursson, T., Barkardottir, R. B., Egilsson, V., Ingvarsson, S. High frequency of allelic imbalance at chromosome region 16q22–23 in human breast cancer: correlation with high PgR and low S phase. *Int. J. Cancer,* **64,** 112–116 (1995).
28) Vleminckx, K., Vakaet, L. Jr., Mareel, M., Fiers, W., van

Roy, F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell,* **66,** 107–119 (1991).
29) Oka, H., Shiozaki, H., Kobayashi, K., Inoue, M., Tahara, H., Kobayashi, T., Takatsuka, Y., Matsuyoshi, N., Hirano, S., Takeichi, M. and Mori, T. Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. *Cancer Res.,* **53,** 1696–1701 (1993).
30) Risinger, J. I., Berchuck, A., Kohler, M. F. and Boyd, J. Mutations of the *E-cadherin* gene in human gynecologic cancers. *Nat. Genet.,* **7,** 98–102 (1994).
31) Kanai, Y., Oda, T., Tsuda, H., Ochiai, A. and Hirohashi, S. Point mutation of the E-cadherin gene in invasive lobular carcinoma of the breast. *Jpn. J. Cancer Res.,* **85,** 1035–1039 (1994).
32) Yoshiura, K., Kanai, Y., Ochiai, A., Shimoyama, Y., Sugimura, T. and Hirohashi, S. Silencing of the E-cadherin invasion-suppressor gene by CpG methylation in human carcinoma. *Proc. Natl. Acad. Sci. USA,* **92,** 7416–7419 (1995).
33) Graff, J. R., Herman, J. G., Lapidius, R. G., Chopra, H., Xu, R., Jarrard, D. F., Issaacs, W. B., Pitha, P. M., Davidson, N. E. and Baylin, S. B. E-Cadherin expression is silenced by DNA hypermethylation in human breast and prostate carcinomas. *Cancer Res.,* **55,** 5195–5199 (1995).
34) Adams, S. M., Helps, N. R., Sharp, M. F. G., Brammar, W. J., Walker, R. A., Varley, J. M. Isolation and characterization of a novel gene with differential expression in benign and malignant human breast cancer. *Hum. Mol. Genet.,* **1,** 91–96 (1992).
35) Pullman, W. E. and Bodmer, W. F. Cloning and characterization of a gene that regulates cell adhesion. *Nature,* **356,** 529–532 (1992).
36) Kaupmann, K., Becker-Follmann, J., Scherer, G., Jockusch, H. and Starzinski-Powitz, A. The gene for the cell adhesion molecule M-cadherin maps to mouse chromosome 8 and human chromosome 16q24.1–q ter and is near the E-cadherin (uvomorulin) locus in both species. *Genomics,* **14,** 488–490 (1992).