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Allelotype Analysis of Common Epithelial Ovarian Cancers with Special Reference to Comparison between Clear Cell Adenocarcinoma with Other Histological Types

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Determination of the histological type of epithelial ovarian cancer is clinically important to predict patient prognosis. To estimate accurately the chromosomal regions that frequently show loss of heterozygosity (LOH) in each histological type, LOH at 55 loci on 38 chromosomal arms was examined by means of laser capture microdissection and PCR-LOH analysis in 45 epithelial ovarian cancers composed of clear cell adenocarcinoma (CCA), serous adenocarcinoma (SEA), endometrioid adenocarcinoma (EMA) and mucinous adenocarcinoma (MUA). In addition, *p53* (exons 5–8) gene mutations and the nuclear immunoreactivity of p53 proteins in these tumors were examined by PCR-SSCP and immunohistochemistry. In CCA, LOH was detected primarily on 1p (69%) followed by 19p (45%) and 11q (43%). On the other hand, in SEA, LOH was detected in at least 50% of cases on 1p, 4p, 5q, 6p, 8p, 9q, 12q, 13q, 15q, 16p, 17p, 17q, 18p, 18q, 19p, 20p and Xp. The incidences of LOH on 5q, 12q, 13q and 17p were significantly lower in CCA than in SEA (*P*=0.019, 0.031, 0.0035 and 0.012). EMA showed a tendency for frequent LOH on 7p, whereas MUA showed significantly high occurrence of LOH at 17p13.1. The incidences of *p53* mutation and p53 nuclear immunoreactivity also differed between CCA and SEA: 0% and 7% in the former and 64% and 45% in the latter (*P*=0.0006 and 0.039). These findings clarify that there are differences in LOH distribution patterns among different histological subtypes of epithelial ovarian cancer. In CCA, *p53* tumor-suppressor gene (TSG) is not involved in carcinogenesis and tumor-suppressor genes located on 1p are considered to play an important role in tumor development.

Key words: Loss of heterozygosity — Laser capture microdissection — Histological subtypes — Ovarian cancer

Malignant epithelial ovarian cancers account for 80–90% of all malignancies of the ovary.[1,2] Common epithelial ovarian neoplasms consist of four different histological types: serous adenocarcinoma (SEA), mucinous adenocarcinoma (MUA), endometrioid adenocarcinoma (EMA) and clear cell adenocarcinoma (CCA). These four types also differ clinically in terms of prognosis and response to systemic chemotherapies.[3,4] However, almost all of the previous studies have focused mainly on SEA; few reports have dealt with genetic abnormalities of CCA.

CCA is reported to constitute 5–12% of ovarian adenocarcinomas, and is more frequent in Japanese than in Western countries.[5,6] CCA tends to be resistant to conventional systemic platinum-based chemotherapy, and has a worse prognosis than other histological subtypes.[6–9]

Like many other solid tumors, ovarian carcinoma may develop through a multi-step process involving activation of oncogenes and inactivation of tumor-suppressor genes (TSGs).[10] According to Knudson's hypothesis,[11] the second hit leads to loss of TSG function in tumor cells. Typically, the inactivation of TSGs is detected as allelic loss around the TSG locus by means of identification of loss of heterozygosity (LOH) in tumor DNA compared with normal DNA.

Numerous studies have been performed to examine the extent and variation of LOH at loci on every chromosomal arm in ovarian cancers. This type of analysis is termed an allelotype.[12] In addition to allelotyping, recent studies have focused on identifying LOH on specific chromosomal regions that harbor potentially relevant TSGs in ovarian cancer. They reported that chromosome regions 1p, 4p, 5q, 6p, 6q, 7p, 8p, 8q, 9p, 9q, 11p, 11q, 12p, 12q, 13q, 14q, 15q, 16p, 16q, 17p, 17q, 18q, 19p, 21q, 22q and Xp were frequently lost.[12–21] Mutations of *p53* TSG, located at 17p13.1, are also reported to occur frequently in ovarian cancers.[22] However, almost all of these studies mainly examined serous adenocarcinoma and did not

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focus on differences in LOH distribution patterns among different tumor types. If abnormalities of different TSGs and chromosomal losses play particular roles in the different histological tumor types, the chromosomal loci most frequently showing LOH would differ among these types.

Laser capture microdissection (LCM) is a rapid and reliable method to obtain pure populations of targeted cells from specific microscopic regions of tissue sections for subsequent analysis, particularly by sensitive amplification methods based on polymerase chain reaction (PCR).[23, 24] In the present study we used LCM to collect pure cancer DNA from 45 epithelial ovarian cancers, and identified LOH accurately with a set of 55 polymorphic markers partially representing almost all chromosome arms. To screen tumor-type-specific chromosomal changes, we analyzed the patterns of LOH frequencies in 16 CCAs,13 SEAs, 11 EMAs and 5 MUAs. By focusing especially on a comparison of LOH loci between CCA and SEA, we tried to determine the chromosomal changes that occur characteristically in CCA, the most aggressive type of ovarian cancer.

MATERIALS AND METHODS

Tissue samples Forty-five paired samples of epithelial ovarian cancers and non-cancerous fimbria or uterine serosa were obtained from patients who underwent surgery

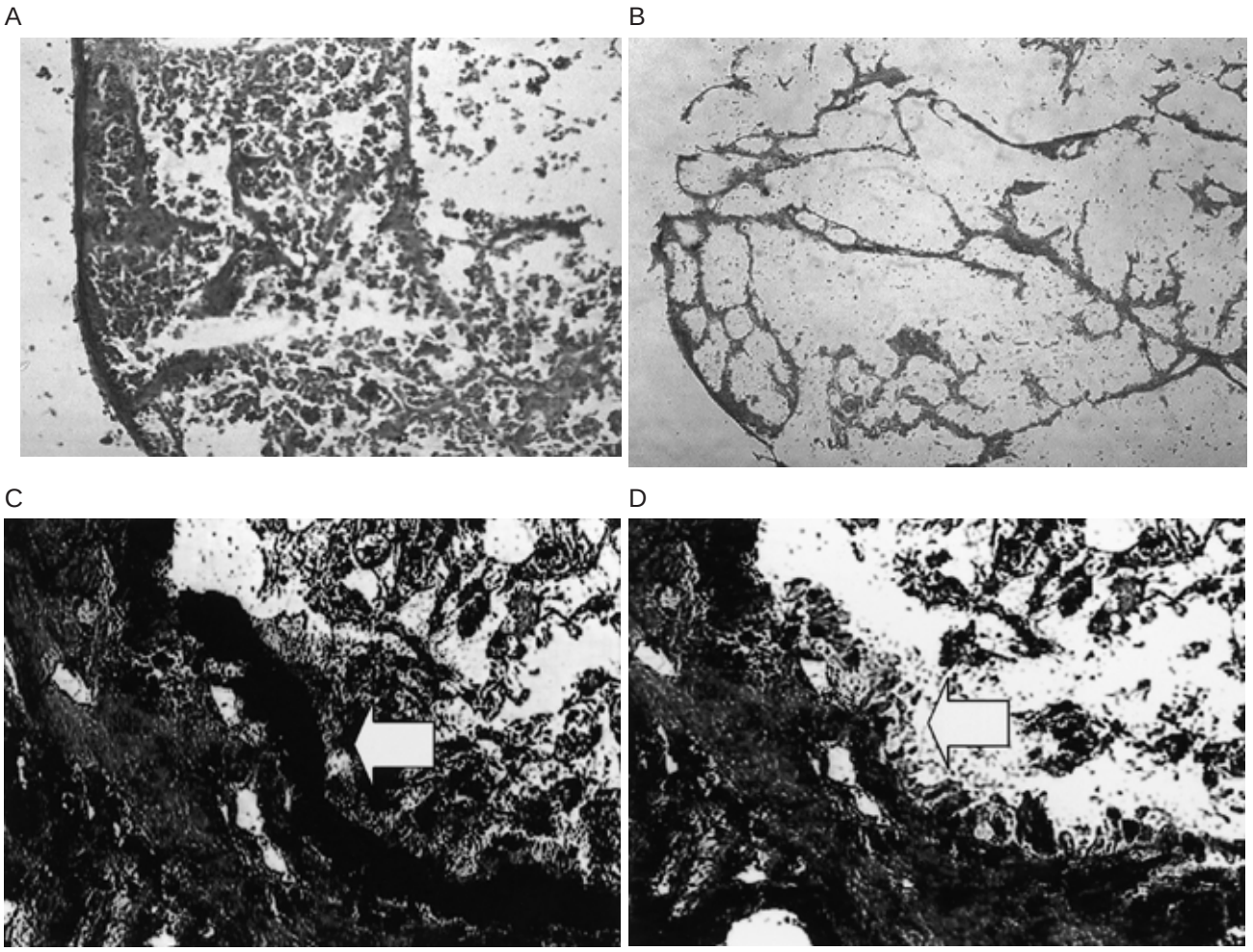

Fig. 1. Selective isolation of the carcinoma cell component from a section of clear cell adenocarcinoma by laser capture microdissection (LCM) transfer. Tissue sections 5–10 μm thick were dehydrated, mounted on a glass slide, and stained with hematoxylin and eosin (HE). A and B: Tissue sections before LCM (A) and after LCM (B) at lower magnifications (×40). Note the vacant spaces left by the selective removal of cancer cell nests. C and D: Tissue sections before LCM (C) and after LCM (D) at higher magnifications (×200). A single layer of carcinoma cells (arrow) in C was removed by LCM transfer.

for primary epithelial ovarian cancer at the National Cancer Center Hospital, Tokyo.

The tumors were classified histopathologically according to the World Health Organization (WHO) classification system[25]: 16 CCAs, 13 SEAs, 11 EMAs and 5 MUAs. The final diagnosis was based on the agreement of two or three pathologists. The diameter of tumors was 12 cm or smaller. Intratumoral heterogeneity in histological subtype was observed in four tumors: two cases of EMA were accompanied with a component of SEA in 20% or 30% of the area, one case of EMA had a component of MUA in 20% of the area, and one case of CCA had a component of serous tumor with low malignant potential in 10% of the area. The other 41 were composed of carcinoma cells of a single histological type. We excluded ovarian carcinomas that are difficult to differentiate from metastatic cancer, e.g., tumors with the involvement of endometrium or other mesonephric tumors. All four MUA cases were composed uniformly of carcinoma cells and were not surrounded by less malignant lesion. Staging was done according to the International Federation of Gynecology and Obstetrics (FIGO) system.[1] Twenty-six patients were stage I, 5 patients were stage II, 12 were stage III and 2 were stage IV. After resection, the tissue samples were immediately frozen and stored at −80°C until DNA extraction.

Table I. Polymorphic Markers Utilized and LOH in Epithelial Ovarian Cancer

Chromosome locus	Primer	Number of cases with allelic loss / number of informative cases (%)			
		SEA (*n*=13)	CCA (*n*=16)	EMA (*n*=11)	MUA (*n*=5)
1p33-p36	#1	**7/11 (64)**	**9/13 (69)**	1/11 (9)	0/5 (0)
1q31	*D1S249*	3/8 (38)	2/11 (18)	2/8 (25)	0/3 (0)
2p16	*D2S123*	0/3 (0)	2/9 (22)	1/7 (14)	0/2
3q27	*D3S1314*	4/9 (44)	0/8 (0)	1/6 (17)	1/2
4p15-p16	*D4S404*	**2/4 (50)**	0/4 (0)	1/6 (17)	0/2
5p14	*D5S810*	0/5 (0)	0/2	1/6 (17)	0/4 (0)
5q21	*D5S409*	**5/5 (100)**	1/9 (11)	2/6 (33)	0/4 (0)
6p21.3-p22.3	*D6S265*	**2/3 (67)**	0/5 (0)	0/6 (0)	0/5 (0)
6q25.2-q27	*D6S264*	0/1	0/6 (0)	1/3 (33)	0/2
7p15-p21	*D7S507*	1/4 (25)	1/9 (11)	**3/5 (60)**	0/4 (0)
7q31	*D7S522*	2/6 (33)	1/6 (17)	1/3 (33)	0/3 (0)
8p21-p23	#2	**6/10 (60)**	3/9 (33)	4/10 (40)	0/3 (0)
9q13-q22.3	*D9S303*	**3/6 (50)**	2/10 (20)	1/11 (9)	0/5 (0)
10q24-q26	*D10S221*	3/10 (30)	1/8 (13)	2/6 (33)	0/4 (0)
11q23.3	*D11S490*	1/4 (25)	3/7 (43)	2/7 (29)	0/5 (0)
12p13.2-pter	*D12S341*	1/6 (17)	1/5 (20)	1/3 (33)	0/3 (0)
12q24-qter	*D12S343*	**6/8 (75)**	1/6 (17)	1/9 (11)	0/1 (0)
13q12-q21	#3	**10/11 (91)**	5/14 (36)	2/10 (20)	1/4 (25)
15q26.1	*FES*	**5/8 (63)**	2/8 (25)	0/6 (0)	0/4 (0)
16p13.13-p13.55	*D16S292*	**2/3 (67)**	0/2	1/6 (17)	ND
16q24-qter	#4	2/6 (33)	2/11 (18)	2/8 (25)	0/1
17p13.1	#5	**6/9 (67)**	1/11 (9)	3/9 (33)	**4/4 (100)**
17q21	*D17S1322*	**4/6 (67)**	1/8 (13)	1/8 (13)	1/5 (20)
18p11	*D18S483*	**4/7 (57)**	1/6 (17)	0/4 (0)	0/3 (0)
18q21.1-q21.3	*D18S69*	**2/3 (67)**	1/3 (33)	0/5 (0)	0/2
19p13.3	#6	**9/11 (82)**	5/11 (45)	3/11 (27)	1/5 (20)
19q13.2	*APOC2*	1/2	1/8 (13)	1/7 (14)	0/2
20p12-p13	*D20S95*	**4/8 (50)**	1/6 (17)	2/7 (29)	0/4 (0)
21q21	*D21S1436*	1/9 (11)	0/6 (0)	0/8 (0)	0/1
Xp22.2	*DXS999*	**4/6 (67)**	2/8 (25)	0/4 (0)	1/2
Xq26.1	*DXS1062*	2/2	0/6 (0)	1/5 (20)	0/1

Bold figures are high frequencies of LOH (>50%).
#1 to #6: Combined information among multiple DNA markers on the chromosomal locus (markers utilized are recorded in the text).
SEA, serous adenocarcinoma; CCA, clear cell adenocarcinoma; EMA, endometrioid adenocarcinoma; MUA, mucinous adenocarcinoma.

Sectioning and staining The cancer tissue was embedded in OCT compound, and cut with a cryostat into 5 to 10 μm-thick sections. Frozen sections on plain untreated glass slides were air-dried for 5 min, washed in deionized water, stained with Mayer's hematoxylin for 30 s, washed with water, then phosphate-buffered saline solution (PBS) for 1 min, followed by water, and finally stained with eosin for 30 s. The slides were then dehydrated with 100% ethanol, placed in xylene and air-dried for 5 min. Before LCM, we checked those tissue sections by fixing one slide in formalin and staining it with hematoxylin and eosin. There was no histological heterogeneity in each sample collected for LCM.

LCM and DNA extraction Using LM200 LCM system (Arcturus Engineering, Inc., Mountain View, CA), the epithelial component of the tumor tissue was collected from the hematoxylin-eosin-stained tissue sections. An example of successful microdissection is presented in Fig. 1. From the collected tissue, DNA was isolated using a QiaAmp DNA Mini Kit (Qiagen, Basel, Switzerland) according to the supplier's recommendation. The DNA was dried and dissolved in 10 m*M* Tris-HCl (pH 8.0)-1 m*M* EDTA, and the concentration was adjusted to 0.1 μg/μl.

Normal genomic DNA was extracted from the non-cancerous frozen tissues by the phenol-chloroform method and the concentration was adjusted to 0.1 μg/μl.[26]

PCR amplifications and analyses of LOH Genomic DNA was amplified by PCR using pairs of sense and antisense primers of 55-oligonucleotide microsatellite markers corresponding to previously reported sequences. These primers were synthesized by Research Genetics (Mappairs, Huntsville, AL) and Perkin Elmer (Applied Biosystems, Foster City, CA) (Table I).

PCR amplifications were performed in a final volume of 20 μl containing 1 μl of DNA extraction solution, 10× GeneAmp PCR Gold buffer (Perkin Elmer, Applied Biosystems), 2.5 mmol/liter $MgCl_2$, 150 μmol/liter dNTP, 0.5 unit of Ampli *Taq* Gold DNA polymerase (Perkin Elmer), and 0.4 μmol/liter primers (forward and reverse). The 5′ ends of the forward primers were labeled with 6-carboxyfluorescein (6-FAM).

The samples were amplified in 35 cycles, each containing a denaturation step (30 s at 94°C), an annealing step (1 min at 55°C) and an extension step (1.5 min at 72°C), in a GeneAmp PCR system 9600 thermocycler (Perkin Elmer). For all samples, PCR cycles were preceded by an initial denaturation step (10 min at 95°C) and followed by a final elongation step (5 min at 72°C).

Subsequently, the PCR products were fractionated by gel electrophoresis using an ABI 377A or ABI 310 sequencer (Applied Biosystems) according to the manufacturer's protocol. These data were analyzed by using the computer program GeneScan (Applied Biosystems).

Non-cancerous tissue samples with two different amplified bands were defined as informative cases for LOH analysis. LOH was recorded when the relative intensity of one allele in tumor DNA was reduced by more than 50% from that in informative cases (Fig. 2).[27] We re-examined samples showing a significant reduction in relative intensity in the initial PCR in order to confirm that the data for LOH were reproducible.

Mutational analysis by single strand conformation polymorphism (SSCP)

To amplify coding exons 5–8 of the *p53* gene, oligonucleotide primers were designed using published sequence data[28] and the 5′ ends of the forward primers were labeled with rhodamine (TaKaRa, Otsu). PCR-SSCP analyses for the detection of *p53* mutations were conducted in the same way as described by Hui *et al*.[28] After electrophoresis, gels were visualized with an FMBIO II Multi-View fluorescence image analyzer (TaKaRa).

Immunohistochemical analyses Five-micrometer-thick sections from formalin-fixed and paraffin-embedded tissue blocks were deparaffinized in xylene, and rehydrated in a

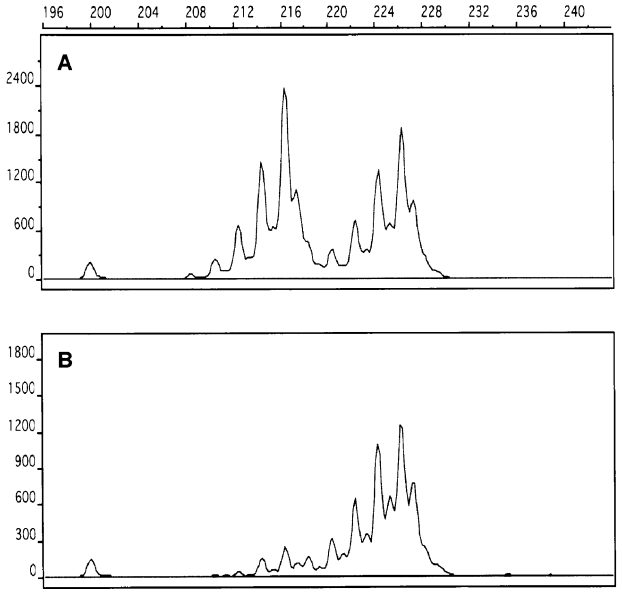

Fig. 2. An example of LOH at the *D13S153* locus on chromosome 13q in serous adenocarcinoma of the ovary. A, Non-cancerous tissue DNA; B, cancer DNA. DNA was amplified by PCR using the *D13S153* primers and analyzed by the ABI310 or 377A sequencer with the Gene Scan program. Allele sizes (in bp) are indicated on the top horizontal axis. Fluorescence intensity units are on the vertical axis. LOH was identified as the reduction of the relative intensity of one of the two alleles in tumor DNA to less than 50% of that in the corresponding normal DNA. In B, the shorter allele (216 bp) was deleted and only the longer allele (226 bp) was retained.

descending ethanol series, ending in water. They were then incubated for 30 min in 0.3% hydrogen peroxide in methanol. Sections were placed in 5% zinc sulfate heptahydrate and treated to unmask antigens by heating in an autoclave for 10 min at 121°C. After having been cooled to room temperature, slides were washed with PBS, and preincubated in 5% normal swine serum. Immunohistochemical examination was performed by the avidin-biotin-complex method as described by Iwaya *et al*.[29]

The primary antibodies used in this study were p53 (DO7; 1:100, Novocastra Laboratories, Newcastle upon Tyne, UK). If evident nuclear staining was observed in more than 10% of the tumor cells, positive immunoreactivity was recorded.

Statistical analysis Differences among four histological types were evaluated by Fisher's exact test. A value of $P<0.05$ was considered to indicate a significant difference.

RESULTS

Multiple allelic losses in ovarian cancer LOH data derived from the analysis of four types of ovarian carcino-

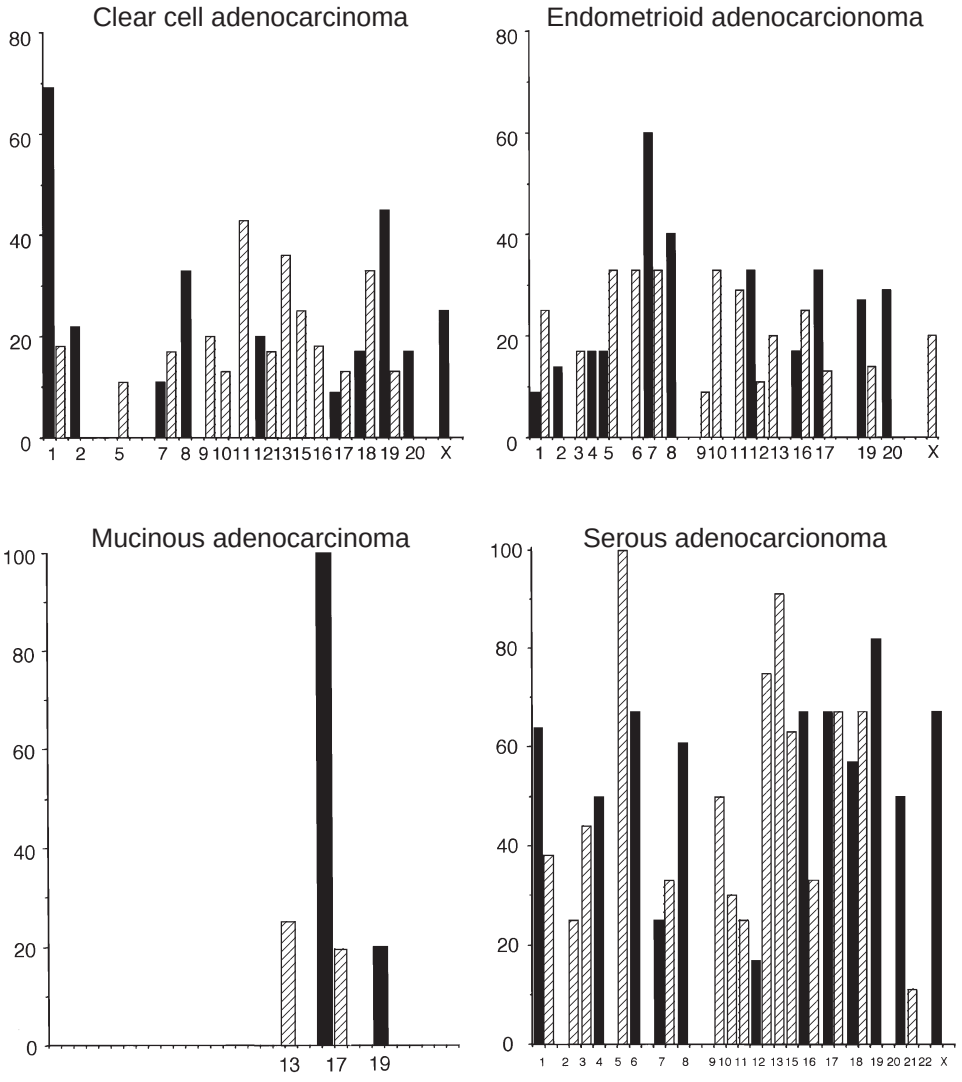

Fig. 3. Different distribution patterns among four histological types of ovarian carcinomas detected by PCR-LOH analysis. Chromosome numbers are indicated on the horizontal axis and the percentage of LOH frequency is on the vertical axis. Black bars represent short chromosome arms and slit bars represent long chromosome arms. There is a striking difference in the distribution pattern of LOH among the four types of tumor.

mas are summarized in Table I and Fig. 3. We used one marker to detect LOH on each of most chromosomal arms. Multiple microsatellite markers were utilized: *D1S201*, *D1S2734*, *D1S199*, *D1S1612*, *D1S2663*, *D1S468* and *D1S243* on 1p, *D8S261*, *D8S254* and *D8S550* on 8p, *D13S267*, *D13S153* and *D13S165* on 13q, *D16S393* and *D16S520* on 16q, *TP53CA*, *D17S786* and *D17S578* on 17p and *D19S406*, *D19S413*, *D19S894*, *D19S216* and *D19S209* on 19p (Table I). The markers for *D2S111* (2q24), *D3S1300* (3p14), *D8S257* (8q22), *D9S171* (9p22), *D11S922* (11p15), *MYH7* (14q11), *IL2RB* (22q13) are not listed in Table I because only one or two cases were informative with these markers.

In all 45 tumors, 40% or higher incidence of LOH was detected on 1p (41%, 17 of 41), 8p (42%, 13 of 31), 13q (44%, 17 of 39), 17p (50%, 10 of 20), 19p (47%, 18 of 38), and Xp (40%, 8 of 20). When the tumors were divided into early stages (I–II) and more advanced stages (III–IV), the incidences of LOH on these six loci were higher in the latter than the former, but were still more than 30% in the tumors of the earlier stages. When all informative loci were taken together for the tumors of each stage, the incidences of LOH were 20% (91 of 447) in stage I tumors, 24% (17 of 72) in stage II tumors, 50% (97 of 195) in stage III tumors, and 10% (4 of 40) in stage IV tumors.

In CCA, the frequency of LOH was 50% or more on only 1p (69%, 9/13), and between 30% and 50% on 8p (33%, 3 of 9), 11q (43%, 3 of 7), 13q (36%, 5 of 14), 18q (33%, 1 of 3) and 19p (45%, 5 of 11). In SEA, LOH was detected with ≥50% frequency on 1p (64%, 7 of 11), 4p (50%, 2 of 4), 5q (100%, 5 of 5), 6p (67%, 2 of 3), 8p (60%, 6 of 10), 9q (50%, 3 of 6), 12q (75%, 6 of 8), 13q (91%, 10 of 11), 15q (63%, 5 of 8), 16p (67%, 2 of 3), 17p (67%, 6 of 9), 17q (67%, 4 of 6), 18p (57%, 4 of 7), 18q (67%, 2 of 3), 19p (82%, 9 of 11), 20p (50%, 4 of 8) and Xp (67%, 4 of 6). The frequency of LOH was between 50% and 30% on 1q (38%, 3 of 8), 3q (44%, 4 of 9), 7q (33%, 2 of 6), 10q (30%, 3 of 10) and 16q (33%, 2 of 6). The incidences of LOH on 5q, 12q, 13q and 17p were significantly lower in CCA than those in SEA (P=0.0019, 0.031, 0.0035 and 0.012, respectively). In summary, the characteristic LOH pattern of CCA compared with SEA was frequent LOH on 1p33-p36 and infrequent LOH on 5q, 12q, 13q and 17p.

In EMA, the frequency of LOH was 50% or more on 7p (60%, 3 of 5), and between 30% and 50% on 5q (33%, 2 of 6), 6q (33%, 1 of 3), 7q (33%, 1 of 3), 8p (40%, 4 of 10), 10q (33%, 2 of 6), 12p (33%, 1 of 3) and 17p (33%, 3 of 9). In EMA, the occurrence of LOH on 7p tended to be higher than in CCA, but the difference was not significant (P=0.094). The incidences of LOH on 12q, 13q, 15q and 19p were significantly lower in EMA than those in SEA (P=0.013, 0.0017, 0.0027 and 0.014).

In MUA, LOH on 17p was observed in all of the four informative samples. LOH on 3p, 9p and 11p was detected in one informative MUA case each and LOH on 3q and Xp was detected in each of the two informative MUA cases. The incidence of 17p LOH was significantly higher than that in CCA (P=0.0064) or that in EMA (P=0.048). In contrast, 1p LOH was less frequent than in SEA (P=0.028) and in CCA (P=0.014), and LOH on 5q, 13q and 19p was less frequent than in SEA (P=0.0079, 0.032 and 0.035).

***p53* gene mutations** The majority of *p53* gene mutations occurs in exons 5, 6, 7 and 8. We analyzed mutation of the *p53* gene in exons 5–8 to reveal the role of these mutations in CCA and SEA. Upon SSCP analysis, positive control DNAs carrying *p53* gene mutations previously reported by Hui *et al.*[28] clearly showed a shift of mobility, whereas DNA extracted from normal tissues used as a negative control showed no mobility shift (Fig. 4). Mutations in exons 5–8 were not detected in CCAs but were detected in 7 (64%) SEAs (P=0.0006). Five of six (83%) SEA cases with LOH on 17p13 revealed *p53* mutations on the retained allele (Table II).

Immunohistochemical analysis of p53 The accumulation of p53 protein in ≥10% of cancer cell nuclei was detected in only 7% (1 of 14) of CCAs, but in 45% (5 of 11) of SEAs (P=0.039, Table II and Fig. 5). Nuclear p53 accumulation is considered to result from the extended half-life of the mutant p53 protein[29] and was actually correlated with both the presence of mutation of the *p53* gene and allelic loss on 17p13 (Table II). In three cases with the mutation of *p53* gene, nuclear accumulation of p53 protein was not observed.

DISCUSSION

In the present study, we observed striking differences in the frequencies of LOH among the four major histological subtypes of epithelial ovarian cancer. Although the fre-

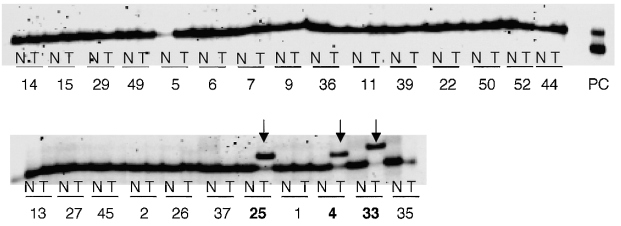

Fig. 4. Detection of *p53* gene mutations in exon 8 in ovarian carcinomas by means of PCR-SSCP analysis: upper, clear cell adenocarcinomas; lower, serous adenocarcinomas. Numbers are case numbers. N, normal tissue DNA; T, cancer DNA. Mobility shift was detected in tumor DNA of cases 25, 4 and 33 (arrow).

quencies of LOH on representative chromosomes tended to be higher in ovarian carcinomas at the advanced stage than in those at the earlier stage, there was no specificity of LOH for advanced-stage disease. Thus, different LOH distribution patterns suggested that different TSGs were involved in the carcinogenesis of each histological type.

Our allelotype study clarified that LOH on 1p33-p36 was especially high (69%) in CCA, and LOH on chromosome arms 8p, 11q, 13q, 18q and 19p also occurred in 30% or more of CCAs. A TSG located on 1p33-p36 might be involved in the development of CCA. Because *p73* TSG is mapped to 1p36.33, we performed immunohistochemical analysis of p73 nuclear expression using a monoclonal antibody (Alpha-Diagnostic, San Antonio, TX). Only two of 14 CCAs and one of 11 SEAs showed nuclear staining (data not shown). We could not find a correlation of the status of p73 nuclear staining and LOH on 1p36.

In SEA, we detected LOH in at least 50% of cases on as many as 17 chromosomal arms, which is one of the largest numbers of abnormal chromosomes and the most frequent incidence of LOH in reports to date.[12, 15, 16] Sato *et al.* and Osborne and Leech widely screened LOH and showed that >30% of LOH was detected in about 40% of chromosome arms, and >50% of LOH was detected in only 6% at most in ovarian SEA.[12, 15] In our study, >30% of LOH and

Table II. Correlations among LOH on 17p13.1, *p53* Gene Mutation and Nuclear Immunoreaction of p53 Protein in CCA and SEA

Sample number	LOH on 17p13.1	*p53* mutation	p53 nuclear immunoreaction
14	○	○	○
15	NI	○	○
29	○	○	○
49	NI	○	●
5	○	○	○
6	○	○	○
7	○	○	○
9	○	○	○
36	NI	○	○
11	○	○	○
39	○	○	○
22	○	○	○
50	●	○	○
52	○	○	○
Total CCA	9% (1/11)	0%	7% (1/14)
13	○	○	○
27	●	●	●
45	○	○	○
2	●	●	●
26	NI	●	○
37	●	○	●
25	NI	●	●
1	●	●	○
4	●	●	●
33	●	●	○
35	○	○	○
Total SEA	67% (6/9)	64% (7/11)	45% (5/11)

The upper half of the samples (No.14–52) are CCAs, and the lower half (No.13–35) are SEAs.
○, no LOH on 17p13.1 or no mutation or negative nuclear immunoreaction; ●, LOH or mutation positive or positive immunoreaction; NI, non informative case.

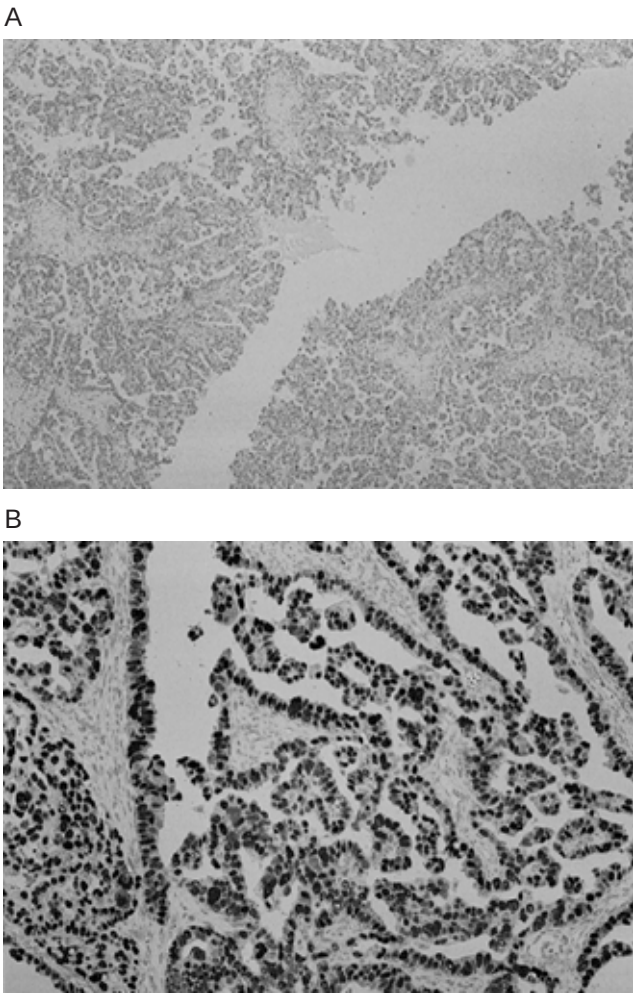

Fig. 5. Immunohistochemical analysis of the p53 protein in ovarian carcinoma. A: Case 14, clear cell adenocarcinoma. Nuclear staining of the p53 protein is absent. B: Case 37, serous adenocarcinoma. Nuclear immunoreactivity is evident in most tumor cells. ×200 (A) and ×400 (B).

>50% of LOH were detected in 54% and 39% of chromosome arms, respectively. It is probable that the incidence of LOH in ovarian cancers could have been underestimated in the previous reports because of contamination by non-cancerous tissue. Our approach of using LCM to analyze fresh cancer tissue samples overcomes the limitations that have traditionally impeded accurate evaluation of genetic alterations in ovarian tumors.

In SEA, frequencies of LOH on 5q, 9q, 12q, 13q, 17p, 17q and 18q were more than 50% in both of the previous studies[12, 15, 16] and ours. LOH on these chromosome arms would be important to inactivate TSGs that are involved in the tumorigenesis and progression of ovarian SEA.

Pieretti *et al.* showed that LOH on chromosome 17 is a common event in epithelial ovarian tumors and almost exclusively associated with high-grade and advanced-stage SEAs.[30] Otis *et al.* showed that LOH at the *TP53* locus on 17p and at the *BRCA1* locus on 17q was frequent in advanced-stage SEA, but not in CCA or serous tumors of low malignant potential.[31] The present result on 17p13.1 confirms the study of Otis *et al.*[31] Chromosome region 17p13.1 contains the *p53* gene that is frequently mutated in epithelial ovarian cancers as well as in other epithelial cancers.[28–30] Low incidences of *p53* gene mutation and nuclear p53 immunoreactivity were also characteristic findings in CCA.

EMA showed higher frequency of 7p LOH and lower frequency of LOH on 12q, 13q, 15q and 19p. MUA was characterized by higher frequency of 17p LOH and lower frequency of LOH on 5q, 13q and 19p. It was reported that LOH on 6p, 12q, 16q and 17q occurred in >30% of MUAs. However, LOH on 17p was detected in one of six MUAs.[12] Pieretti *et al.* showed that the occurrence of LOH on 17p was relatively frequent in high-grade MUAs.[30] Sample sizes of EMA and MUA are not large, so more cases should be examined.

In summary, we performed an allelotype study by using LCM to collect pure cancer DNA, and identified frequent allelic loss on the largest number of chromosomes to date in SEA. In comparison with the previous study by Sato *et al.*,[12] we could estimate more accurately the incidence of LOH in ovarian cancers using LCM, and we identified chromosomal regions that show LOH frequently and specifically in CCA. The different LOH distribution among different histological types of epithelial ovarian cancer suggested that different TSGs were involved in the carcinogenesis of each cancer type. Frequent allelic deletions on 1p33-p36 were common in CCA and SEA, but low incidences of LOH on 17p13.1 and *p53* gene mutation were characteristic findings of CCA. This suggests that *p53* is not involved in the carcinogenesis of CCA, and TSGs on 1p33-p36 would especially play an important role in tumorigenesis and development of CCA. Further studies on these regions are necessary to detect TSGs specific for CCA.

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