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Allelotype of Human Ovarian Cancer[1]

Takaaki Sato, Hiroko Saito, Ryoji Morita, Sumiko Koi, Je H. Lee, and Yusuke Nakamura[2]

Departments of Biochemistry [T. S., H. S., R. M., Y. N.] and Gynecology [S. K.], Cancer Institute, Tokyo, 1-37-1, Kami-Ikebukuro, Toshima-ku, Tokyo 170, Japan, and Department of Biochemistry, Korea Cancer Institute Hospital [J-H. L.], Seoul, Korea

ABSTRACT

In order to determine which chromosome(s) carries a tumor suppressor gene(s) for human ovarian cancer, we examined loss of heterozygosity in 37 tumors with a set of polymorphic DNA markers which cover each autosomal chromosome arm partially.

Frequent losses were observed in chromosomes 4p (42%), 6p (50%), 7p (43%), 8q (31%), 12p (38%), 12q (33%), 16p (33%), 16q (38%), 17p (46%), 17q (39%), and 19p (34%). In addition to these chromosomes, frequent losses of alleles on chromosomes 6q, 13q, and 19q were observed uniquely in serous and serous papillary cystadenocarcinomas; loss of heterozygosity was detected only rarely on these chromosomal arms in nonserous types of tumors. The average (0.12) of fractional allelic loss seen in mucinous cystadenocarcinoma, which usually has a better prognosis than other types, was much lower than that of other tumor phenotypes including serous cystadenocarcinoma (0.31) and clear cell carcinoma (0.20).

These results suggested that (*a*) a large number of tumor suppressor genes might play a role in ovarian cancer, (*b*) losses of alleles in different chromosomal regions could account for differences in histopathological features and/or prognoses among patients, and (*c*) this kind of analysis can contribute to an improved understanding of tumor development and/or progression in human ovarian cancer.

INTRODUCTION

In recent years, tumor suppressor genes involved in a number of human cancers, such as colon (1), breast (2–9), kidney (10, 11), liver (12), lung (13–15), and bladder (16) carcinomas, have been identified through LOH[3] for polymorphic genetic markers. In all these types of cancer, multiple genetic alterations including loss of chromosomes could contribute to tumor development and/or progression as appears to be the case in colorectal cancer (1, 17).

Lee *et al.* (18, 19) have reported the frequent loss of chromosomes 6q, 11, and 17 in 19 ovarian carcinomas, and Ehlen and Dubeau (20) also reported frequent loss of heterozygosity on chromosomes 3p, 6q, and 11p by RFLP analysis of 12 different patients with ovarian carcinomas. However, in those studies, the number of tumors was small and the loci tested with RFLP markers did not cover all chromosomes. Furthermore, neither of these reports correlated LOH with histopathological features or clinical parameters.

The histopathological classification of human ovarian cancer is sometimes very difficult because malignant phenotypes can be complicated. To identify loci of potential tumor suppressor gene(s) for ovarian cancer and to examine correlation between chromosomal loss and histopathological features, we tested loss of heterozygosity in 37 ovarian tumors with a set of 46 polymorphic markers representing all 22 autosomes. Twenty-nine of the 46 were variable number of tandem repeat markers (21).

We report here the frequent loss of alleles on chromosomes 4p, 6p, 7p, 8q, 12, 16, 17, and 19p in those tumors. LOH in certain other chromosomal regions was observed specifically in serous types of carcinomas.

MATERIALS AND METHODS

Materials. Tumors and normal tissues from 37 patients with ovarian carcinomas were analyzed for allelotypes. The tumors were classified as follows: 33 surface epithelial tumors (12 serous cystadenocarcinomas, 4 serous papillary cystadenocarcinomas, 8 mucinous cystadenocarcinomas, 5 clear cell carcinomas, 2 endometrioid carcinomas, 1 undifferentiated carcinoma, 1 malignant adenocarcinofibroma); 3 germ cell-derived tumors (2 endodermal sinus tumors, 1 dysgerminoma); and 1 granulosa cell tumor. The histopathological classification was done according to the typing scheme of the Japanese Obstetrics and Gynecology Society (22) which is basically the same as the typing scheme for ovarian tumors recommended by WHO.

DNA Extraction from Tissues. Extractions of DNA from tumors, normal tissues, and blood were carried out according to the method of Sato *et al.* (8). Briefly, the fine powder in liquid nitrogen, ground by a mortar and pestle, was transferred to a 15-ml tube and suspended in 4 ml of 50 mM Tris-HCl (pH 7.5)-150 mM NaCl-50 mM EDTA with 1% sodium dodecyl sulfate-1 mg/ml proteinase K for 4–16 h at 37°C. Genomic DNA was purified by phenol:chloroform:isoamyl alcohol (25:24:1).

Probes. The probes used in this study are listed in Table 1. All of the probes have been described in the Human Gene Mapping Workshops (23) and cCl3-373 (*D3S659*), cCl3-515 (*D3S685*), and cCl11-237 (*D11S454*) were published in a recent report from our laboratory (24, 25).

Hybridization Conditions. Nylon membranes (Pall Biodyne) were neutralized in 2× standard saline-citrate (0.15 M NaCl-0.015 M sodium citrate) after alkali transfer in 0.1 N NaOH-0.1 M NaCl and fixed by UV cross-linking with Stratalinker. All hybridizations were done under conditions of high stringency according to the method of Sato *et al.* (8).

Statistical Analysis. The χ^2 test and Fisher's exact test were used for statistical analysis of the results.

RESULTS

Fig. 1 shows results of typical Southern blots and demonstrates LOH at several loci. Loss or significant reduction of one allele was observed clearly in each case pictured. Table 1 summarizes the frequency of LOH on each chromosomal arm. Forty-six RFLPs including 29 variable number of tandem repeat markers were tested for the LOH on each autosomal chromosome arm, except for 5p, 8p, 9p, and the short arms of the acrocentric chromosomes. Some chromosomal arms were examined for LOH at several loci. Fig. 2*A* is a schematic summary of LOH for marker alleles on all chromosomes in ovarian cancer. Frequent losses (>30%) were observed on chromosomes 4p (42%), 6p (50%), 7p (43%), 8q (31%), 12p (38%), 12q (33%), 16p (33%), 16q (37%), 17p (46%), 17q (39%), and 19p (34%). None of the four tested RFLP markers on the short arm of chromosome 3 (cCl3-373 and YNZ86.1 at 3p14.2–13, or CCl3-515 and EFD145 at 3p21.3–23) detected LOH in

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[2] To whom requests for reprints should be addressed, at Department of Biochemistry, Cancer Institute, Tokyo, 1-37-1, Kami-Ikebukuro, Toshima-ku, Tokyo 170, Japan.

[3] The abbreviations used are: LOH, loss of heterozygosity; FAL, fractional allelic loss; RFLP, restriction fragment length polymorphism.

Table 1 *Loss of heterozygosity in human ovarian cancer*

Chromosome location	Probe	Locus	Enzyme	No. of patients tested	Allelic loss/ informative case (%)
1p	YNZ2[a]	*D1S57*	*Msp*I	24	1/14 (7)
q	HHH106	*D1S67*	*Msp*I	24	4/14 (29)
2p	TBAB5.7[a]	*D2S47*	*Msp*I	20	0/11 (0)
q	YNH24[a]	*D2S44*	*Taq*I	37	4/29 (14)
3p	EFD145	*D3S32*	*Taq*I	30	3/17 (18)
p	cCl3-373	*D3S659*	*Pvu*II	13	0/6 (0)
p	cCl3-515[a]	*D3S685*	*Msp*I	21	3/18 (17)
p	YNZ86.1	*D3S30*	*Msp*I	32	1/14 (7)
Total (chromosome 3p)				36	6/33 (18)
q	EFD64.2[a]	*D3S46*	*Msp*I	22	1/18 (6)
4p	YNZ32[a]	*D4S125*	*Msp*I	33	10/24 (42)
q	EFD139[a]		*Msp*I	17	1/16 (6)
5q	MC5.61	*D5S84*	*Taq*I	30	0/15 (0)
6p	THH157	*D6S29*	*Bam*HI	22	6/12 (50)
q	JCZ30[a]	*D6S37*	*Taq*I	35	5/29 (17)
7p	RMU7-4	*D7S370*	*Msp*I	21	3/7 (43)
p	THH28	*D7S371*	*Msp*I	21	0/2 (0)
Total (chromosome 7p)				23	3/7 (43)
q	JCZ67[a]	*D7S396*	*Msp*I	24	4/19 (21)
8q	MCT128.2[a]	*D8S39*	*Taq*I	37	5/16 (31)
9q	MCT112	*D9S15*	*Msp*I	16	0/4 (0)
q	EFD126.3[a]	*D9S17*	*Taq*I	37	2/20 (10)
10p	TBQ7[a]	*D10S28*	*Taq*I	32	3/27 (11)
q	EFD75.1[a]	*D10S25*	*Taq*I	36	5/24 (21)
11p	pINS310[a]	*INS*	*Pvu*II	29	7/27 (26)
p	cCl11-237[a]	*D11S454*	*Taq*I	36	0/11 (0)
Total (chromosome 11p)				36	7/29 (24)
q	SS6 (int2)	*INT2*	*Taq*I	34	1/19 (5)
12p	THH14	*D12S16*	*Taq*I	36	3/8 (38)
q	YNH15	*D12S17*	*Msp*I	22	5/15 (33)
13q	MHZ47[a]	*D13S52*	*Taq*I	37	6/27 (22)
14q	CMM101[a]	*D14S13*	*Msp*I	28	5/28 (18)
15q	YNZ90.1	*D15S28*	*Bam*HI	21	1/9 (11)
16p	CMM65[a]	*D16S84*	*Taq*I	36	7/21 (33)
q	p79-2-23[a]	*D16S7*	*Taq*I	34	11/30 (37)
17p	YNZ22[a]	*D17S30*	*Taq*I	37	12/25 (48)
p	pBHP53	*P53*	*Bam*HI	22	3/12 (25)
Total (chromosome 17p)				37	13/28 (46)
q	HHH202	*D17S33*	*Rsa*I	13	1/7 (14)
q	CMM86[a]	*D17S74*	*Taq*I	32	10/26 (39)
q	THH59[a]	*D17S4*	*Pvu*II	31	2/21 (10)
q	RMU-3[a]	*D17S24*	*Pvu*II	13	0/3 (0)
Total (chromosome 17q)				35	12/31 (39)
18p	B74	*D18S3*	*Msp*I	19	0/6 (0)
q	MCT108.2	*D18S24*	*Taq*I	36	0/8 (0)
19p	JCZ3.1[a]	*D19S20*	*Taq*I	36	8/24 (33)
p	YNZ21[a]		*Msp*I	24	5/19 (26)
Total (chromosome 19p)				36	11/32 (34)
q	EFD4.2[a]	*D19S22*	*Taq*I	36	4/16 (25)
20	CMM6[a]	*D20S19*	*Taq*I	35	4/32 (13)
21	MCT15[a]	*D21S113*	*Msp*I	23	0/12 (0)
22	EW7.20[a]		*Msp*I	36	2/10 (20)

[a] Variable number of tandem repeat markers.

more than 18% of informative cases. On chromosome 11, results were very different for two loci in the same chromosomal segment (11p15.5): frequent LOH was observed at the *INS* locus (7 of 27, 26%), but not at the locus (*D11S454*) defined by cCl11-237 locus (0 of 11, 0%).

Six RFLP markers, including the *p53* gene, were used to examine LOH on chromosome 17. Frequent losses of both the short and the long arms of chromosome 17 were observed at *D17S30* (YNZ22; 12 of 25, 48%) and *D17S74* (CMM86; 10 of 26, 39%). Interestingly, two tumors (tumors 38 and 45 in Fig. 2*B*) showing retention at the *P53* locus revealed LOH at *D17S30* on chromosome 17p13, and eight tumors (including tumors 37 and 40 in Fig. 2*B*) showing LOH at the *D17S74* locus on 17q21 retained both alleles at *D17S4* (THH59) on 17q23–25. Thus, two common regions of deletion could be identified on this chromosome. We detected no LOH on chromosomes 2p, 5q, or 21 even though at least 10 informative cases were available for the markers on each of these chromosomal arms.

Although the average frequencies of LOH on chromosomes 6q, 13q, and 19q in all tumors were not high, alleles on these chromosomes were often lost in serous types of tumors (serous cystadenocarcinoma and serous papillary cystadenocarcinoma). As summarized in Table 2, serous types showed frequent LOH on chromosomes 6q (42%), 13q (56%), and 19q (67%), while in nonserous tumors the incidence of LOH was very rare (6q, 0%; 13q, 6%; 19q, 0%). These differences were statistically significant.

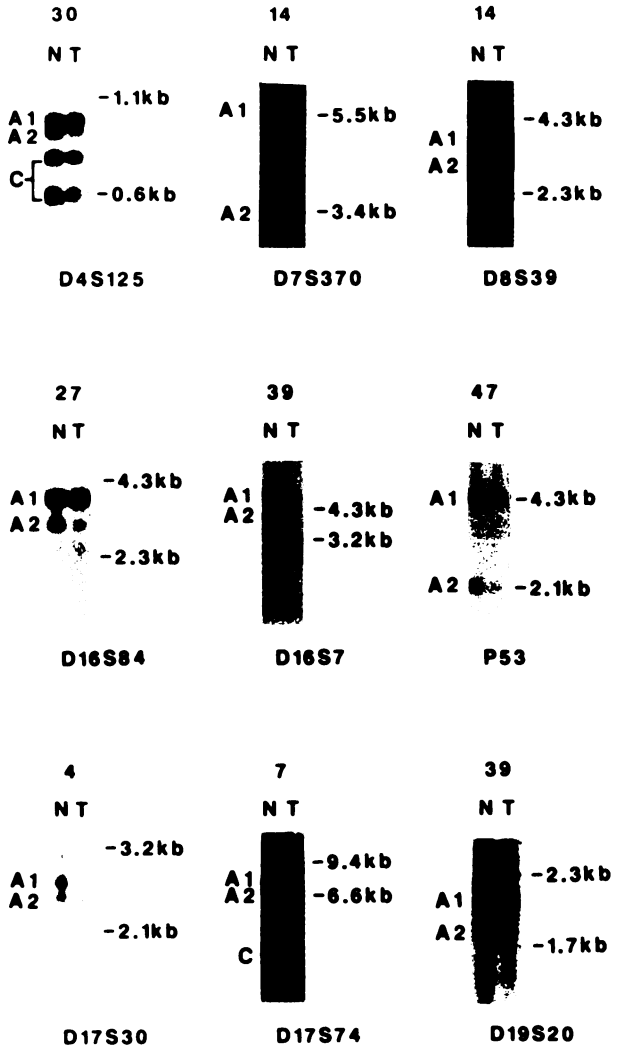

Fig. 1. Autoradiograms from Southern blot analyses, demonstrating LOH from tumor (*T*) and normal (*N*) tissues in patients with ovarian cancer. The probes used are YNZ32 (*D4S125*), RMU7-4 (*D7S370*), MCT128.2 (*D8S39*), CMM65 (*D16S84*), p79-2-23 (*D16S7*), pBHP53 (*P53*), YNZ22 (*D17S30*), CMM86 (*D17S74*), and JCZ3.1 (*D19S20*). *Abscissa*, tumor number; *kb*, kilobases.

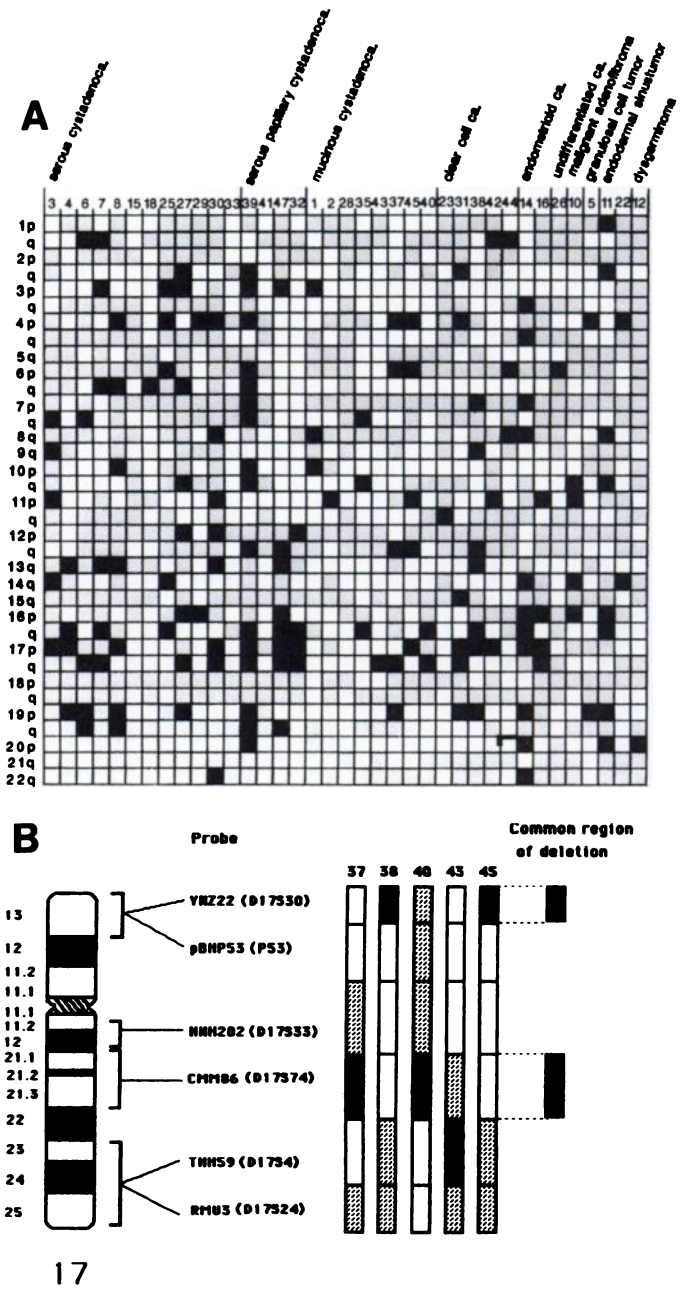

Fig. 2. Schematic representations of LOH in ovarian cancer. ■, LOH; □, retention of both alleles; ▢, uninformative or not examined. (*A*) The results of LOH on each autosomal chromosome. Types of tumors and case numbers of patients are shown above. The probes used for each chromosome are shown in Table 1. *ca.*, carcinoma. (*B*) Schematic representation of the common region of deletion on chromosome 17 in ovarian cancer. The common regions of deletions are shown on the *right*.

To examine the association between FAL and histopathological features or clinical data, the FAL value on each phenotypically different tumor was calculated as the ratio of the number of allelic losses *versus* the number of cases informative in each chromosomal arm, as described by Vogelstein *et al.* (26). The average FALs for each phenotypically different tumor were: serous cystadenocarcinomas, FAL = 0.31; mucinous cystadenocarcinomas, FAL = 0.12; and clear cell carcinomas, FAL = 0.20 (see Table 3). Four tumors showed no LOH although each of them was informative for at least 10 loci. The average FAL for all 37 ovarian tumors was 0.23.

DISCUSSION

Among 37 human ovarian cancers tested for LOH, we observed allelic losses in >30% of informative tumors on chromosomes 4p, 6p, 7p, 8q, 12p, 12q, 16p, 16q, 17p, 17q, and 19p. Frequent LOH on chromosomes 11p and 17p has been reported by others, in smaller studies (18–20). Although Ehlen and Dubeau (20) reported frequent LOH with probe pBH302 (*ERBA2*) on chromosome 3p21–25 in ovarian cancer, in four of seven tumors (57%), the four loci on chromosome 3p used

Table 2 *Frequency of loss of heterozygosity in serous and nonserous phenotypes*

Chromosome	Allelic loss/informative cases		
	Serous types[b]	Nonserous types[c]	*P*[a]
6q	5/12	0/17	0.0224
13q	5/9	1/18	0.0371
19q	4/6	0/10	0.0433

[a] Calculated by Fisher's exact test.
[b] Serous types include serous cystadenocarcinomas and serous papillary cystadenocarcinomas.
[c] Nonserous types include mucinous cystadenocarcinomas, clear cell carcinomas, and others described in "Materials and Methods."

Table 3 *Fractional allelic loss of phenotypically different tumors*

Histological types	Tumor no.[a]	Allelic loss/ informative cases	FAL
Serous types	3	5/11	0.46
	4	4/19	0.21
	6	5/17	0.29
	7	6/17	0.35
	8	7/14	0.50
	15	0/16	0.00
	18	1/21	0.05
	25	5/9	0.56
	27	8/14	0.57
	29	2/21	0.10
	30	8/13	0.62
	33	0/22	0.00
	41	16/22	0.73
	47	0/18	0.00
	32	4/27	0.15
Av.			0.31
Mucinous types	1	3/21	0.14
	2	1/20	0.05
	28	0/14	0.00
	35	3/24	0.13
	43	1/24	0.04
	37	5/17	0.29
	45	5/23	0.22
	40	2/20	0.10
Av.			0.12
Clear cell types	23	1/14	0.07
	31	6/20	0.30
	38	7/22	0.32
	42	3/18	0.17
	44	3/22	0.14
Av.			0.20

[a] Corresponds to numbering in Fig. 2.

in this study detected LOH no greater than 18%. Similarly, in contrast to the results of Lee *et al.* (19), who reported frequent LOH at the *HRAS1* locus [5 of 10, 50%] on chromosome 11p15.5 largely in metastatic tumors, we observed lower frequencies of LOH at loci on chromosome 11p: at the *INS* locus 28% [7 of 26] and at *D11S454* [cCl11-237 (25)] 0% [0 of 11]. These results might be due to the different loci tested. Furthermore, 11p allelic losses occur more frequently at the *HRAS1* locus and might be a late genetic change associated with tumor progression.

We found two common regions of deletion on chromosome 17 in ovarian cancer, one in the region distal to *P53* and the other near CMM86 (*D17S74*). The region distal to *P53* also has been identified as a candidate locus for a tumor suppressor gene in breast cancer (8, 27). Furthermore, Hall *et al.* (28) recently reported linkage of a gene associated with early-onset familial breast cancer to the CMM86 (*D17S74*) locus on chromosome 17q21. Whether the two putative tumor suppressor genes for ovarian cancer on chromosome 17 are identical to the breast cancer genes that appear to be in the same chromosomal segments is only conjectural at present.

Many of the epithelium-derived serous cystadenocarcinomas and serous papillary cystadenocarcinomas in our tumor panel had lost alleles on chromosomes 6q, 13q, and 19q, although nonserous types of tumors only rarely showed LOH on these chromosomal arms. Wake *et al.* (29) reported frequent observations of cytogenetical abnormalities on the long arm of chromosome 6 among serous papillary cystadenocarcinomas. Such losses of specific chromosomal regions may have something to do with the development or progression of serous types of tumors, and/or they may correlate with the expression of the serous phenotype.

The FALs in tumors we studied varied among tumors with different histopathological features. The average FAL (0.12) in mucinous cystadenocarcinoma was much lower than that in serous cystadenocarcinoma (FAL = 0.31) or in clear cell carcinoma (FAL = 0.20). This observation may reflect the fact that as a group, mucinous cystadenocarcinomas have a better prognosis relative to other malignant phenotypes of ovarian cancer. Higher FAL value in colorectal cancer (>0.20) seem to be correlated with poorer prognosis for patients, regardless of tumor size or Dukes' classification (26). In renal cell cancer, a direct correlation has been observed between FAL > 0.20 and higher histopathological grade or larger tumor size (10). FAL might therefore be a valuable prognostic tool for the clinical management of human cancers.

Our results suggest that multiple tumor suppressor genes are involved in the development, the progression, and possibly the metastatic proclivity of ovarian cancer. The detection of mutations in tumor suppressor genes through LOH analysis might well be a useful approach to diagnosis, especially for tumors of borderline malignancy in which histopathological features are often ambiguous.

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