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Advances in Brief

A Deletion Unit on Chromosome 17q in Epithelial Ovarian Tumors Distal to the Familial Breast/Ovarian Cancer Locus

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Abstract

Linkage analysis in familial breast and ovarian cancer and studies of allelic deletion in sporadic ovarian tumors have suggested that chromosome 17q may be the location of a gene of importance in ovarian carcinogenesis. We have examined tumor and normal DNA samples from 120 patients with ovarian tumors for allelic deletion at 12 loci on chromosome 17q. Allelic deletion was observed in 64 cases (53%) of which 56 showed loss of heterozygosity at all loci analyzed on 17q. The pattern of allele loss at metastatic sites was consistent with loss of heterozygosity having occurred prior to metastasis. A common region of deletion, defined by 6 cases of invasive epithelial ovarian cancer and a benign serous cystadenoma, spanned 16 cM and was delimited by *nm23* and *GH*. This region is distal to the region on chromosome 17q to which the familial breast/ovarian cancer susceptibility gene has been mapped. The results suggest that a tumor suppressor gene involved in sporadic ovarian carcinogenesis is located on the distal portion of chromosome 17q and is distinct from the gene linked to familial cases.

Introduction

Although ovarian cancer is the most common cause of death from gynecological cancer, relatively little is known about the natural history of the disease or the genetic events in ovarian carcinogenesis. Amplification of *HER-2/neu* and c-*myc* and overexpression and mutation of *p53* have been demonstrated in a proportion of cases (1–5). Recent reports suggest that chromosome 17q may be the location of a tumor suppressor gene of importance in ovarian carcinogenesis. Linkage to a locus on 17q has been demonstrated for early onset breast cancer and for familial breast and ovarian cancer (6, 7). In each of 9 cases studied from 17q-linked breast/ovarian cancer families, the allele losses affected the wild-type chromosome, consistent with a retinoblastoma-like model in which the predisposing gene is a suppressor gene (8). Furthermore, loss of heterozygosity has been consistently documented at a high frequency on 17q in sporadic ovarian cancer (9–12). As the studies of loss of heterozygosity in ovarian cancer have been limited to a small number of loci on 17q it is unclear whether the deletion unit in sporadic ovarian cancers is within the region linked to familial ovarian cancer or may be associated with a distinct tumor suppressor gene. In order to map the deletion unit more precisely we have performed a detailed study of allelic deletion on chromosome 17q in a panel of 120 ovarian tumors.

Materials and Methods

Tissue Samples and DNA Extraction. Ovarian tumor tissue samples were obtained either at the time of surgery (76 cases) or from archival paraffin-embedded tissue (44 cases). The histological type, International Federation of Gynecology and Obstetrics stage (13), and grade of the tumors are shown in Table 1. In addition, samples of between 4 and 7 separate tumor sites were obtained at the time of surgery from 10 individuals. Tissue obtained at surgery was stored at −80°C and peripheral blood leukocytes were obtained from each patient as a source of normal DNA. DNA was prepared from blood and frozen tissue according to standard protocols. Tumor and normal DNA samples were extracted from archival paraffin-embedded ovarian tumor tissue blocks and matched normal tissue blocks by incubating 5-μM histological sections in 0.5 ml buffer containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 μg/ml bovine serum albumin, 0.45% Tween 20, 0.45% Nonidet P-40, and 100 μg/ml proteinase K. The sections were incubated for at least 8 h at 55°C and then transferred to a boiling water bath for 10 min and cooled on ice. Aliquots of 1–5 ml were used in PCR.[4]

Loci Analyzed. The initial analysis of all 120 normal/tumor DNA pairs was performed using a panel of 5 PCR microsatellite polymorphisms (*D17S250*, *D17S579*, *D17S588*, *nm23*, and *GH*; Table 2). In addition, Southern analysis using *D17S509*, *D17S74*, and *D17S4* was performed for cases in which sufficient DNA had been extracted from freshly frozen tumor. Cases with loss of heterozygosity at all loci in the initial analysis were not studied further. Cases with loss of heterozygosity involving one or more but not all loci in the initial analysis were analyzed further using additional polymorphic markers summarized in Table 2. Allelic losses were scored as decreases in intensity of one allele relative to the other, determined from visual comparison of tumor with normal allele intensities (21, 22).

Southern Analysis of Restriction Fragment Length Polymorphisms. Paired samples of normal and tumor DNA were digested with appropriate restriction endonucleases, size fractionated on a 1% agarose gel, denatured, neutralized, and transferred to nylon filters. Hybridization to random primed ^{32}P-labeled probes (Table 2) was conducted at 65°C for 12–18 h. The filters were then washed at 22°C in 2 × SSC for 30 min, at 22°C in 2 × SSC containing 0.1% sodium dodecyl sulfate for 30 min, and at 65°C in 2 × SSC containing 0.1% sodium dodecyl sulfate for 20 min, and then autoradiographed at room temperature for 1–10 days.

PCR Analysis of Microsatellite Length Polymorphisms. Primers used for the analysis of microsatellite polymorphisms are detailed in Table 2 and were used in assays at a concentration of 1.0 μM. PCR reactions were performed with approximately 50 ng of genomic DNA in 20-μl volumes using 0.5 units/reaction of Taq DNA polymerase (Promega). PCR products were either labeled by including [α-^{32}P]dATP in the PCR reaction or by end-labeling one primer prior to the PCR reaction. For end-labeling, 2 μM of primer, 50 μCi of [γ-^{32}P]dATP (Amersham), and 10 units T4-kinase (Promega) were incubated with T4-kinase buffer (Promega) at 37°C for 20 min and then at 65°C for 10 min in a 20-μl reaction and subsequently diluted to 50 μl. PCR reactions were then performed with 0.5 μl of the labeled primer/reaction and 200 μM dNTPs. Alternatively, PCR was performed with [α-^{32}P]dATP (3000 Ci/mmol, Amer-

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[4] The abbreviations used are: PCR, polymerase chain reaction; SSC, standard saline citrate.

Table 1 *Relationship between loss of heterozygosity on chromosome 17q and tumor grade, stage, and histological type*

	No. with LOH[b] on 17q	% with LOH on 17q
Tumor differentiation[a]		
Benign	1/2	
Borderline	1/6	17
Well differentiated	4/23	17
Moderately differentiated	19/34	56
Poorly differentiated	39/55	71
Figo stage[c]		
Benign	1/2	
Stage I	10/35	29
Stage II	5/10	50
Stage III	41/61	66
Stage IV	7/12	58
Histological type[d]		
Benign	1/2	
Serous	37/57	65
Endometrioid	5/20	25
Mucinous	5/14	36
Clear cell	3/9	33
Undifferentiated	13/18	72

[a] $X^2 = 16.4$; $P < 0.0001$ (trend test, 1 d.f.).
[b] LOH, loss of heterozygosity.
[c] FIGO, International Federation of Gynecology and Obstetrics. $X^2 = 10.3$; $P = 0.001$ (trend test, 1 d.f.).
[d] $X^2 = 15.3$; $P = 0.004$ (heterogeneity test, 4 d.f.).

sham) at 1.0 μCi/reaction, dATP at 2.5 μM, and dTTP, dCTP, and dGTP at 200 μM. Reaction conditions consisted of 1 min at 94°C, 2 min at 55°C, and 1 min at 72°C for 27 cycles followed by a final extension for 7 min at 72°C. Reaction products were diluted 1:2 in loading buffer (90% formamide, 10 mM EDTA, 0.3% bromophenol blue, 0.3% xylene cyanol), heated at 90°C for 5 min, and loaded (2–5 μl) onto 5% denaturing polyacrylamide gels. After electrophoresis, gels were dried at 80°C and exposed to X-ray film for 4–48 h at room temperature.

Statistical Analysis. The relationship between allelic deletion on chromosome 17q and tumor stage, grade, and histological type was analyzed using the χ^2 test.

Results

Allelic deletion at one or more loci on 17q was observed in 53% of tumors (64 of 120) and was more frequent in association with poorly differentiated, advanced stage, and serous tumors (Table 1). In 56 tumors both alleles were retained at all informative loci. In an additional 56 tumors allelic deletion was observed at all informative loci. Loss of a limited region of 17q was observed in 8 tumors of which 7 were invasive epithelial ovarian carcinomas and 1 was a benign serous cystadenoma. The pattern of loss of heterozygosity in these 8 tumors is shown in Fig. 1 and representative results are illustrated in Fig. 2. Analysis of tumor D57 revealed loss of heterozygosity proximally on 17q at *D17S250* but retention of both alleles at all informative distal loci. In tumors D58-D62 both alleles were retained at proximal loci but allelic deletion was observed at distal loci suggesting that the loss was due to somatic recombination. In tumors D63 and D64 there were interstitial losses that are likely to represent deletions. The pattern of loss in tumors D58-D64 defined a common region of loss that spanned 16 cM (Fig. 1) and was flanked by, but did not include, *nm23* and *GH*.

It is possible that the high frequency of allelic deletion on 17q could reflect random losses associated with advanced stage disease which had occurred in the primary tumor after metastasis. To investigate this, the samples obtained from metastatic sites in 10 cases (3–6 metastatic deposits/case) were assayed for loss of heterozygosity at the *D17S579* locus. Of the 10 cases, 8 were informative for this polymorphism. In 6 of 8 informative cases allelic deletion of *D17S579* was observed and in each of these 6 tumors the same allele was lost in the primary tumor and in all of the metastatic deposits (3 deposits, 2 cases; 4 deposits, 1 case; 5 deposits, 1 case; 7 deposits, 1 case; 8 deposits, 1 case). In the remaining 2 informative cases both alleles were retained at the *D17S579* locus in the primary tumor and in metastatic deposits.

Discussion

This study has documented a high frequency of loss of heterozygosity on chromosome 17q in sporadic ovarian cancer. Previous reports of allelic deletion on 17q in ovarian cancer have involved analysis at a maximum of 4 loci. Eccles *et al.* (9) and Russell *et al.* (10) observed loss of heterozygosity at *D17S4* in 77% of informative cases and Lee *et al.* (11) observed loss of heterozygosity at *D17S26* in 31% of informative cases. Sato *et al.* (12) analyzed the *D17S33*, *D17S74*, *D17S4*, and *D17S24* loci and observed allelic loss at these loci in 13, 32, 31, and 13% of informative cases, respectively. Foulkes *et al.* (24) reported loss of heterozygosity at the *D17S74*, *D17S308*, and *D17S4* loci in 65, 83, and 65% of informative cases, respectively. Taken together with our findings these studies are consistent with a high frequency of allelic deletion on 17q but also highlight inconsistencies in the reported proportion of tumors with loss of heterozygosity. Our observation that the frequency of allelic deletion on 17q is related to the grade, stage, and histological type of the tumors studied may explain these inconsistencies.

Table 2 *Summary of the PCR microsatellite polymorphisms and Southern blot restriction fragment length polymorphisms analyzed*

Locus	Probe/primer	Position	Primer sequence	Ref.
D17S250	Mfd15	17q11–12	GGAAGAATCAAATAGACAAT GCTGGCCATATATATATTTAAACC	14
THRA1	THRA1	17q11–12	CTGCGCTTTGCACTATTGGG CGGGCAGCATAGCATTGCCT	15
D17S579	Mfd188	17q12–21	AGTCCTGTAGACAAAACCTG CAGTTTCATACCAAGTTCCT	16
D17S509	LB17.1[a]	17q12–21	DNA probe	
GIP	GIP	17q21	CACAATGGGGCTCGACTTAGCATAA CTTGCTGGATCAGACAAACCTCTG	17
HOX2	HOX2	17q21	GAAATTGGAAGCCTGGAC AGTTCGGGAGTAAAATTCTT	18
D17S293	6C1	17q21	ACAGTGCCAGAGATATACCG GCTATGAGCCTGGCAGACC	16
D17S588	42D6[b]	17q21	CCTGGTCTAGGAAGAGTGTCA GTGTAAGCATCTGTGTATACTAC	
nm23	nm23	17q21–22	TTGACCGGGGTAGAGAACTC TCTCAGTACTTCCCGTGACC	16
D17S74	CMM86	17q22	DNA probe	19
GH	GH	17q23	TCCAGCCTCGGAGACAGAAT AGTCCTTTCTCCAGAGCAGGT	20
D17S4	THH59	17q23–25	DNA probe	19

[a] Kindly provided by Dr. R. White.
[b] Kindly provided by Dr. M. Skolnick.

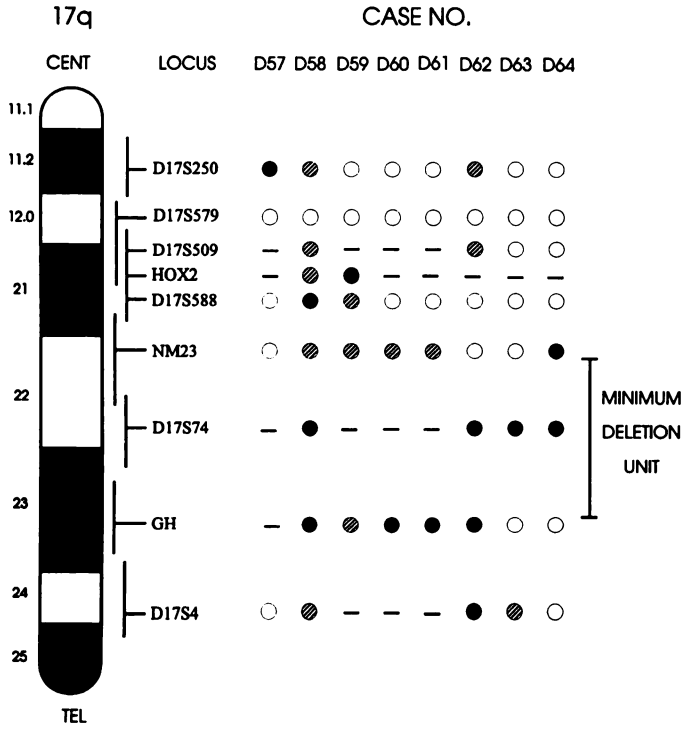

Fig. 1. Allelic deletion pattern for 8 ovarian tumors with loss of a limited region of chromosome 17q. The other 112 tumors analyzed had loss of heterozygosity at all informative loci or had retained both alleles at all informative loci. The pattern of loss in tumors D58-D64 defined a minimum deletion unit flanked by, but not including, *nm23* and *GH*. Tumor D63 is a benign serous cystadenoma and the other tumors are invasive epithelial carcinomas. ○, both alleles retained; ●, loss of heterozygosity; ◍, not informative; –, not analyzed. Cases were not analyzed for some polymorphic markers either because DNA was obtained from paraffin-embedded material and was not suitable for Southern analysis with LB17.1 (*D17S509*), CMM86 (*D17S74*), and THH59 (*D17S4*) or because the *HOX2* polymorphism was not included in the initial screen and subsequent analysis was only performed for the 2 cases (D58 and D59) in which it could potentially add useful information. The regional assignments of loci/genes referred to in the text but not shown are: *D17S26*/17q; *D17S33*/17q11.2-q12; *D17S24*/17q; *D17S308*/17q21-qter; *THRA1*/17q11.2-q12; *RARA*/17q21.1; prohibitin/17q21; *WNT3*/17q21-q22 (23).

The high rate of allele loss on 17q may be related to the existence of a tumor suppressor gene on this chromosome, the loss or inactivation of which is an important step in the development of epithelial ovarian tumors. However, at least 3 other factors which may have contributed to the high rate of loss should be considered. (*a*) Allelic deletions at some chromosomal locations may represent random or late events in malignancy associated with metastatic, advanced stage disease. It is unlikely that this is the explanation for the losses we have observed in view of their frequency and our observations in metastatic ovarian cancer. The same allele of *D17S579* was lost in the primary tumor and at all metastatic sites in 6 cases of ovarian cancer. These results suggest that loss of heterozygosity on 17q in the cases studied occurred prior to metastasis. (*b*) At least some of the cases in which allelic deletion was observed at all informative loci on 17q may be related to primary events on the short arm of chromosome 17 and in particular to mutation and deletion of the *p53* gene. Overexpression of *p53* has been observed in 50% of ovarian cancers and allelic deletion at the *p53* locus in 25–55% of epithelial ovarian cancers (3, 9, 12). (*c*) It is possible that the tendency for extensive rather than localized losses reflects the presence of more than one tumor suppressor gene on chromosome 17q.

The observation of an interstitial deletion in a benign serous cystadenoma which was located within the minimum deletion unit for invasive disease raises the possibility that loss of heterozygosity on 17q is a relatively early event in ovarian neoplasia. Russell *et al.* (10) also reported allelic deletion on 17q in a benign ovarian tumor. A well defined premalignant condition of the ovary has not been described. It is possible that benign epithelial ovarian tumors represent a precursor lesion for invasive disease. The application of current techniques for molecular genetic analysis may resolve this issue.

The minimum deletion unit on 17q defined in this study lies between (but does not include) the *nm23* and *GH* loci, a region spanning approximately 16 cM (16). This region is compatible with the common region of deletion on 17q identified by Sato *et al.* (12) in sporadic ovarian cancer but is distinct from a common region of deletion recently reported for sporadic breast cancer which lies between *D17S250* and *D17S579* (25). There is now evidence from several studies for linkage of early onset breast cancer and familial breast/ovarian cancer to chromosome 17q (6, 7, 16). Smith *et al.* (8) have

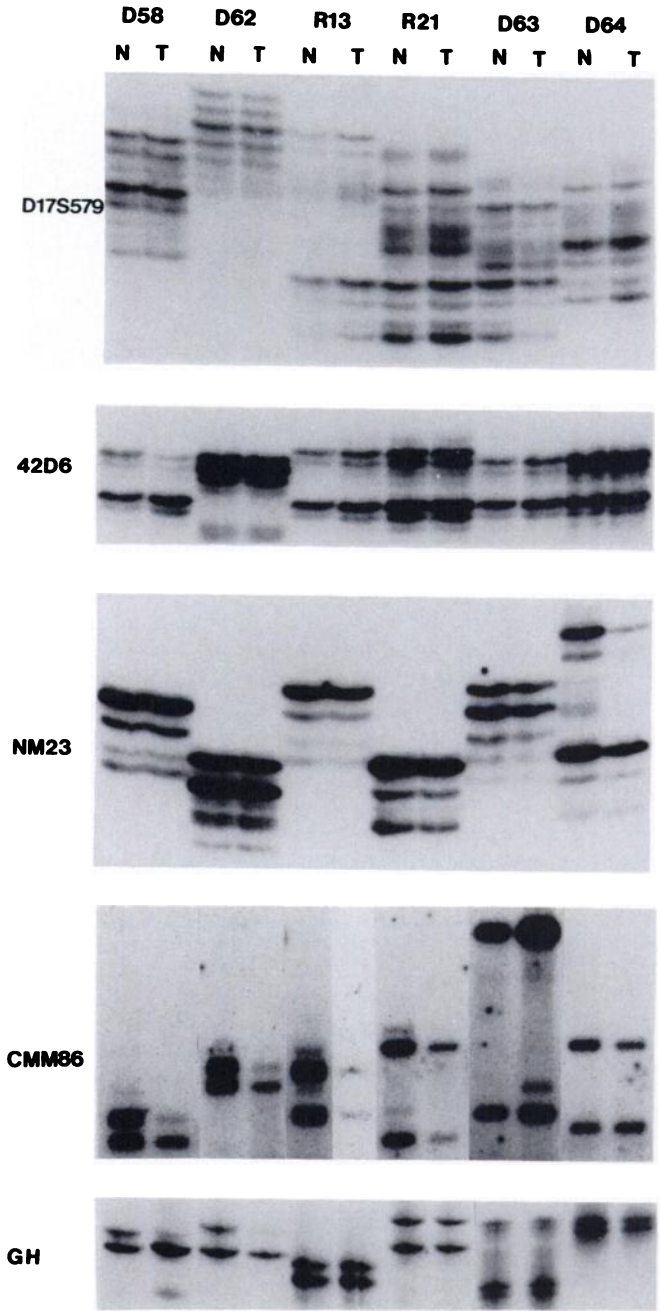

Fig. 2. Examples of results obtained with 4 PCR microsatellite polymorphisms *D17S579* (Mfd188), 42D6 (*D17S588*), *NM23*, and *GH* and a Southern blot restriction fragment length polymorphism CMM86 (*D17S74*) in cases D58, D62, R20, R21, D63, and D64. Tumors D58 and D62 have retained both alleles at proximal loci but have loss of heterozygosity at distal loci. Tumors R13 and R21 have retained both alleles at all informative loci. Tumors D63 and D64 have interstitial deletions of CMM86 alone and *nm23* and CMM86, respectively. N, peripheral blood leukocytes; T, tumor cells.

recently established that in tumors from breast/ovarian cancer families with linkage to 17q, allele losses affect the wild-type chromosome. This observation suggests that the putative "breast-ovarian" cancer gene (*BRCA1*) is a tumor suppressor gene and that the development of some tumors involves a "two-hit" mechanism with germ line and somatic inactivation of the first and second alleles, respectively. Recombinant cases from families with early onset breast cancer linked to 17q suggest that *BRCA1* lies between *D17S250* and *GIP* (16). If this is correct, it is likely that the gene linked to familial breast/ovarian cancer is distinct from the putative tumor suppressor gene in the deletion unit defined in this study of ovarian cancer. We cannot exclude the possibility that the familial gene plays a significant role in sporadic ovarian cancer since 56 of 120 cases in this study had loss of heterozygosity at all loci on 17q. Furthermore, tumor D57 had a proximal deletion in a region which may include the familial breast/ovarian cancer gene.

There are a number of candidate tumor suppressor genes on 17q including *nm23*, *THRA1*, *WNT3*, *RARA*, prohibitin, and the *HOX 2* cluster. The pattern of allelic deletion in our study appears to exclude *nm23* and current gene maps suggest that the other candidate genes are outside our minimum deletion unit. Further studies of allele loss using a panel of markers within the minimum deletion unit will be required to define more precisely the region containing the putative tumor suppressor gene.

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