Characterization of Chromosome 9 in Human Ovarian Neoplasia Identifies Frequent Genetic Imbalance on 9q and Rare Alterations Involving 9p, Including CDKN2


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ABSTRACT

We have examined 41 forms of ovarian cancer for genetic alterations on chromosome 9 using a combination of five RFLP DNA probes and 15 simple tandem repeat polymorphisms. Genetic imbalance (i.e., loss of heterozygosity, microsatellite instability, amplification) for one or more informative markers on chromosome 9 was observed in 66% (27 of 41) of our tumor panel. Genetic imbalance was observed on 9q in 59% (24 of 41) of tumors informative for at least one locus. In contrast, only 13% (5 of 40) of informative tumors demonstrated a genetic alteration involving 9p. Furthermore, allelic loss on 9q was more common in late stage tumors (63%, 17 of 27) and poorly differentiated tumors (75%, 15 of 20) as compared to benign and early stage tumors (30%, 3 of 10). Evaluation of 15 tumors showing limited regions of genetic imbalance has identified 2 candidate tumor suppressor regions on 9q and 1 on 9p. Interestingly, the regions defined to 9p21–p24, 9q31, and 9q32–q34 all overlap with several known disease loci. In this aspect, the potential role of the CDKN2 gene at 9p21–p22 in ovarian carcinogenesis was assessed in an extended panel of ovarian tumors, 11 human ovarian carcinoma cell lines, and 1 cervical tumor cell line. With the use of comparative multiplex PCR, homozygous deletions were detected in 16 of 115 (14%) fresh tumors and 3 of 12 cancer cell lines. For those tumors demonstrating allelic loss for markers on 9p no somatic mutations were observed in the retained allele of CDKN2, as determined by single-strand conformation polymorphism analysis, but a mutation was observed in an additional cell line. Furthermore, CDKN2 mRNA levels were similar in the 9 cancer cell lines that retain CDKN2, as compared to normal human ovarian surface epithelial cell lines. Overall, our results suggest the potential involvement of a gene or genes on chromosome 9q and de-emphasize a significant role for the CDKN2 gene on 9p in the initiation and progression of ovarian cancer.

INTRODUCTION

Ovarian cancer is the most lethal gynecological malignancy and the fifth most common cause of cancer related deaths in women in the United States. By the end of 1994 it is estimated that 22,000 new cases of ovarian cancer will have been diagnosed and that approximately 13,000 deaths will have resulted from this disease. These numbers translate to an average lifetime risk of 1.4% for women in the United States. Furthermore, more than two-thirds of the women with ovarian cancer are diagnosed with advanced disease, when existing therapeutic measures are often ineffective. Thus, understanding the genetic events that lead to initiation and progression of disease remains an important challenge in gynecological research and ultimately may enable the development of better approaches for earlier diagnosis, when current therapeutic strategies have a high cure rate.

It is well accepted that cancer, particularly adult cancer, is a multistep process resulting in an accumulation of genetic lesions in proto-oncogenes or tumor suppressor genes. In ovarian cancer, cytogenetic and loss of heterozygosity studies have implicated many regions of the genome, suggesting that a large number of genes may be involved in ovarian cancer development and/or progression (2–11). We have chosen to characterize chromosome 9 in ovarian cancers, based on the clinical manifestation of ovarian fibromas and cysts of the ovary in patients with nevoid basal cell carcinoma syndrome (NBCCS) and the relationship of this disease with chromosome 9q. A candidate tumor suppressor gene for this syndrome has previously been genetically mapped to 9q22.3–q31. In addition to this putative tumor suppressor gene, genes for multiple self-healing squamous epitheliomata (ESSI), familial dysautonomia (DYS), Fukuyma-type congenital muscular dystrophy (FCMB), and tuberous sclerosis (TSC1) have also been localized to chromosome 9q. Secondly, we have reported previously that rat ovarian surface epithelial cells spontaneously transformed in vitro possess deletions on chromosome 5p, in a region syntenic to human chromosome 9p where the HNF gene cluster resides. It was reported recently that an inhibitor (CDKN2) of cyclin D/cyclin-dependent kinase 4 complexes maps to 9p21 and is homozygously deleted in nearly 30% of ovarian cancer cell lines (12).

In this report we describe the detailed deletion mapping of 41 paired ovarian tumors and corresponding peripheral blood lymphocyte DNA samples for genetic alterations on chromosome 9, using a total of 20 polymorphic DNA markers. In addition, gene dosage studies were performed on 115 fresh ovarian tumors, 11 ovarian carcinoma cell lines, and 1 cervical tumor cell line for deletions of the CDKN2 gene.

MATERIALS AND METHODS

Tumor Samples and Histology. One hundred fifteen ovarian tumors (58 common epithelial tumors, 3 mixed Müllerian tumors, 3 stromal tumors, 37 benign tumors, 7 tumors of low malignant potential, and 7 tumors of some other pathology) were collected from consenting patients undergoing surgery for ovarian cancer at the American Oncological Hospital and the Lankenau Hospital in Philadelphia, or were obtained from the Gynecological Oncology Group/Cooperative Human Tissue Network ovarian tissue bank (Columbus, OH) which is funded by the National Cancer Institute. A portion of the debulked tumor mass was used immediately for DNA isolation, while the remaining tumor sample was either frozen in liquid nitrogen and stored at −70°C or frozen in isopentane. The histopathological classification was based on the typing criteria of the WHO.

Cell Lines. Ovarian cancer cell lines A2780, 1847, PEO1, and SKOV3 were derived from patients prior to treatment with chemotherapy (13–15). The OVCAR ovarian cancer cell lines -2, 3, 4, 8, and 10 were obtained from OVCAR ovarian cancer cell lines -2, 3, 4, 8, and 10 were obtained from patients who were refractory to platinum-based combination chemotherapy, while OVCAR-5 and OVCAR-7 are cell lines derived from ovarian cancer patients prior to treatment. CVCAR-13 was derived from a cervical carcinoma. HOSE1 cell lines were derived from individuals who underwent prophylactic...
of streptomycin, 100 units of penicillin, 0.3 mg of glutamine, and 0.3 unit of porcine insulin/ml. HOSE cells were maintained in MCDB105/199 (1:1) medium (Sigma Chemical Co., St. Louis, MO) supplemented with 4% (v/v) FCS. All cells were grown at 37°C in 5% CO2.

**Southern and Microsatellite Analysis.** Isolation of DNA from tumor and matching blood samples, typing of RFLPs, and PCR typing of STRPs was as described previously (6). The following loci were evaluated: D5S324, 0pter-p22; IFN, 9p21-p22 (16); D5S126, 9p21 (17); MCTI12, 9q21 (18); D5S201, 9q21.3 (19); D5S12, 9q22.2-23 (20); D5S109, 9q31 (21); D9S27, 9q31 (22); D9S53, 9q31 (23); LAMP92, 9q31 (24); D9S58, 9q22.3-23 (25); D9S59, 9q31 (25); HXB, 9q34-36 (26); MCOA12, 9q32 (27); GSN, 9q33 (28); ASS1, 9q34.1 (29); ABL1, 9q34.1 (25); D9S64, 9q34 (26); MCTI364, 9q34.3 (29); and EF D126.3, 9q34 (30). Allelic loss was interpreted by D. C. S., L. V., and A. K. G. LOH was characterized by the absence or reduction in the signal intensity of one allele relative to the other in tumor versus constitutional samples. DNA RFLPs or STRPs were classified as heterozygous with no loss, heterozygous with loss, or homozygous/uninformative.

**Gene Dosage Analysis of CDKN2.** A total of 41 assays of CDKN2 genotypes were amplified in three segments with the following primers: exon 1, 5'-GAAAGAAGGGAGGGCTGACCTG-3' and S'-AACTTGCTTTTAAGCCATAAC-S'); exon 2, 5'GGAATTTG-GAAAATGGAAG-3' and 5'-TCTGAATCTTGGAGACTCCT-3'; exon 3, 5'-GGATTCTCCACTTGTGTTTGG-3' and 5'-ATGATTACGAAAAGGCGG-3'. Amplification of exons was performed in a final volume of 50 µl containing 50 ng of DNA template, 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin, 5% DMSO, 100 µM each dNTP, 2.5 units of AmpliTaq DNA polymerase (Perkin Elmer Cetus), and 1 µM each primer. DNA was amplified with an initial denaturation step of 2 min at 94°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, elongation at 72°C for 1 min, and a final elongation step for 10 min at 72°C.

**RESULTS**

**Loss of Heterozygosity Analysis.** Forty-one ovarian tumors (29 common epithelial tumors, 2 recurrent adenocarcinomas, 2 mixed Müllerian tumors, 1 clear cell tumor, 1 teratoma, 1 Granulosa cell tumor, 1 Brenner tumor, 2 mucinous cystadenomas, and 2 thecomas) were examined for genetic alterations on chromosome 9. DNA isolated from these tumors was analyzed with the use of 5 RFLP DNA probes (all on 9q) and 15 STRP PCR-primer pairs (3 for 9p and 12 for 9q). Concordant results were observed between the two methods as characterized in a direct comparison between D9S29 (LAMP 92) and D9S53 (Figs. 1 and 2). Loss of heterozygosity for at least one informative marker on chromosome 9 was observed in 63% (26 of 41) of our tumors (Table 1). Allelic loss was observed in 56% (23 of 41) of tumors informative for markers on 9q, in contrast to 13% (5 of 40) for informative 9p markers. It should be noted that for 2 of the tumors that show LOH for markers on 9q, LOH was concordant on 9q. The other three cases that demonstrated allelic loss for 9p markers, tumors 83, 89, and 160 retain all informative markers for 9q (Fig. 2). Of the 41 tumors studied, 6 were obtained from patients with a family history of ovarian cancer or breast cancer. When we examined 2 early stage and 4 late stage familial ovarian cancers for LOH at these loci, we observed a higher frequency of allelic loss on chromosome 9q (83%) and 9p (17%), as compared to the sporadic tumors (Table 1).

Loss of heterozygosity was characterized according to the histopathological subtypes of the tumors. LOH for informative markers on 9q was observed for 88% (7 of 8) and 67% (12 of 18) of the undifferentiated and serous papillary adenocarcinomas, respectively, as compared to 12% (3 of 26) for both subtypes at informative 9p markers (Table 1). Furthermore, Table 1 also illustrates that only one of four endometrioid adenocarcinomas and the only mucinous adenocarcinoma demonstrate loss of heterozygosity for an informative marker on chromosome 9q. Table 2 demonstrates that allelic loss on 9q was more common in late stage tumors (63%; 17 of 27) and high grade (III) tumors (75%; 15 of 20).

Retention of heterozygosity at all informative loci on chromosome 9 was observed in 15 of the 41 tumors evaluated. Of the 23 tumors that demonstrate LOH for 9q markers, 9 of these tumors showed allelic loss for all informative markers on 9q and retention of heterozygosity for 9p markers. Fifteen of the tumors evaluated revealed limited regions of genetic imbalance. The pattern of genetic imbalance for these tumors is illustrated in Fig. 2. Analyses of tumors 57, 60, 81, and 167 reveal large interstitial deletions with telomeric retention. Tumor 82 revealed allelic loss for all informative markers distal to D9S109. Tumors 78, 79, 93, 120, 142, and 173 reveal deletions telomeric to the HXB (hexabraxion) locus and retention of heterozygosity informative proximal markers. In addition to the telomeric loss, tumor 78 also revealed the amplification of an allele for the marker MCOA12 (D9S16), as represented in Fig. 3A. Of particular interest is tumor 66, a stage I mixed Müllerian tumor. This tumor does not show LOH for any of the loci typed on chromosome 9, but microsatellite instability was observed for the markers D9S109, D9S127, and D9S53, exclusively (Fig. 3B). The mutated alleles were subcloned and sequenced to verify a 2-bp addition in the lower allele for D9S109 and D9S53, and a 4-bp deletion in the lower allele for D9S127. Furthermore, genome wide allelotypeing of this tumor with more than 60 different dinucleotide and tetranucleotide repeat polymorphisms revealed no other genetic alterations (Fig. 3C and data not shown), suggesting...
Fig. 1. Representative autoradiograms demonstrating allelic loss on chromosome 9q at (A) D9S126, D9S53, D9S59, ASS and (B) LAMP 92 (D9S29). DNA isolated from peripheral blood lymphocytes and ovarian tumor tissue were assayed as described in "Materials and Methods." Allelic loss was scored by either the absence or reduction in signal intensity of one allele relative to the other in tumor versus constitutional samples. N, constitutional DNA isolated from peripheral blood lymphocytes; T, tumor DNA; Om, omentum DNA.

Regional genetic instability in this area of chromosome 9 for this tumor.

Deletion Mapping of Minimum Regions of Overlap. The patterns of genetic imbalance observed in these tumors define two candidate tumor suppressor regions on chromosome 9q and one on chromosome 9p. As illustrated in Fig. 2, a region at 9q32–q34 is defined by a minimal region of imbalance spanning the markers HXB and ASS1 (arginosuccinate synthetase) and a genetic distance of approximately 23 cM. Analysis of our tumors reveals that 53% (21 of 40) show allelic loss in the this region. Of the 23 tumors in which allelic loss was observed on 9q, 6 tumors (78, 79, 93, 120, 142, and 173) exclusively demonstrated LOH for markers at 9q32–q34. If the microsatellite instability data is used as a mapping tool, a second region at 9q31 is defined by a minimal region of instability of approximately 1.5 cM, which is bounded by, but does not include, the anonymous markers D9S127 and D9S53 (Fig. 2). In our panel of ovarian tumors, 39% (16 of 41) show genetic imbalance for informative markers localized to 9q31. A third candidate tumor suppressor region has been identified on 9p, and this region extends distal to the marker D9S126 but proximal to INFA, as defined by tumors 81 and 83.

Mutational Analysis of CDKN2. On the basis of the recent observation that an inhibitor (CDKN2) of the cyclin-dependent kinase 4 mapped to 9p21 and was homozygously deleted in approximately 50% of cell lines derived from various tumor types and nearly 30% of ovarian cancer cell lines evaluated, we examined the gene dosage of CDKN2 in the 41 initial ovarian neoplasms, as well as an additional 74 ovarian tumors by comparative...
multiplex PCR. In addition 11 ovarian cancer cell lines and 1 cervical cancer cell line were also analyzed for CDKN2 deletions. As determined by comparative multiplex PCR, homozygous deletions of the CDKN2 gene were observed in 14% (16 of 115) of fresh ovarian tumors (Fig. 4) and 3 of 12 cancer cell lines (Fig. 5A). Furthermore, of the 5 fresh tumors demonstrating LOH on 9p, 2 demonstrated reduction to hemizygosity for CDKN2. For those tumors showing allelic loss for markers on 9p, none possessed a somatic mutation in the retained allele of CDKN2 as determined by SSCP. However, a nonsense mutation was discovered in exon 2 of OVCAR10 (G to A at nucleotide position 324), resulting in a termination of translation at codon 102. Furthermore, as expected, expression of CDKN2 was absent in the 3 cell lines showing homozygous deletion of CDKN2, while the 9 cancer cell lines that retain CDKN2 were similar to the mRNA levels observed in 6 normal human surface epithelial cell lines (Fig. 5B).

Table 1 Relationship between loss of heterozygosity on chromosome 9 and histopathological subtype in sporadic versus familial ovarian cancer

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<td>35</td>
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<td>Overall (%)</td>
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<td>26/41 (63%)</td>
<td>5/40 (13%)</td>
<td>23/41 (56%)</td>
<td>16/41 (39%)</td>
<td>21/40 (53%)</td>
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* LOH with any informative marker on chromosomal arm out of the total number of informative tumors in that histopathological subtype.
- The number of cases of LOH affecting informative markers within the chromosomal region 9q31 or 9q32–q34 out of those tumors with any LOH on the long arm of chromosome 9.
  - Serous papillary adenocarcinomas include serous papillary cystadenocarcinomas, serous carcinomas, and papillary carcinomas.
DISCUSSION

It is becoming increasingly clear that multiple genetic alterations are required for the initiation and progression of cancer. In ovarian cancer much attention has been focused on chromosome 17 as a consequence of many studies involving the familial breast/ovarian cancer susceptibility gene, BRCA1, on 17q21 (31). However, the potential role of this gene in the development of sporadic ovarian cancers remains to be seen (32). Moreover, numerous additional genes and chromosomal regions have been implicated in ovarian tumorigenesis (2–11).

We have chosen to characterize chromosome 9 for genetic alterations in a panel of human ovarian tumors, based on the development of ovarian fibromas and cysts in the ovary in patients with the autosomal dominant NBCCS and the relationship of this disease to chromosome 9q (33). Second, we have reported previously that rat ovarian surface epithelial cells spontaneously transformed in vitro have deletions on chromosome 5, in a region syntenic to human chromosome 9p where the IFN gene cluster resides (34).

In this study we demonstrate that 59% (24 of 41) of our tumors display genetic imbalance (i.e., loss of heterozygosity, microsatellite instability, amplification) on the long arm of chromosome 9 (Table 1 and Fig. 2). This result is in agreement with LOH results reported by Cliby et al. (54%; Ref. 10) and Osborne and Leech (48%; Ref. 11) for 9q in large allelotyping studies of common epithelial ovarian tumors. Moreover, the high frequency of allele loss for markers on 9q suggests that this alteration is a nonrandom phenomenon. Furthermore, allelic loss on chromosome 9q was observed in 75% (15 of 20) of high grade (III) tumors, and 63% (17 of 27) of late stage (III and IV) tumors (Table 2), which is similar to the values reported by Dodson et al. (35). It should be noted that in a previous study (6) in which chromosome 17 alterations were evaluated, all tumors that showed 9q alterations were also observed to have chromosome 17 changes. Thus, similar to other molecular abnormalities suggested previously (10, 36–38), acquisition of genetic alterations on chromosome 9q may be instrumental in acquiring the aggressive tumor phenotype.

In order to better define the regions on chromosome 9q in which a candidate growth or tumor suppressor gene(s) may reside, we have created a high density deletion map of 9q using 17 highly polymorphic markers. Limited regions of genetic imbalance were observed in 15 of the tumors evaluated. The pattern of genetic imbalance in these tumors identifies two potential subchromosomal regions that may harbor candidate genes. Our frequent observation of LOH for markers at 9q32–q34 (53%; 21 of 40) is suggestive of a candidate tumor suppressor locus on chromosome 9q. Moreover, nearly 100% (23 of 24) of the tumors that show genetic imbalance for any informative 9q
CHROMOSOME 9 ALTERATIONS IN OVARIAN NEOPLASIA

Fig. 4. Comparative multiplex PCR analysis of CDKN2 (p16) in ovarian cancers. N, 6-ng amplified normal DNA; T, 12-ng amplified tumor DNA; Tp, 6-ng amplified tumor DNA; Tp, 3-ng amplified tumor DNA. Tumors 81 and 89 demonstrate homozygous deletion of CDKN2, confirmed by Southern blotting (data not shown).

marker include alterations for 9q32-q34 markers, suggesting that this region may in fact be closer to the gene of interest. Several candidate genes that have been mapped to this subchromosomal region include the c-Abelson proto-oncogene (c-ABL), hexabrachion (HXB), gelso-lin (GSN), and Xeroderma pigmentosum, complementation group A (XPA). Although considered by many to be a proto-oncogene, overexpression of c-ABL has been shown to inhibit growth by causing cell cycle arrest. Overexpression of a dominant negative c-ABL disrupts cell cycle control and enhances phenotypic transformation (39). Furthermore, hexabrachion/tenascin is an extracellular glycoprotein that has been proposed as a candidate tumor suppressor gene. It has been reported that HXB can support epithelial tumor growth in serum-free medium and is abnormally expressed in common epithelial tumors (40, 41). However, deletion mapping in our tumor panel has placed this gene outside the minimal region of deletion observed for tumors 78, 79, 93, 120, 142, and 173. An interesting candidate gene in this region may be the gene for Xeroderma pigmentosum, complementation group A (XPA), the gene product of which participates in the process of nucleotide excision repair of mutagen-damaged DNA. Recently, mutations in the DNA mismatch repair enzymes hMSH2, hMLH1, PMS1, and PMS2 have been implicated in a subset of sporadic colorectal cancers and most tumors developing from hereditary nonpolyposis colorectal cancer (42-44). If the XPA gene is genetically altered in ovarian tumors, it would be additional support for the concept that genes responsible for maintaining the integrity of the genome are important in the initiation or progression of human cancers.

In addition to the potential tumor suppressor locus at 9q32-q34, a second candidate locus on 9q can be defined by several tumors. Tumors 57, 60, and 82 demonstrate allelic loss distal to the markers D9S127, D9S201, and D9S109, respectively. Tumor 160 demonstrates a large deletion extending proximal to D9S53, and no mutation was observed in CDKN2. In addition to the partial deletions observed in these 4 tumors, tumor 66 exhibits microsatellite instability for the closely linked markers D9S109, D9S127, and D9S53, as shown in Fig. 3B. It is important to note that no other genetic alterations were observed for more than 60 different di- and tetranucleotide repeat polymorphisms typed genome wide in this tumor (data not shown). In general, utilizing these 4 tumors, we have identified a minimal region of imbalance, spanning markers D9S127 and D9S53 at 9q31 and a distance of approximately 1.5 cM. Furthermore, this region of chromosome 9 was genetically altered in 41% (17 of 41) of the tumors examined. With regard to tumor 66, it is noteworthy that reports suggest that approximately 8% of human cancers examined demonstrate a pattern of microsatellite instability that differs from that responsible for the replication error-positive phenotype observed in

A.

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Fig. 5. Analysis of CDKN2 in ovarian carcinoma cell lines. (A) Ethidium bromide-stained agarose gels of PCR amplified exon 1 (top), 2 (middle), and 3 (bottom) in 11 ovarian carcinoma cell lines, and 1 cervical carcinoma cell line. Homozygous deletions of the 350-bp exon 1 and 500-bp exon 2 PCR products were observed in OVCAR-5, PEO-1, and SKOV3. The 200-bp exon 3 PCR product is homozygously deleted in OVCAR-5 and PEO-1. (B) Reverse transcriptase PCR amplification of CDKN2 (p16) mRNA in human ovarian surface epithelial cell lines and ovarian cancer cell lines (top). The human Xeroderma pigmentosum complementation group A gene (XPA) mRNA served as a control (bottom). M.W., molecular weight.

B.

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colorectal tumors (45). However, the phenotype observed in tumor 66 may be the first reported example of regional chromosomal instability observed in epithelial tumors of the ovary.

It was originally reported that the gene predisposing to NBCCS and sporadic basal cell carcinomas maps to chromosome 9q22.3–q31 and is believed to be a tumor suppressor gene, as would be predicted by genetic linkage and somatic loss of the wild-type allele (33, 46–48). This autosomal dominant disorder predisposes to basal cell carcinomas of the skin and other neoplasms, including ovarian fibromas. Furthermore, we have observed a higher than expected incidence of basal cell carcinomas in breast and ovarian cancer syndrome families which are not linked to BRCA1 on chromosome 17q.4 However, recent studies of large kindreds affected with NBCCS revealed recombinants that have refined the localization of the NBCCS gene distal to D9S12 but proximal to D9S109 (49, 50). Thus, our data have identified the locus of a possible novel gene in this region of chromosome 9q.

A third region of imbalance has been identified on the short arm of chromosome 9. Previous studies have identified 9p13–p22 as a frequent site of chromosomal loss in leukemias, gliomas, melanomas, lung carcinomas, bladder carcinomas, and malignant mesotheliomas. Overall, 13% of the tumors evaluated showed allelic loss for a 9p marker. Although this finding is much lower than the 30% LOH reported for 9p markers in large allelotyping studies of ovarian tumors (9–11, 51), it appears that genetic alterations of genes on 9p play a less frequent role in the initiation and/or progression of ovarian cancer.

It has been reported recently that an inhibitor (CDKN2) of cyclin-dependent kinase 4 maps to 9p21 and is deleted homozygously in approximately 50% of cell lines derived from several tumor types including ovarian cancer (12). If the protein referred to as p16 is truly a classic tumor suppressor gene, it would establish a link between cell cycle control and cancer genes. Although it was reported that nearly 30% of ovarian cancer cell lines show homozygous deletions for the CDKN2 gene, we have observed homozygous deletions of the CDKN2 gene in 14% (16 of 115) of fresh tumors (Fig. 4), and homozygous deletions were observed in 3 of 12 cancer cell lines evaluated (Fig. 5A). It could be argued that homozygous deletions might be overlooked by PCR amplification due to the logarithmic coamplification of contaminating constitutional DNA. However, comparative multiplex PCR enables one to control for amplification of contaminating constitutional DNA and loading differences (52). Furthermore, no somatic mutations in the retained allele of CDKN2 were observed in those tumors showing allelic loss for chromosome 9p markers. However, a nonsense mutation that has been reported previously was discovered at codon 102 (Trp to Stop) in OVCAR10, making alterations of CDKN2 twice as prevalent in cell lines (33%) as compared to fresh tumors (14%). Detection of message for CDKN2 was consistent between the 9 cell lines that retain CDKN2 when compared to the expression of CDKN2 mRNA in 6 human surface epithelial cell lines. The absence of expression of CDKN2 in OVCAR-5, PEO-1, and SKOV3 was expected, based on the observation that the gene is deleted homozygously in these cell lines (Fig. 5A and B). Thus, the apparent normal expression of CDKN2 in ovarian cancer cell lines, and the infrequent observation of alterations in gene dosage, emphasizes the involvement of CDKN2 in the pathogenesis of ovarian cancer.

Although the role of tumor suppressor genes in the development of ovarian cancers has not been delineated completely, the high frequency of genetic imbalance observed for chromosome 9q markers in our panel of ovarian tumors adds to the growing list of human neoplasms in which the dysfunction of 9q genes have been implicated. It has been reported that greater than 50% of bladder tumors show LOH for chromosome 9 markers (53–56). Cairns et al. (53) have identified a common region of deletion on 9q, mapping proximal to 9q22.3 in a large cohort of bladder tumors. Deletion mapping studies in small cell lung cancers have identified a similar region of allelic loss at 9q22.3 (57). More recently, it was suggested that a candidate tumor suppressor gene on 9q for a subset of superficial bladder tumors resides in the 9q34.1–q34.3 (58). Furthermore, a subgroup of non-Hodgkin’s lymphoma has been characterized cytogenetically to have consistent deletion involving 9q31–32 (59). On the basis of our observation of frequent imbalance for markers distal to 9q22.3, it appears that we have identified two regions which could harbor novel gene(s) important in the pathogenesis of ovarian cancer.

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Characterization of Chromosome 9 in Human Ovarian Neoplasia Identifies Frequent Genetic Imbalance on 9q and Rare Alterations Involving 9p, Including *CDKN2*


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