

Analysis of DNA Copy Number Alterations in Ovarian Serous Tumors Identifies New Molecular Genetic Changes in Low-Grade and High-Grade Carcinomas

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Abstract

Ovarian serous carcinoma, the most common and lethal type of ovarian cancer, is thought to develop from two distinct molecular pathways. High-grade (HG) serous carcinomas contain frequent TP53 mutations, whereas low-grade (LG) carcinomas arise from serous borderline tumors (SBT) and harbor mutations in KRAS/BRAF/ERBB2 pathway. However, the molecular alterations involved in the progression from SBT to LG carcinoma remain unknown. In addition, the extent of deletion of tumor suppressors in ovarian serous carcinomas has not been well studied. To further address these two issues, we assessed DNA copy number changes among affinity-purified tumor cells from 37 ovarian serous neoplasms including SBT, LG, and HG tumors using high-density 250K single nucleotide polymorphism arrays. Chromosomal instability index as measured by changes in DNA copy number was significantly higher in HG than in LG serous carcinomas. Hemizygous ch1p36 deletion was common in LG serous carcinomas but was rarely seen in SBT. This region contains several candidate tumor suppressors including miR-34a. In contrast, in HG serous carcinomas, significant numbers of amplifications and deletions, including homozygous deletions, were identified. Among homozygous deletions, loci containing Rb1, CDKN2A/B, CSMD1, and DOCK4 were most common, being present in 10.6%, 6.4%, 6.4%, and 4.3%, respectively, in independent 47 affinity-purified HG serous carcinomas. Except for the CDKN2A/B region, these homozygous deletions were not present in either SBT or LG tumors. Our study provides a genome-wide homozygous deletion profile in HG serous carcinomas, which can serve as a molecular foundation to study tumor suppressors in ovarian cancer. [Cancer Res 2009;69(9):4036–42]

Introduction

In the United States, ovarian cancer is responsible for more cancer deaths than any other neoplasms of the female reproductive organs, with an estimated 15,520 deaths in 2008 (1). Ovarian cancer is a heterogeneous group of diseases, and among them, serous

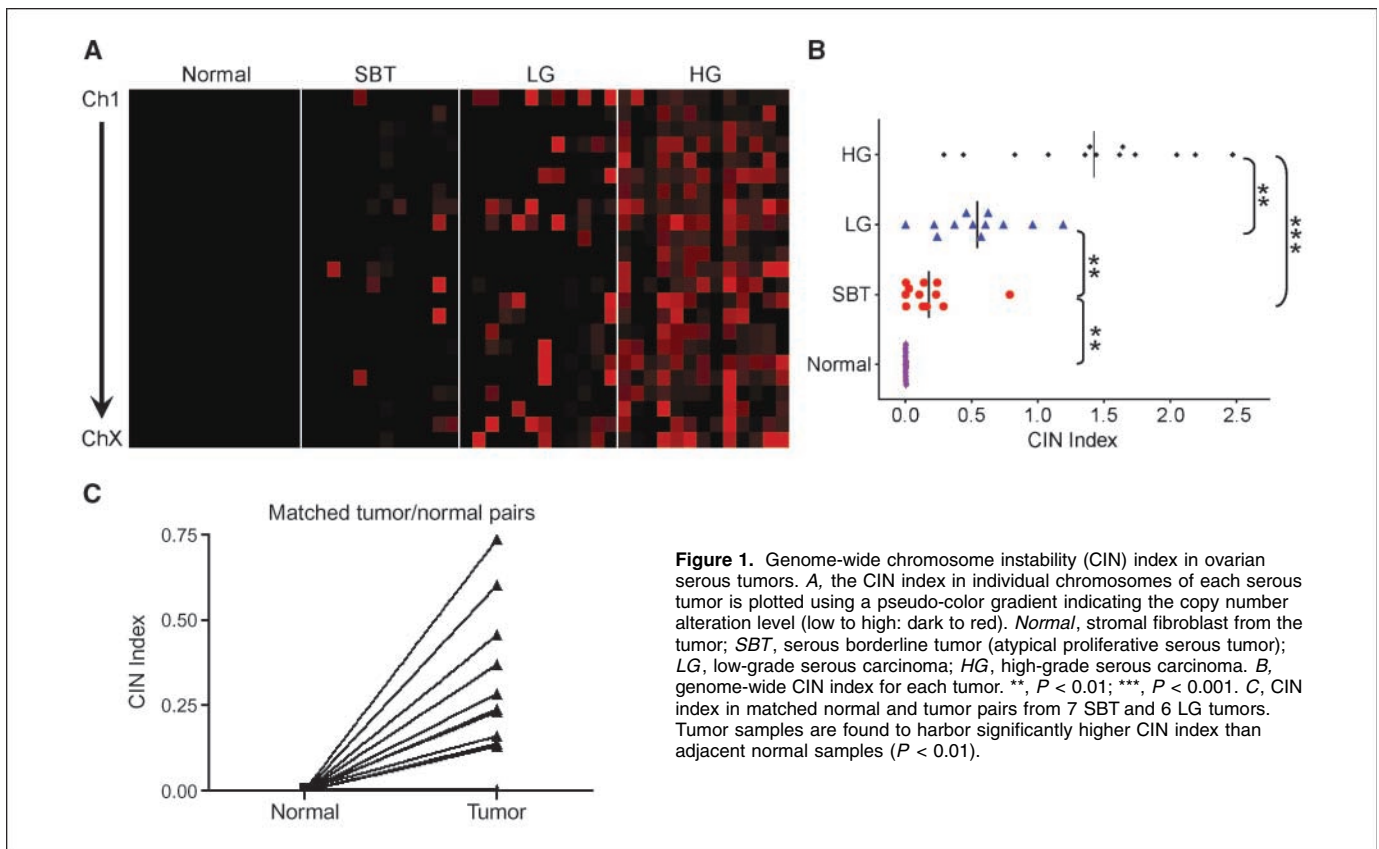
carcinoma is the most common type, representing approximately half of ovarian cancers. The majority of serous carcinomas are high-grade and have been thought to arise in a progressive fashion from benign serous cystadenoma to serous borderline tumor (SBT; also known as serous tumor of low malignant potential or atypical proliferative serous tumor), to low-grade (LG) serous carcinoma, and then finally to high-grade (HG) serous carcinoma (2). However, clinicopathologic observations and recent molecular genetic studies from several research groups have challenged this paradigm. Two distinct pathways are now thought to lead to the development of LG and HG serous carcinomas (3–10). LG carcinomas are now thought to develop from SBT and progress in a stepwise fashion because histologic transitions can be found in SBT and LG serous carcinomas from the same specimen; furthermore, both SBT and LG lesions share similar molecular genetic changes (3). They are slow-growing, indolent tumors that have a relatively good prognosis compared with HG carcinomas. Molecular genetic analysis has shown that SBT/LG serous carcinomas typically display sequence mutations in *KRAS/BRAF/ERBB2* but with infrequent mutations in *TP53* (11–13). In contrast, HG serous carcinomas often present in advanced stages (stages III and IV) and rarely harbor mutations in *KRAS/BRAF/ERBB2*. HG serous carcinomas grow rapidly and are highly aggressive and >75% of these tumors harbor *TP53* mutations (14–18).

DNA copy number alterations including chromosomal amplification, deletion, and aneuploidy are the hallmarks of neoplasia (19). Amplification is one of the mechanisms that lead to an increase in activity of oncogenes and development of drug resistance (20, 21), whereas allelic deletion results in inactivation of tumor suppressors. Identification and characterization of genes within the amplified and deleted chromosomal loci not only provide new insights into the pathogenesis of cancer but may also lead to new approaches to diagnosis and therapy. Previous studies have applied different approaches to analyze DNA copy number changes on a genome-wide scale (22–28), but most combined different histologic types of ovarian carcinomas such as serous, endometrioid, clear cell, and mucinous tumors in the analysis, and very few included LG serous carcinomas and SBTs. A head-to-head comparison between HG and LG serous carcinomas and between LG serous carcinomas and their precursor lesions, SBTs, has not been published. Furthermore, although studies have identified several convincing amplification events in ovarian cancer, detection of deletions, especially homozygous ones, has been challenging because (a) it requires technologies with a sufficient resolution and (b) it requires samples that are highly enriched with tumor cells because the presence of normal stromal cells or endothelial cells can mask deletions. To overcome these limitations in this study, we

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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applied high-density 250K single nucleotide polymorphism (SNP) arrays and used affinity-purified tumor cells from fresh specimens to address two biological questions that are related to the pathogenesis of ovarian cancer and have not yet been elucidated: (a) What is the cardinal molecular genetic alteration(s) in the transition from SBT to LG serous carcinoma and (2) What are the deleted tumor suppressor genes in HG serous tumors?

Materials and Methods

Tissue samples. Tissue samples from 12 SBTs, 12 LG ovarian serous carcinomas, and 13 HG ovarian serous carcinomas were collected from the Department of Pathology, Johns Hopkins Hospital. The acquisition of the anonymous tissue specimens for this study was approved by the Institutional Review Board at the Johns Hopkins Medical Institutions.

The status of primary/recurrence and the mutational status of *TP53/KRAS/BRAF* of each sample are listed in Supplementary Table S1. Fresh tumor specimens were digested with collagenase (1 mg/mL), washed with RPMI, and affinity-purified using the anti-Ber-EP4-conjugated magnetic beads (Invitrogen) as described previously (29, 30). The purity of the isolated tumor cells was confirmed by immunostaining using an anti-cytokeratin-8 antibody (CAM5.2; Becton Dickinson). The negatively sorted cellular fraction, which represented the tumor adjacent normal stromal cells, was separately cultured. The stromal fibroblasts showed distinct morphology and their purity was confirmed by positive vimentin immunoreactivity and negative Ber-EP4 and CD31 immunoreactivities. Anti-vimentin was purchased from Sigma, anti-Ber-EP4 was from Abcam, and anti-CD31 was from Invitrogen.

SNP array. SNPs were genotyped using 250K *Sty1* arrays (Affymetrix) in the Microarray Core Facility at the Dana-Farber Cancer Institute. A detailed protocol is available on the Core Center Web page.⁶ Briefly, genomic DNA

was fragmented using the restriction enzyme, *Sty1*, ligated with linkers, followed by PCR amplification. The PCR products were purified and then digested with DNase I to a size ranging from 250 to 2,000 bp. Fragmented PCR products were labeled with biotin and hybridized to the arrays. Arrays were then washed with the Affymetrix fluidics stations. The bound DNA was fluorescently labeled using streptavidin-phycoerythrin conjugates and scanned using the Gene Chip Scanner 3000.

Analysis of SNP array data was done using the dChip 2006 program (24, 31). Data were normalized to a baseline array with median signal intensity at the probe intensity level using the invariant set normalization method. A model-based (PM/MM) method was employed to obtain the signal values for each SNP in each array. Signal values for each SNP were compared with the average intensities from 15 normal samples, among which 13 samples are tumor adjacent stromal fibroblasts purified from the same patients whose tumor samples were analyzed by SNP array. The remaining two samples are normal fallopian tubes. To infer the DNA copy number from the raw signal data, we used the Hidden Markov Model (31) based on the assumption of diploid for normal samples. Mapping information of SNP locations and cytogenetic bands were based on curation of Affymetrix and University of California Santa Cruz hg17. In this study, we used an arbitrary cutoff of >3 copies in >6 consecutive SNPs to define amplification, a cutoff of <0.5 copy to define homozygous deletion, and a copy number between 0.5 and 1.5 to define hemizygous deletions. If there were intermittent, nonconsecutive probes with inferred copy numbers that deviated ± 0.1 copies from the cutoff criteria, this probe was considered within the cutoff range. The DNA copy number changes in all samples were analyzed using the same criteria.

⁶ <http://biosun1.harvard.edu/complab/dchip/>

Index of DNA copy number changes (chromosome instability index).

To facilitate the quantification of subchromosomal copy number alterations, we applied the Circular Binary Segmentation algorithm (32) to count copy number changes and used a chromosome instability (CIN) index to determine the levels of DNA copy number changes. In this study, amplification was defined as a region with a copy number >3 in at least six contiguous SNPs, whereas homozygous deletion was defined as a region with a copy number <0.5 in at least six contiguous SNPs. Hemizygous deletion was defined as a region where the copy number fell between 0.5 and 1.5 in at least six contiguous SNPs. We further computed the CIN index for each chromosome at a genome-wide scale based on the total amount of gain and loss. The chromosome-specific CIN index was defined as the sum of amplitudes of all gain/loss segments divided by the total number of SNPs in the chromosome, and the genome-wide CIN index was defined as $\log(C_1 + 1) + \dots + \log(C_i + 1) + \dots + \log(C_{23} + 1)$, where C_i is the CIN index of chromosome i . For a gain segment, the amplitude was the average intensity of SNP signals within the segment. For a loss segment, to match the effect of losses to the same scale of gains, the amplitude was calculated by $2.5 + (A - 2.5)(1.5 - a) / 1.5$, where a was the average intensity of SNP signals within the loss segment and A was the maximum gain amplitude across all cases on the same chromosome.

Western blot analysis. Western blot analysis was done on representative HG tumors to test if tumors with apparent homozygous deletions expressed the gene products. Similar amounts of protein were loaded in 10% to 12% Tris-

acrylamide gels, and after transblotting, the membranes were probed with either anti-pRb antibody (clone IF8; Santa Cruz Biotechnology) or anti-p16 antibody (clone G175-1239; BD Biosciences). GAPDH was used as the loading control. After secondary antibody incubation, membranes were developed using chemiluminescence and detected using Chemidoc XRS (Bio-Rad).

Detailed methods for quantitative real-time PCR, statistic analysis, and transfection and functional study of miR-34a are described in Supplementary Materials and Methods.

Results

Global DNA copy number alterations detected in ovarian serous tumors. The genome-wide DNA copy number profiles in 12 SBTs, 12 LG, and 13 HG serous carcinomas are shown in Supplementary Fig. S1. Compared with SBTs and LG serous carcinomas, HG serous carcinomas showed widespread DNA copy number gains and losses involving all chromosomes. To compare the overall levels of DNA copy number changes in different serous neoplasms, we used the CIN index based on counting the total numbers of discrete DNA fragments showing either gain or loss in each sample. First, we compared the CIN index between tumor adjacent stromal fibroblasts (isolated from 7 SBTs and 6 LGs) and their corresponding tumors. Figure 1A shows CIN index at each chromosome and Fig. 1B shows genome-wide CIN index (the combined CIN index from all chromosomes) of each specimen. The results showed that the CIN index of normal samples was around zero in all chromosomes, whereas their tumor counterparts showed an elevated CIN index. Paired t test was done to show a statistically significant difference in the CIN index between normal samples and matched tumors ($P < 0.01$). In contrast, serous LG and HG carcinomas showed an elevated CIN index (Fig. 1). In LG serous carcinomas, the chromosomes with the highest CIN index were chromosomes 1, 22, and X, whereas chromosomes 2, 3, 5, 7, 11, and 12 had the lowest CIN index (Supplementary Fig. S2). In HG tumors, all chromosomes had a high CIN index (Supplementary Fig. S2). Compared with SBTs and LG tumors, HG tumors showed the highest level of DNA copy number alterations as their overall CIN index was significantly higher than the CIN index of either LG serous carcinomas or SBTs (Fig. 1B). LG serous carcinomas also exhibited a significantly higher CIN index than SBTs, indicating that there were more chromosomal rearrangements in LG serous carcinomas than in SBT tumors (Fig. 1B).

Frequent deletions in the ch1p36 and ch9p21 loci in LG tumors. To identify subchromosomal regions that are potentially involved in the progression from SBT to LG serous carcinoma, we compared the profiles of DNA copy number changes between both types of ovarian tumors. Our results showed that the most significant DNA copy number changes in LG carcinomas were deletions at ch1p36 and ch9p21.3, which occurred in 7 of 12 and 6 of 12 of LG tumors, respectively (Fig. 2). Of note, ch1p36 deletions were all hemizygous, whereas 2 specimens of ch9p21.3 deletions were homozygous and 4 were hemizygous. One of the SBTs also harbored ch1p36 hemizygous deletion, but none of the SBTs contained detectable ch9p21.3 deletions. Minimal mapping of the ch1p36 deletion in these 7 LG serous carcinomas revealed that there were two immediately adjacent deleted regions: one spanning the interval from 5,579,980 to 16,540,200 bp and the other spanning the interval from 17,035,400 to 31,700,900 bp (Fig. 3). Notably, *CHD5* and *miRNA-34a*, previously reported tumor suppressor genes, are located within the first common deleted region in LG carcinomas. To determine if *CHD5* was inactivated by point mutations, we performed DNA sequence

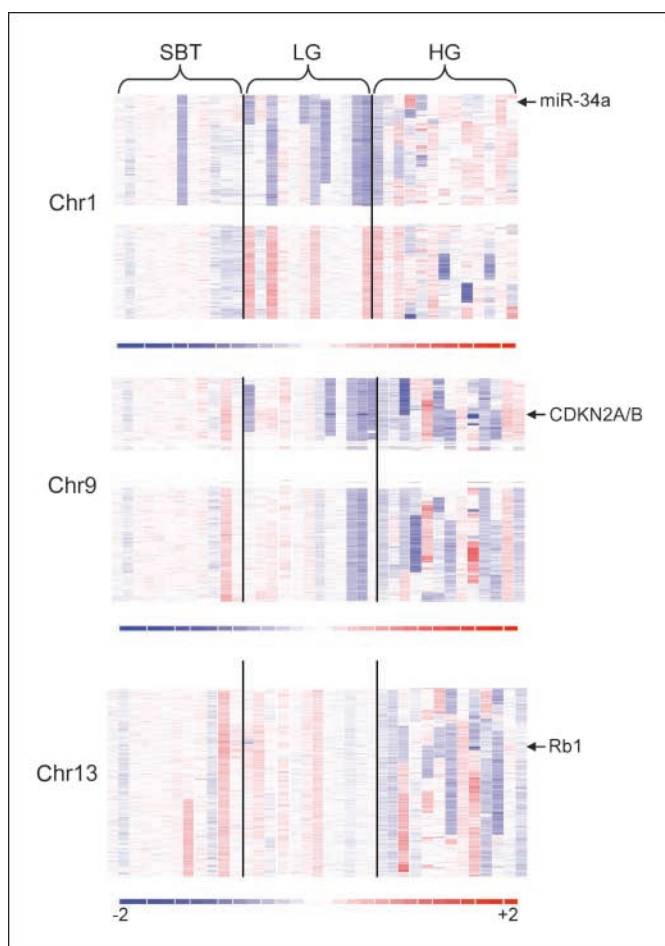


Figure 2. Copy number alteration in chromosome 1, 9, and 13. DNA copy number changes are represented as pseudo-color gradients corresponding to the copy number increase (red boxes) and decrease (blue boxes) as compared to pooled normal samples. Each column represents an individual tumor sample. Arrow indicates candidate tumor suppressor gene at each region.

analysis on the 12 LG carcinomas used in the SNP array analysis and on the SBT that also harbored the ch1p36 hemizygous deletion. However, we did not identify somatic mutations in *CHD5*.

Minimal mapping of the two LG serous carcinomas with homozygous deletion in ch9p21.3 region showed that the core deletion, spanning 21,945,000 to 22,009,700 bp and which harbors the *CDKN2A/B* locus, encodes p16, p15, and Arf (p14; Fig. 4A). To determine if additional mutations were present in both *CDKN2A* and *CDKN2B*, we performed DNA sequence analysis of these genes among 10 LG serous carcinomas and 9 SBTs but did not detect somatic point mutations in any of these specimens.

Additional genomic alterations present in LG serous carcinoma but not in SBTs included a 3-fold gain at ch1q and a hemizygous deletion of the entire X chromosome. These regions may contain genes important in the progression from SBT to LG serous carcinoma.

Profiles of DNA copy number changes in HG serous carcinomas. The current 250K SNP array analysis revealed that the most frequently amplified regions in HG serous carcinomas were the loci at ch3q, ch12p, and ch19p, all of which have been reported in our previous study using a 10K SNP array platform (27). Supplementary Table S2 summarizes the most common amplicons identified among HG serous carcinomas in this study. Because genomic amplifications in the ovarian cancer have been previously characterized in several studies, including our own (9, 26, 27, 33–37), in this report, we focused on analyzing homozygous deletions that were difficult to be discovered by previous approaches. In this regard, we took advantage of affinity purification of tumor cells, which would enhance the sensitivity of detecting homozygous deletions. Furthermore, we used the relatively high-density 250K SNP platform because our pilot study has shown that it can reliably detect a previously reported 450 kb homozygous deletion in DiFi cells (32). Supplementary Table S3 summarizes the most common regions carrying deletions identified in this study. Among the loci that were deleted were *Rb1* (ch13q14.2), *CDKN2A/B* (ch9p21.3), *CUB* and *sushi multiple domains 1* (*CSMD1*; ch8q23.1–23.3), and *dictator of cytokinesis 4* (*DOCK4*; ch7q31.1), all of which have been previously reported to be homozygously deleted in human cancers (Fig. 4B). Ten of the homozygous deletion loci were randomly selected for validation by quantitative real-time PCR. We were able to confirm each of the selected deletions (data shown in Supplementary Table S3). None of the deletions were present in the matched normal tissues, indicating that these homozygous deletions in HG serous carcinomas were somatic changes acquired during tumor progression. To further assess the frequency of deletions of *Rb1*, *CDKN2A*, *CSMD1*, and *DOCK4*, we used genomic quantitative real-time PCR on an independent panel of 47 affinity-purified HG serous carcinomas. The frequency of homozygous deletion of *Rb1*, *CDKN2A*, *CSMD1*, and *DOCK4* was 10.6%, 6.4%, 6.4%, and 4.3%, respectively. Specific antibodies against two of the known tumor suppressors, pRb and p16 (encoded by *CDKN2A*), were available, which permitted us to determine if the deletion affected protein expression. Eleven representative HG serous carcinomas with known DNA copy number status in both regions were included in Western blot analysis. As expected, the results were entirely consistent with the deletional status of each locus. As shown in Fig. 5, two HG serous carcinomas with homozygous deletions in the *Rb1* locus completely lost pRb protein expression. Two tumors of the four tumors harboring hemizygous deletions at the *Rb1* locus exhibited low pRb expression. Other tumors with retained *Rb1* alleles showed a robust pRb protein band. Three HG serous carcinomas harboring homozygous deletions of the *CDKN2A* locus did not express

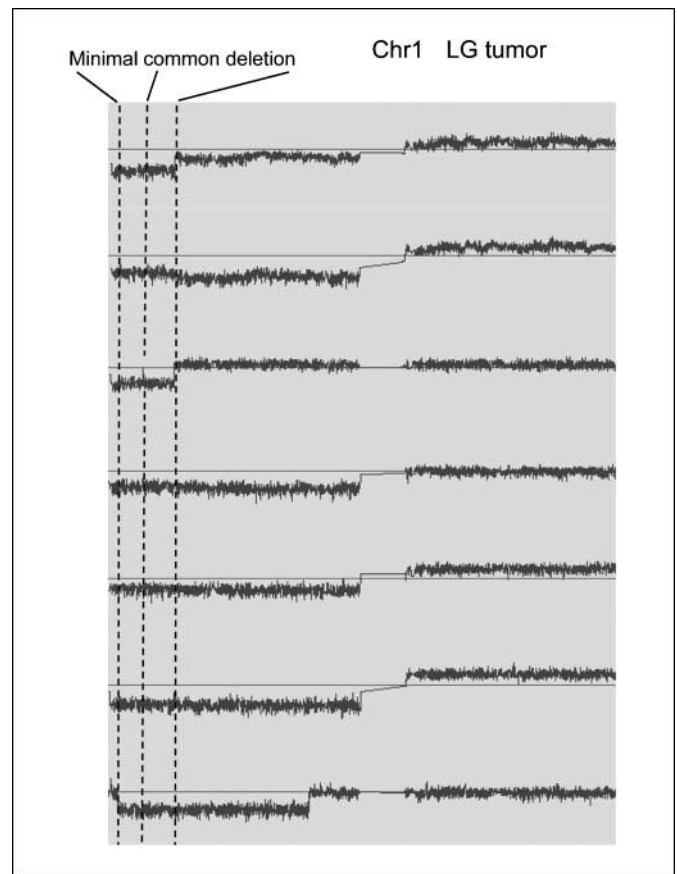


Figure 3. Minimal mapping of chromosome 1p hemizygous deletions in LG tumors. Copy number of chromosome 1 of all seven LG tumors harboring ch1p36 deletion is plotted against chromosomal location. The minimally deleted region (delineated by dotted lines) contains two adjacent deletion fragments. Red horizontal lines indicate normal copy number: 2. Candidate tumor suppressor genes, *CHD5* and miRNA-34a, are located within the first deletion fragment.

detectable p16 protein. The remaining 8 tumors, which retained one or both *CDKN2A* alleles, expressed a variable but detectable amount of p16 protein.

Growth inhibition by miR-34a in LG carcinomas. miR-34a has been shown as a potential tumor suppressor in several tumors including neuroblastoma, colorectal cancer, pancreatic cancer, and non-small cell lung cancer (38–43). Given the frequent hemizygous deletion at the miR-34a locus in LG carcinomas, we decided to determine if miR-34a participated in the pathogenesis of LG serous carcinomas. MPSC-1, a LG carcinoma cell line that harbors a hemizygous deletion at the miR-34a locus and expresses a low level of endogenous miR-34a compared with normal ovarian surface epithelial cells, was selected for functional study (Fig. 6A and B). MPSC-1 cells were transfected with synthetic miR-34a mimic and cell growth was monitored. We found that miR-34a mimic significantly suppressed the cell growth of MPSC-1 when compared with the control miRNA-treated cells (Fig. 6C). Furthermore, miR-34a mimic treatment was associated with a higher percentage of apoptotic cells in MPSC-1 cells than the mimic control ($P < 0.05$, t test; data not shown), a result consistent with previous reports (39, 41). To examine if miR-34a can regulate mRNA stability of its target genes, following miR-34a transfection, we performed quantitative RT-PCR to determine mRNA levels of 11 reported miR-34a target genes (listed in Supplementary Table S4). Our

results showed that *CCDN1*, *CDK6*, and *BCL2* mRNAs were significantly down-regulated by miR-34a 24 h after transfection compared with the control miRNA (Fig. 6D).

Discussion

Ovarian carcinoma is a complex disease composed of a variety of neoplasms that have been termed surface epithelial tumors. These tumors have vastly different clinical, histopathologic, and molecular features and their pathogenesis differs significantly. A major challenge, in so far as their pathogenesis is concerned, has been to identify precursor lesions and shed light on the molecular pathways that lead to the development of invasive ovarian carcinoma. Among the various types of surface epithelial tumors, the serous group is by far the most common, accounting for approximately half of all surface epithelial tumors. They are also arguably the most lethal. Accordingly, in this study, we focused exclusively on the serous tumors. Although several previous reports have applied genome-wide molecular analyses to study ovarian carcinomas (9, 34–36), there are several unique approaches in this report that aim to answer these fundamental questions. First, we applied high-resolution SNP arrays on affinity-purified tumor cells, an approach that provides high sensitivity and specificity in detecting subtle DNA copy number alterations, especially hemizygous and homozygous deletions. Second, the focus on a single histologic subtype of ovarian cancer rather than grouping different tumor subtypes together permitted a comprehensive view of molecular genetic changes in serous ovarian tumors. Third, we performed quantitative PCR to validate the homozygous deletions detected by SNP arrays and showed high specificity of the current approach in detecting homozygous deletions.

Several conclusions can be drawn from this study in terms of the molecular etiology of ovarian SBT/LG carcinoma. First, the observation that ch1p36 hemizygous deletions and ch9p21 homozygous or hemizygous deletions occurred far more frequently in LG serous carcinomas than in SBTs suggests that these regions

may harbor potential tumor suppressors, the loss of which contributes to tumor progression from SBT to LG carcinoma. The ch1p36 region contains several potential tumor suppressor genes (44, 45). We chose miR-34a to investigate its function and found that transfected miR-34a can suppress cellular proliferation and induce apoptosis in LG carcinoma cells. The functional effects of miR-34a reported here are similar to previous studies (38–43). Furthermore, in LG carcinoma cells, miR-34a was found to down-regulate several of the miR-34a target genes including *CCDN1*, *CDK6*, and *BCL2*. The results support the role(s) of miR-34a in regulating cell proliferation and apoptosis in LG serous carcinoma. It should be noted that the ch1p36 deletion encompasses a large chromosomal region containing more than 80 genes; therefore, further studies are required to delineate the culprit tumor suppressor gene(s) located in this region.

Second, frequent deletion at the ch9p21 region corresponding to the *CDKN2A/B* locus encodes three well-known tumor suppressor proteins, p14 (Arf), p16 and p15, observed in LG serous carcinoma. Arf is a potent tumor suppressor that blocks cell cycle progression by interfering with the p53 negative regulator, MDM2, thereby stabilizing p53 protein expression. *CDKN2A* and *CDKN2B* share similar, if not redundant, function in inhibiting cyclin-dependent kinase, which regulates the pRb pathway. Besides, the expression level of *CDKN2A* was enhanced in response to oncogene-induced stress such as by the activation of RAS-RAF-MEK signaling pathway. This observation is of considerable interest because SBTs and LG carcinomas frequently contain activating *KRAS* and *BRAF* mutations but rarely contain *TP53* mutations. Thus, retained *CDKN2A/B* in SBTs may prevent progression to LG carcinoma despite the presence of activating *KRAS* and *BRAF* mutations frequently observed in SBTs. When *CDKN2A/B* locus is deleted, the checkpoint becomes defective and tumor cells may escape from cell cycle arrest and exploit the oncogenic functions of mutated *KRAS/BRAF*, leading to LG tumor progression.

The genome-wide analysis from this study uncovered a handful of homozygously deleted genes that may be implicated in

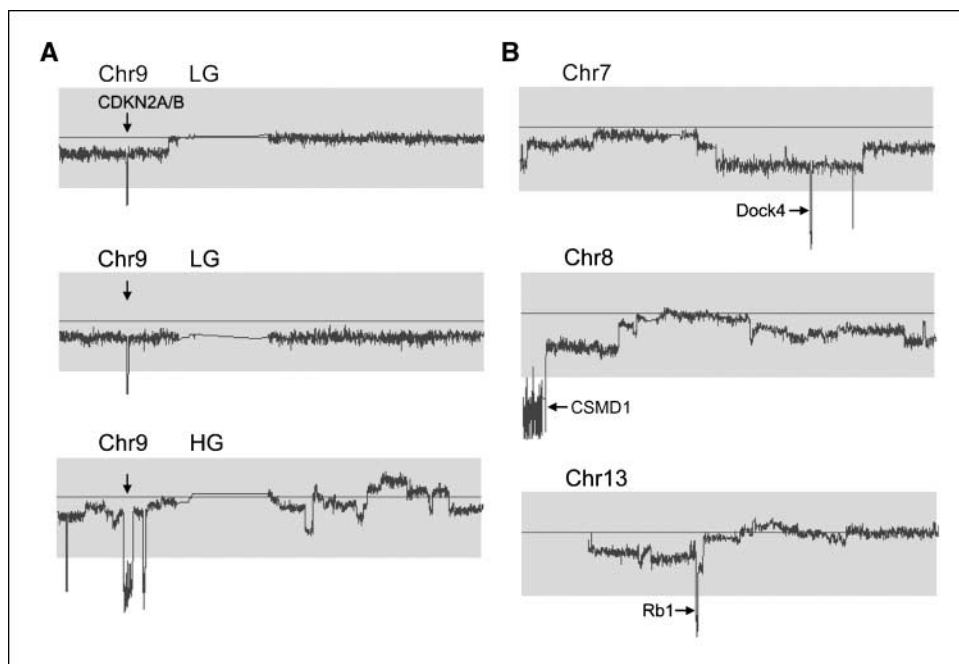


Figure 4. Representative chromosomes containing homozygous deletions. A, two LG serous carcinomas and one HG serous carcinoma harbor the ch9p21.3 homozygous deletion. Red horizontal lines represent normal copy number: 2. The deletions in LG serous carcinomas are small and discrete, but the deletion in the HG tumor is larger and encompasses 2.92 Mb. B, representative homozygous deletions in ch7, ch8, and ch13 in HG serous carcinomas. Candidate tumor suppressor genes residing within the deleted regions are indicated by arrows.

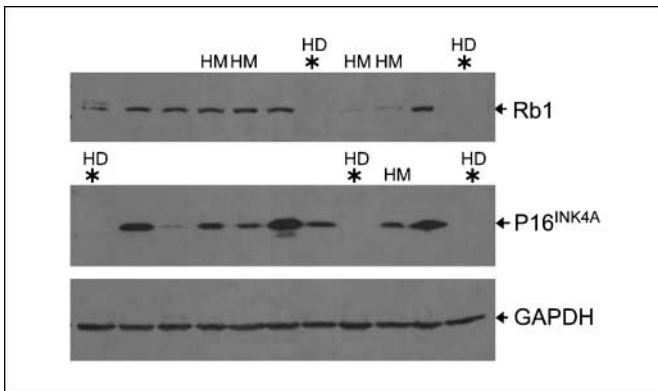


Figure 5. Loss of p16 and Rb1 protein expression in tumors with homozygous deletion at 9p21.3 and 13q14.2, respectively. Western blot analysis was performed using HG serous tumors with known DNA copy number at 9p21.3 and 13q14.2 regions. Tumors with homozygous deletions (HD) at those loci were found to completely lose protein expression of p16 or Rb1, respectively. Hemizygous deletion (HM) was found to variably affect protein expression of p16 and Rb1. The remaining tumors contain normal copy number (2 copies). GAPDH was used as the sample loading control.

tumorigenesis of HG serous carcinomas. These include relatively frequent homozygous deletions of *Rb1* and *CDKN2A/B*. Homozygous deletions in either of these regions accounted for 17% (8 of 47) of HG serous carcinomas. Because both genes are probably silenced by additional molecular mechanisms such as promoter hypermethylation, the inactivation in either gene in HG serous carcinomas is expected to be very frequent. In fact, we observed that >50% (6 of 11) of HG serous carcinomas had a significant reduction in either pRb or p16 protein expression based on Western blot analysis supporting this interpretation. pRb and p16 proteins both participate in regulating progression of the cell cycle from G₁ to S phase. It is well known that HG serous carcinomas harbor frequent *TP53* mutations; however, the molecular genetic

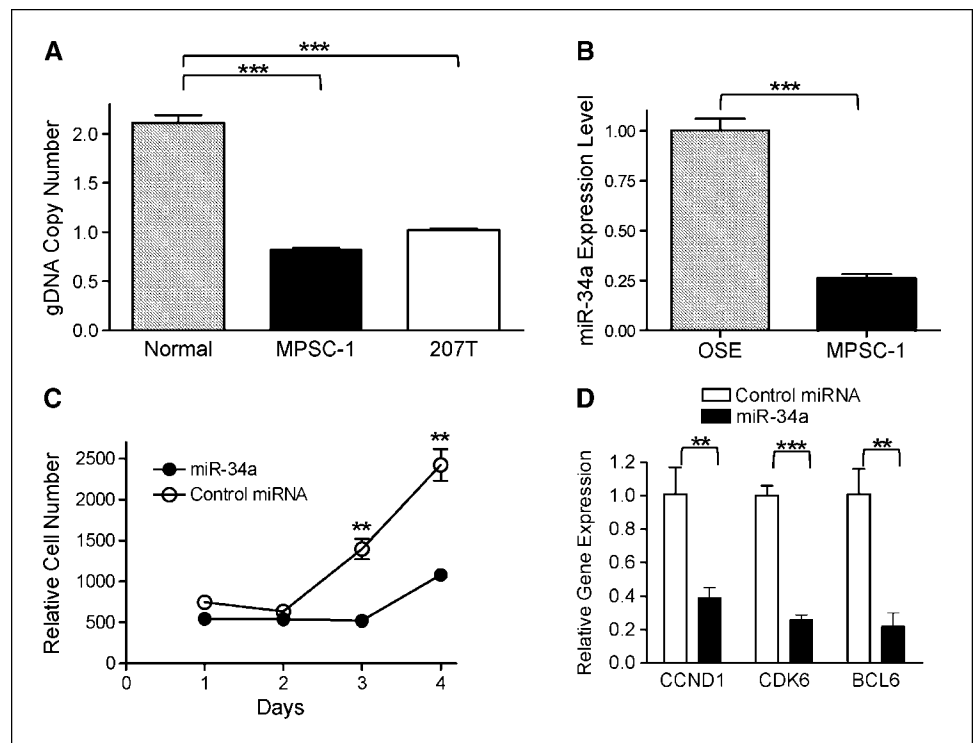
changes in *Rb1* and *CDKN2A* were previously not recognized because somatic mutations in these genes are rare in HG serous carcinomas. In addition, our results showing that deletions and down-regulation of the Rb/p16 pathway often coexist with *TP53* mutations, suggest that *Rb1* and *TP53* act independently, and perhaps are equally important in the development of HG serous carcinomas. The genetic analysis reported here provides an explanation why a mouse ovarian cancer model generated by inducible deletion of *Rb1* and *TP53* in ovarian epithelium results in tumors with morphology that closely resembles its human counterpart (46).

In addition to the well-characterized tumor suppressors discussed above, in HG serous carcinoma, we also identified candidate tumor suppressor genes that are less known to be implicated in human cancers. These include *CSMD1*, which is located in ch8p23.1-23.3 (47), and *DOCK4*, which is located in ch7q31.1 (48). Deletions in ch8p23 have been detected in several other types of human cancer, including squamous cell carcinoma. Also, comprehensive sequencing analysis has revealed somatic mutations of *CSMD1* in colon and breast cancers (49, 50). *DOCK4* encodes a regulator of small GTPase and was originally identified as a homozygously deleted tumor suppressor (48). The function of both *CSMD1* and *DOCK4* are less clear and future studies are required to define their role(s) in the pathogenesis of HG serous carcinoma.

The current data using 250K SNP array has validated our previous study using 10K SNP array in profiling amplicons in 33 HG and 10 LG ovarian serous carcinomas (27). In addition, the much higher resolution of 250K SNP arrays permitted reliable detection of small subchromosomal deletions. However, it should be noted that the sample sizes in the current study are relatively small and the observed mutation rate should be confirmed in future large-scale studies.

Our quantitative measurement of DNA copy number changes as defined by the CIN index, provides cogent evidence that HG serous

Figure 6. Biological effects of miR-34a in a LG carcinoma cell line, MPSC-1. **A**, quantitative PCR demonstrates a reduced genomic DNA (gDNA) copy number at the miR-34a locus in a LG carcinoma cell line, MPSC-1. The calculated copy number in normal tissue, MPSC-1, and 207T is 2.1, 0.8 and 1.0, respectively. 207T is a LG carcinoma with hemizygous deletion at the miR-34a locus based on SNP array analysis. **B**, miR-34a expression measured by the TaqMan microRNA assay. OSE: normal ovarian surface epithelial cells. **C**, miR-34a inhibits cell proliferation as measured by the cell-titer blue assay. **D**, mRNA levels of candidate miR-34a target genes measured by quantitative RT-PCR. **, $P < 0.01$; ***, $P < 0.001$.



carcinomas are characterized by a much higher level of DNA copy number changes compared with LG serous carcinomas and SBTs. In addition, LG serous carcinomas harbor higher numbers of subchromosomal alterations than do SBTs. In 13 pairs of matched tumors and tumor stromal fibroblasts, the CIN index was found to be significantly higher in the tumors than the tumor-associated stromal fibroblasts, refuting the proposal of coevolution of tumor stromal cells during tumorigenesis.

In summary, the results from this study shed new light on the possible tumor suppressor roles of miR-34a and CDKN2A/B in the progression of SBT to LG carcinoma. For HG tumors, deletions in the Rb-p16 pathway may contribute to their development, and *CSMD1* and *DOCK4* may represent new tumor suppressor genes. The reported unique molecular genetic landscape in different types of ovarian serous neoplasms can serve as a roadmap for future

studies aimed at elucidating the molecular pathogenesis of these tumors may assist in the development of and new diagnostic tests and target-based therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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