



ELSEVIER

Cancer Genetics and Cytogenetics 161 (2005) 170–173

CANCER GENETICS
AND
CYTOGENETICS

Short communication

Ovarian cancer is a heterogeneous disease

Vivian Wang^a, Cheng Li^b, Ming Lin^b, William Welch^c, Deborah Bell^d, Yuk-Fu Wong^e,
Ross Berkowitz^a, Samuel C. Mok^a, Christina A. Bandera^{a,*}

^aDivision of Gynecologic Oncology, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Dana Farber Harvard Cancer Center, Harvard Medical School, Boston, MA 02115

^bHarvard School of Public Health, Harvard Medical School, Boston, MA

^cDepartment of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

^dDepartment of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA

^eChina University of Hong Kong, Hong Kong, Hong Kong SAR, China

Received 5 October 2004; received in revised form 15 December 2004; accepted 16 December 2004

Abstract

The purpose of this study was to systematically evaluate the molecular profiles of different histologic types of epithelial ovarian cancer before the disease has metastasized beyond the ovary. Stage I epithelial ovarian cancers were chosen for analysis of early genetic events associated with different cell types. Allelotyping of 47 cases was performed using 224 polymorphic markers. Analysis with Fisher's exact test found markers specific for grade 3 tumors and clear cell histology. Hierarchical clustering analysis using dChip software revealed that the pattern of allele loss in eight regions on four chromosomes led to grouping of grade 3 tumors, endometrioid (grades 1 and 2) tumors, and clear cell tumors. We conclude that ovarian cancer is a heterogeneous disease in which histologic phenotypes correlate with distinct genetic patterns. © 2005 Elsevier Inc. All rights reserved.

1. Introduction

Ovarian cancer is the most deadly gynecologic malignancy in the United States. It is estimated that 16,210 deaths will be caused by ovarian cancer in the year 2005 [1]. The vast majority of ovarian cancers are carcinomas of the surface epithelial type [2], and typically present at an advanced stage with metastases beyond the ovary. Most patients initially respond to treatment, but these cancers tend to recur, and when they do, they are usually fatal. Many tumor suppressor genes are implicated in the behavior of ovarian cancer. These include the *TP53* gene, which is associated with disease metastasized beyond the ovary and appears to play a role in chemotherapy resistance [3,4]. Additional tumor suppressor genes implicated in ovarian cancer pathogenesis include *CDKN2A* (alias *p16*), *CDKN1A* (alias *p21*), *CDKN1B* (alias *p27*), *DOC2*, and *DPH2L1* (alias *OVCA1*) [5–9].

Though our knowledge of the pathways important to ovarian cancer pathogenesis is growing, most investigations of the genetic basis of ovarian cancer fail to consider the heterogeneity of the disease. According to World Health

Organization (WHO) criteria, epithelial ovarian cancers are divided into four major histologic subtypes [10]. Serous carcinomas are the most common form of ovarian malignancy, with epithelial cells resembling those of the fallopian tube. They comprise ~50% of primary epithelial ovarian cancers. Mucinous carcinomas are cystic tumors with locules lined with mucin-secreting epithelial cells resembling either endocervical or colonic epithelium. They comprise ~10% of epithelial ovarian cancers. Endometrioid and clear cell lesions each account for ~10% of epithelial ovarian cancers. These neoplasms are thought to arise from foci of endometriosis and endometriotic cysts within the ovary [11]. The clear cell type of ovarian cancer has consistently been shown to behave very differently from the more common serous cancers. Other, less common cell types include Brenner, mixed epithelial type, and undifferentiated carcinomas. Each subtype, with the exception of clear cell, is further divided into three grades [12]. One of the challenges in performing studies correlating molecular factors with clinical outcome in ovarian cancer is collecting adequate specimens to account for the heterogeneity of tumor type.

Genetic research focusing on the molecular basis of different ovarian cancer histologies is further complicated by the fact that most patients present with advanced-stage disease. Study of these advanced cancers cannot isolate early

* Corresponding author. Tel.: (401) 453-7520; fax: (401) 453-7529.
E-mail address: cbandera@wihri.org (C.A. Bandera).

genetic events associated with pathogenesis. For this reason, the genetic exploration of less common stage I cancers, where disease is limited to the ovary, may provide unique information.

The present study compared the allelotypes of different histologic subtypes of early ovarian cancer (before dissemination beyond the ovary), to gain insight into the correlation of genetic events and phenotype.

2. Materials and methods

2.1. Tissue samples

We collected a total of 47 surgically assessed stage I epithelial ovarian cancers from the Brigham and Women's Hospital and Massachusetts General Hospital in Boston, MA, and the Prince of Wales Hospital in Hong Kong. The internal review boards of the three institutions approved the study. For all cases, formalin-fixed paraffin-embedded samples were obtained from pathology archives, and reviewed by a pathologist. The cases comprised 14 serous, 9 mucinous, 12 endometrioid, and 12 clear cell cancers. Excluding clear cell histology, there were 12 grade 1, 10 grade 2, and 13 grade 3 cancers. There were two serous cases of disease that were apparent only with microscopic evaluation, including one grade 1 (case 3317) and one grade 2 (case 7024). For each case in the study, normal tissue was also collected.

2.2. DNA isolation and allelotyping

Sections measuring 6 μm were cut and mounted onto plain glass slides. The tissue was stained with methylene green, then deparaffinized using xylene, and rehydrated using serial ethanol solutions of 100, 95, and 70%. Tumor was isolated using laser capture microdissection of paraffin-embedded samples using the PixCell II capture microdissection system (Arcturus Engineering, Mountain View, CA). Approximately 5,000 tumor cells were collected per case; these digested with proteinase K for 72 hours, then heat-inactivated at 95°C for 10 minutes. Degenerate oligonucleotide primer-polymerase chain reaction (DOP-PCR) was used for whole-genome amplification [13]. For each tumor specimen, normal tissue from the same patient was also processed.

A total of 224 polymorphic markers spanning 23 chromosomes (Applied Biosystems, Foster City, CA) were evaluated for loss of heterozygosity (LOH) using standard PCR methods. Amplified PCR products were run on an ABI PRISM 310 automated DNA sequencer (Applied Biosystems). Allelic products were assessed for peak height and peak area using Genescan and Genotype software (Applied Biosystems), and ratios of the areas of heterozygous normal and tumor alleles were calculated. A 50% decrease in one allele was considered indicative of allele loss. For each case, 72–90% of alleles were informative, with frequency of LOH ranging from 60–90%.

2.3. Statistical analysis

Two statistical approaches were used to analyze the data. First, Fisher's exact test with a false discovery rate (FDR) set at 1–5% to control for multiple comparisons was used to identify markers specific for cell type and grade. The second statistical approach was a novel hierarchical clustering performed using dChip software. This biostatistical package infers LOH from the data set and then performs a clustering analysis based on a chosen threshold LOH score, as has been previously described [14]. The software is available online at <http://www.dchip.org>.

3. Results

3.1. Traditional analysis using Fisher's exact test

The traditional Fisher's exact test with the FDR set at 1% revealed three markers specific for grade 3 tumors on chromosome 17: D17S1795 ($P = 0.000017$), D17S787 ($P = 0.000074$) and D17S836 ($P = 0.000081$). For cell type comparison, the same analysis method with an FDR of 5% revealed D11S901 ($P = 0.000053$) and D17S944 ($P = 0.00039$) to be significant markers for clear cell cancers.

3.2. Clustering analysis

Using the dChip software with a LOH score of -1.0 , leads to grouping of three distinct subtypes including 92% of grade 3 tumor; 100% of endometrioid tumors grades 1 and 2; and 58% of clear cell tumors (Fig. 1). Eight distinct regions on four chromosomes defined this clustering pattern including chromosome regions 5q13.3~5q21.2, 6p25.1~6p21.2, 6q22.3~6q27, 13q12.11~13q31.3, 17p13.3, 17p13.3~17p13.2, 17p13.1~17q11.2, and 17q23.2~17q24.2 (Table 1). This analysis does not associate loss in specific regions with distinct histologic subtypes; rather, these eight regions together produce the clustering of different histologies.

4. Discussion

The present study clearly shows that ovarian cancer is a heterogeneous disease in which histologic phenotypes correlate with distinct genetic events. We draw this conclusion from the evaluation of 47 Stage 1 ovarian cancer cases in which disease is isolated to the ovaries. Our series of specimens included surgically assessed cases of serous, mucinous, endometrioid, and clear cell histologies. Although advanced ovarian cancer is more than three times as common as Stage 1 cancer, we purposely excluded advanced cases from this study, because the genetic fingerprint in widespread disease is likely to be associated more with metastatic potential than with early development of cancer. For example, it is known that the *TP53* gene is more commonly overexpressed in metastatic ovarian cancer than in early ovarian

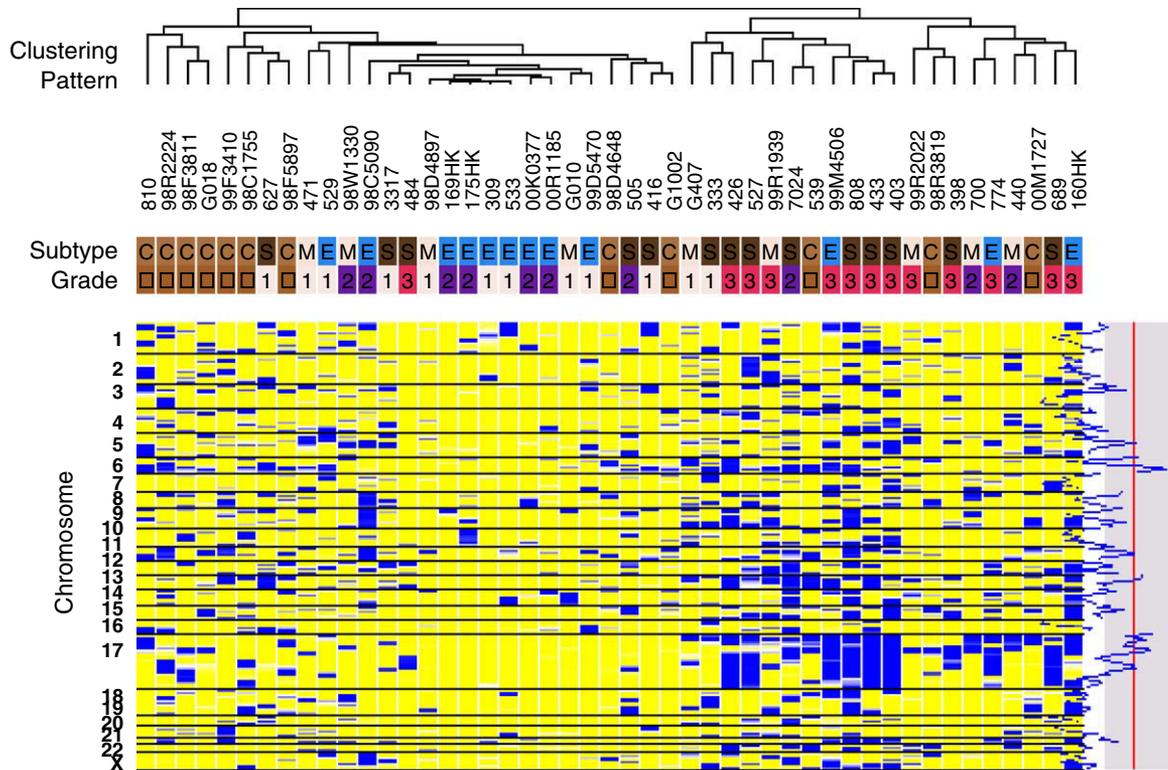


Fig. 1. The LOH map for the entire genome is shown (blue, LOH; yellow, no LOH; gray, uninformative; white, no data). Histopathologic descriptors (C, clear cell; M, mucinous; E, endometrioid; S, serous) and grade (1, 2, and 3, with □ for clear cell) are identified for each case. Along the right, the shaded box shows a LOH score threshold of -1.0 , represented by the red line. The blue line is the LOH score proportional to the frequency of LOH across all samples. Eight chromosomal regions with LOH reaching levels to the right of the red LOH score threshold define the clustering pattern shown at the top of the figure. The eight LOH regions are described in Table 1.

cancer [3,4]. This is the first published study of ovarian cancer genetics in Stage I tumors only.

Allelotyping a cancer involves identifying regions where one copy of a heterozygous marker has been lost. Loss of heterozygosity (LOH) is thought to be a vital component of the inactivation of tumor suppressor genes. To complete the process, the opposing allele must also be lost or mutated. Not every site of LOH exposes a tumor suppressor gene [15]. It is believed that LOH may also be a marker for genetic fragility, with cancers displaying allele loss at weak points throughout the genome. These LOH patterns may contribute to the overall phenotype of the tumor [16]. In the

Table 1
Summary of the eight regions that resulted in clustering of three distinct histologic subtypes

No.	Region	Flanking marker 1	Flanking marker 2
1	5q13.3~5q21.2	D5S424	D5S433
2	6p25.1~6p21.2	D6S1574	D6S1610
3	6q22.3~6q27	D6S287	D6S281
4	13q12.11~13q31.3	D13S175	D13S265
5	17p13.3	D17S849	D17S831
6	17p13.3~17p13.2	D17S831	D17S938
7	17p13.1~17q11.2	D17S182	D17S1824
8	17q23.2~17q24.2	D17S957	D17S1816

present study, we chose to analyze patterns of allele loss in different histologic types of tumors.

We used two statistical packages to analyze allele loss patterns. The traditional Fisher’s exact test revealed specific markers associated with grade 3 tumors and clear cell tumors. Our analysis involved screening of too few individual markers (5–15 per chromosome) to find alleles linked to each subtype and grade. The dChip software is a cutting-edge tool that facilitates the inference of regional LOH from existing data. Furthermore, this software defines several regions, rather than individual alleles, whose LOH pattern allows for clustering of cases.

Our data clearly show that tumor types have distinct complex genetic fingerprints. The clustering groups identified in our data set coincide with loss of alleles in eight different chromosomal regions leading to grouping of grade 3 tumors, endometrioid tumors (grades 1 and 2), and clear cell tumors (Fig. 1). It is notable that 12 of 13 grade 3 tumors, regardless of histology, clustered together. This result complements the clinical observation that grade is a more relevant prognostic indicator than cell type.

Studies that fail to perform analyses accounting for ovarian cancer variety may miss important characteristics of different histologies. Ultimately, this may retard the quest for biochemical markers and targeted treatments. As ovarian

cancer research enters the era of high-throughput technology for evaluation of molecular characteristics, it is important to recognize the diversity of the disease. The genetic heterogeneity of epithelial ovarian cancer also has important clinical implications for strategies of early detection with biomarkers, prevention, and treatment.

Acknowledgments

The study was funded by the Ovarian Cancer Research Foundation, the Women's Reproductive Health Research Fellowship (NIH K12-HD01265), the Morse Family Fund, the Natalie Pihl Fund, the Early Detection Research Network Grant CA86381 from the National Institutes of Health, the Gillette Center for Women's Cancer, the Friends of the Dana Farber Cancer Institute, the Ruth N. White Research Fellowship, the Adler Foundation Inc., Mr. Edgar Astrove, the Elaine M. Schuster Fellowship in Gynecologic Oncology, the Dana-Farber Ovarian Cancer SPORE grant P50CA165009, and the R33CA103595 from the U.S. National Institutes of Health, Department of Health and Human Services. The sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

References

- [1] Cancer facts and figures 2005. Atlanta, GA: American Cancer Society; 2005.
- [2] Boring CC, Squires TS, Tong T, Montgomery S. Cancer statistics, 1994. *CA Cancer J Clin* 1994;44:7–26.
- [3] Schuijjer M, Berns EM. TP53 and ovarian cancer. *Hum Mutat* 2003;21: 285–91.
- [4] Kmet LM, Cook LS, Magliocco AM. A review of p53 expression and mutation in human benign, low malignant potential, and invasive epithelial ovarian tumors. *Cancer* 2003;97:389–404.
- [5] Chen CM, Behringer RR. *Ovca1* regulates cell proliferation, embryonic development, and tumorigenesis. *Genes Dev* 2004;18:320–32.
- [6] Havrilesky LJ, Alvarez AA, Whitaker RS, Marks JR, Berchuck A. Loss of expression of the *p16* tumor suppressor gene is more frequent in advanced ovarian cancers lacking *p53* mutations. *Gynecol Oncol* 2001;83:491–500.
- [7] Mok SC, Chan WY, Wong KK, Cheung KK, Lau CC, Ng SW, Baldini A, Colitti CV, Rock CO, Berkowitz RS. *DOC-2*, a candidate tumor suppressor gene in human epithelial ovarian cancer. *Oncogene* 1998;16:2381–7.
- [8] Bali A, O'Brien PM, Edwards LS, Sutherland RL, Hacker NF, Henshall SM. Cyclin D1, p53, and p21Waf1/Cip1 expression is predictive of poor clinical outcome in serous epithelial ovarian cancer. *Clin Cancer Res* 2004;10:5168–77.
- [9] Katsaros D, Cho W, Singal R, Fracchioli S, Rigault De La Longrais IA, Arisio R, Massobrio M, Smith M, Zheng W, Glass J, Yu H. Methylation of tumor suppressor gene *p16* and prognosis of epithelial ovarian cancer. *Gynecol Oncol* 2004;94:685–92.
- [10] Serov SF, Scully RE. Histological typing of ovarian tumors. International Histological Classification of Tumors 9. Geneva: World Health Organization, 1993.
- [11] Campbell IG, Morland S, Hitchcock A. Endometriosis and the relationship with ovarian cancer. In: Sharp F, Blackett T, Derek J, Bast R, editors. *Ovarian cancer 5*. Oxford: ISIS Medical Media, 1998, pp. 159–70.
- [12] Blaustein A, Kurman RJ, editors. *Blaustein's pathology of the female genital tract*. 5th ed. New York: Springer, 2002.
- [13] Kuukasjarvi T, Tanner M, Pennanen S, Karhu R, Visakorpi T, Isola J. Optimizing DOP-PCR for universal amplification of small DNA samples in comparative genomic hybridization. *Genes Chromosomes Cancer* 1997;18:94–101.
- [14] Lin M, Wei LJ, Sellers WR, Lieberfarb M, Wong WH, Li C. dChipSNP: significance curve and clustering of SNP-array-based loss-of-heterozygosity data. *Bioinformatics* 2004 May 22;20(8):1233–40.
- [15] Tomlinson IP, Lambros MB, Roycastle RR. Loss of heterozygosity analysis: practically and conceptually flawed? *Genes Chromosomes Cancer* 2002;34:349–53.
- [16] Hoque MO, Lee CC, Cairns P, Schoenberg M, Sidransky D. Genome-wide genetic characterization of bladder cancer: a comparison of high-density single-nucleotide polymorphism arrays and PCR-based microsatellite analysis. *Cancer Res* 2003;63:2216–22.