THE POTENTIAL INVOLVEMENT OF THE BREAST CANCER (*BRCA1*) AND RETINOBLASTOMA (*Rb*) TUMOR SUPPRESSOR GENES IN THE HEREDITARY PREDISPOSITION TO OVARIAN CANCER IN JAPANESE WOMEN

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PREFACE

The objective of this study was to determine the incidence of unique germline mutations in the *BRCA1* and retinoblastoma (*Rb*) tumor suppressor genes in Japanese women diagnosed with ovarian cancer by Single-Strand Conformation Polymorphism (SSCP) analysis. This thesis has been prepared as two chapters that have been submitted as two manuscripts. The first manuscript was submitted with collaborators to *Human Mutation* and the second manuscript was submitted to *Cancer*.

The author would like to acknowledge Dr. Gary Ostrander for his guidance, support and inspiration. I would also like to express my gratitude to him for giving me the opportunity to be a part of his lab and work on this project . Thanks are also extended to my committee members, Drs. E.C. Nelson, D.K. Burnham and M.M. Mitas, for their advice and review of these manuscripts.

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NOMENCLATURE

PCR	Polymerase Chain Reaction
SSCP	Single-Strand Conformation Polymorphism
Rb	Retinoblastoma

CHAPTER I

INTRODUCTION

Researchers now recognize that a complex interplay of genetic, hormonal, and environmental factors contribute to cancer susceptibility. Tumor suppressor genes play a major role in hereditary predisposition to cancer. These genes are believed to control cell growth through mechanisms that are not yet fully understood. Two of the most vigorously studied tumor suppressor genes are the retinoblastoma, *Rb,* and the breast cancer susceptibility gene, *BRCA1*.

The *Rb* gene was the first human tumor suppressor gene to be cloned and sequenced (Friend et al., 1986; Fung et al., 1987; Lee et al., 1987). Inherited mutations causing loss of the *Rb* gene's function play an essential role in the etiology of retinoblastoma and osteosarcoma (Benedict et al., 1983; Bishop, 1991). Furthermore, somatic defects leading to loss of the *Rb* gene's function are found in other cancers including breast, ovarian, bladder, and prostate (Bookstein et al., 1990; Horowitz et al., 1990; Lee et al., 1990; Bishop, 1991; Li et al., 1991).

In contrast, the *BRCA1* gene is one of the more recent tumor suppressor genes to be identified. This gene has been positively linked to familial breast and ovarian cancers as well as a lower percentage of sporadic tumors in the same locations (Easton et al., 1993; Futreal et al., 1994; Steichen-Gersdorf et al., 1994).

Ovarian cancer is the leading gynecological malignancy in the United States. Early detection is extremely difficult because of the relative inaccessibility of the ovary (Gallion et al., 1995). As a result, clinical prognosis is generally poor because most tumors are

usually discovered in later stages once metastasis has occurred (Tsao et al., 1991). Ovarian cancer incidence is one-fifth to one-third lower in Japan than in the United States (Herrinton et al., 1994), however, the same obstacles to early detection exist. In light of this difficulty, one major focus in tumor suppressor gene research has been to identify mutations that could be used as a screening test for early detection.

The major objective of this research was to determine whether a sample population of Japanese women with ovarian cancer carry unique germline mutations in the *BRCA1* and *Rb* tumor suppressor genes.

This thesis has been prepared as two chapters that have been submitted as two manuscripts. Chapter II was submitted with collaborators to *Human Mutation* and Chapter III was submitted to *Cancer*.

CHAPTER II

BRCA1 GERMLINE MUTATION IN A JAPANESE OVARIAN CANCER FAMILY

ABSTRACT

We analyzed 21 Japanese women diagnosed with ovarian cancer for *BRCA1* germline mutations using single-strand conformation polymorphism (SSCP). The women were randomly selected without regard for a family history of ovarian cancer. We identified one frameshift mutation, 797delAA, in exon 11 of the *BRCA1* gene in a woman with a family history of ovarian cancer. *BRCA1* mutations have been shown to play a role in breast and ovarian cancer in the United States where approximately 1.4% of women will develop ovarian cancer during their lifetime. In contrast, the cumulative incidence of ovarian cancer in Japan is one-fifth to one-third that of the United States. Since this mutation has only been seen once before, in a Japanese woman with breast cancer, this data suggests that this mutation may only be carried in the Japanese population.

INTRODUCTION

Ovarian cancer is the leading gynecological malignancy in the United States. Approximately 5-10% of ovarian cancers diagnosed in American women result from hereditary predisposition with the remaining 90-95% believed to be of sporadic origin (Houlston et al., 1991; Bewtra et al., 1992; Lynch et al., 1993). Germline mutations in the *BRCA1* gene have been linked to 40-50% of familial breast cancers and 80% of familial breast-ovarian cancers (Easton et al., 1993; Steichen-Gersdorf et al., 1994). Steichen-Gersdorf and coworkers report evidence of linkage to the *BRCA1* gene in an estimated 78% of families with hereditary or "site-specific" ovarian cancer. The criteria for being a "site-specific" ovarian cancer family is three or more cases of epithelial ovarian cancer and no breast cancer cases diagnosed in anyone under the age of 50 (Steichen-Gersdorf et al., 1994). Alternatively, mutations in the *BRCA1* gene in sporadic ovarian tumors have been found to occur only in rare instances (Futreal et al., 1994).

The *BRCA1* gene has been mapped to chromosome 17q21. It is composed of 22 coding exons containing 5592 bp of sequence that is translated into a protein of 1863 amino acids. A highly conserved RING finger motif containing cysteine and histidine residues in the arrangement Cys_3-His-Cys_4, lies at the NH_2-terminus of the protein (Miki et al., 1994; Wu et al., 1996). RING finger motifs containing the same consensus sequence are found in regulatory proteins, suggesting the *BRCA1* protein may function in gene transcription regulation (Wu et al., 1996; Shattuck-Eidens et al., 1995). In addition, the C-terminus contains an excess of negatively charged amino acids that can activate transcription of both yeast and mammalian cells when bound to a GAL4 binding domain (Monteiro et al., 1996). Szabo and coworkers report that the 120 residues of the NH_2-

terminus including the RING finger motif and the 80 residues of the C-terminus are more than 80% conserved between the human, canine, and murine *BRCA1* gene sequences supporting the theory that these regions are critical to gene function (Szabo et al., 1996).

Frameshift and nonsense mutations that lead to premature protein truncation are the most common mutations found in the *BRCA1* gene in breast and breast-ovarian cancer families. Exon 11 contains 60% of the entire coding region of the gene and, to date, 55% of the >100 currently reported mutations (Grade et al., 1996). The other 45% of the reported mutations are spread throughout the remaining 21 exons. The most common mutation found in the *BRCA1* gene is a 2 bp deletion at base 185 in exon 2 (Simard et al., 1994; Shattuck-Eidens et al., 1995; Struewing et al., 1995). This deletion occurs with high frequency in people of Ashkenazi Jewish descent. Recent studies report occurrences in the British population (Berman et al., 1996) and in Iraqi Jews (Sher et al, 1996; Abeliovich et al., 1997). Abeliovich and coworkers believe the 185delAG mutation was transferred to the Iraqi Jews through a common ancestor with Ashkenazi Jews approximately 2500 years ago (Abeliovich et al., 1997). When a genetically distinctive population, such as the Ashkenazi Jews, can trace their ancestors back to a relatively isolated group of founders, unique mutations or characteristics may appear at elevated incidence levels when compared to the general population. This concept of a "founder effect" has been used as a likely explanation for the high incidence of the 185delAG mutation in the *BRCA1* gene in the Ashkenazi Jews.

While the hereditary predisposition to ovarian cancer in Japan is equivalent to the 5-10% found in American women (Inoue et al., 1995), the overall incidence of ovarian cancer in Japan is one-third to one-fifth that of the United States (Herrinton et al., 1994).

Japanese populations lack many of the dietary risks, such as a diet high in saturated fats and frequent red meat consumption (Cramer et al., 1984), commonly attributed to ovarian cancer in the United States. This dietary difference has been used as one explanation for the lower ovarian cancer incidence rates in Japan (Herrinton et al., 1994).

In this study, we used SSCP to screen exons 1, 3, 11, 13, 16, 17, 18, 21 and 22 of the *BRCA1* gene, including the intron-exon boundaries, in genomic DNA from 21 Japanese women diagnosed with various forms of ovarian cancer. Our primary focus on exon 11 was due to its extensive coding region (60%) and the fact that 55% of the currently identified mutations are located in this exon. The other exons were selected randomly across the gene.

MATERIALS AND METHODS

Materials. Buffy coats from 21 Japanese women ranging in age from 19 to 61 and diagnosed with various forms of ovarian cancer were obtained from the Tokyo Medical College, Tokyo, Japan (Table 1).

DNA Extraction. Genomic DNA was extracted from the buffy coats and purified by phenol-chloroform extraction (Sambrook et al., 1989).

PCR-SSCP Analysis. Polymerase Chain Reaction (PCR) amplification of the genomic DNA was performed using primers and primer conditions previously described (Friedman et al., 1994). PCR reactions were carried out in 50 µl volumes containing 50 ng genomic DNA, 10X PCR Buffer (Boehringer-Mannheim, Indianapolis, IN), 200 µM each of dATP, dGTP and dTTP, 20 µM dCTP, 0.5 µCi [α-^{32}P] dCTP [(3000 Ci/mmol); DuPont NEN, Boston, MA], 1 µM of each primer and 1.25 U of *Taq* polymerase (Boehringer-Mannheim, Indianapolis, IN). Amplification was accomplished with 35 cycles of 94°C, 45 s; 55 or 60 °C, 30 s; 72°C, 30s; with a 7 min extension at 72°C following the final cycle. A 4.5 µl aliquot of the PCR product was diluted in 25 µl of denaturing loading buffer (95% formamide, 10 mM Ethylenediaminetetraacetic acid, 0.05% bromophenol blue, 0.05% xylene cyanol FF), heated at 99°C for 2 min then immediately cooled on ice. Aliquots of 4.5 µl were then loaded onto a 0.5X Mutation Detection Enhancement gel (FMC Bioproducts, Rockland, ME) and electrophoresed at room temperature for 18 hours at 8 W constant power. Following electrophoresis, gels were transferred to Whatman filter paper and exposed to film for 24 hours at -70°C.

Sequencing. The aberrant band and a normal control were eluted from the gel and reamplified with the appropriate primer pair. PCR products were purified for sequencing

with a QIAquick Spin PCR Purification kit (Qiagen, Santa Clarita, CA). Purified products were bidirectionally sequenced using a model 373A automated fluorescence-based cycle sequencer (Applied Biosystems, Foster City, CA).

RESULTS AND DISCUSSION

We have screened 21 Japanese women diagnosed with several forms of ovarian cancer using SSCP analysis over 9 of the 22 exons in the *BRCA1* gene including intron-exon boundaries. Variant banding was discovered in patient one following PCR amplification with primer pair 11i (Figure 1). DNA sequence analysis of the aberrant band revealed a heterozygous AA deletion in codon 797 creating a premature stop codon downstream at codon 799 (Figure 2). The premature stop codon would be expected to generate a truncated protein missing the 3' 1065 amino acids. Truncated forms of the *BRCA1* protein may be highly unstable as well as being nonfunctional. In 87% of mutated *BRCA1* proteins, the C-terminus of the protein is absent (Couch et al., 1996) supporting the finding that this area contains an important functional domain (Monteiro et al., 1996; Szabo et al., 1996).

The patient was diagnosed with ovarian cancer at age 61. A limited family history revealed that the subject's mother was diagnosed with ovarian cancer at age 68. In addition, eight of the mother's nine siblings were diagnosed with various forms of cancers. Unfortunately, further study of the inheritance of this mutation is not possible because of unavailable living or archival tissue from the individuals previously mentioned.

This frameshift mutation was previously reported by Katagiri and coworkers in a study of 103 Japanese breast cancer patients (Katagiri et al., 1996). The study samples were selected using criteria to identify women with a likely hereditary predisposition to breast cancer. In that study, one patient, with an unknown family history, displayed the AA deletion at codon 797 (Katagiri et al., 1996).

The identification of this mutation in two Japanese individuals diagnosed with

breast cancer and breast-ovarian cancer may indicate that this is an ethnically isolated mutation similar to the 185DelAG mutation in *BRCA1* predominantly found in the Ashkenazi Jewish population. Additional screening of Americans with Japanese ancestry may provide more information on the incidence of the *BRCA1* mutation reported here, 797delAA, in the Japanese population. Future studies are needed to further determine the existence of unique mutations in cancer-related genes not only in Japanese populations but also other geographic and/or ethnic groups exhibiting distinctive high or low incidence rates of cancer. Identification of mutations in the *BRCA1* gene as well as other cancer related genes that are expressed in specific groups of individuals may be useful in genetic counseling and preventive screening.

REFERENCES

Abeliovich D, Kaduri L, Lerer I, Weinberg N, Amir G, Sagi M, Zlotogora J, Heching N, and Peretz T (1997) The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women. Am J Hum Genet 60:505-514.

Berman DB, Wagner-Costalas J, Schultz DC, Lynch HT, Daly M, and Godwin AK (1996) Two distinct origins of a common *BRCA1* mutation in breast-ovarian families: A genetic study of 15 185delAG-mutation kindreds. Am J Hum Genet 58:1166-1176.

Bewtra C, Watson P, Conway T, Read-Hippee C, and Lynch HT (1992) Hereditary ovarian cancer: a clinicopathological study. Int Jour of Gynecol Pathol 11:180-187.

Couch FJ, Weber BL, and the Breast Cancer Information Core (1996) Mutations and polymorphisms in the familial early-onset breast cancer (*BRCA1*) gene. Human Mutation 8:8-18.

Cramer DW, Welch WR, Hutchison GB, Willett W, and Scully RE (1984) Dietary animal fat in relation to ovarian cancer risk. Obstet Gynecol 63:833-838.

Easton DF, Bishop DT, Ford D, Crockford GP, and the Breast Cancer Linkage Consortium (1993) Genetic linkage analysis in familial breast and ovarian cancer: Results from 214 families. Am J Hum Genet 52:678-701.

Friedman LS, Ostermeyer EA, Szabo CI, Dowd P, Lynch ED, Rowell SE and King M-C (1994) Confirmation of *BRCA1* by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nature Genet 8:399-404.

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, Eddington K, McClure M, Frye C, Weaver-Feldhaus J, Ding W, Gholami Z, Söderkvist P, Terry L, Jhanwar S, Berchuck A, Iglehart JD, Marks J, Ballinger DG, Barrett JC, Skolnick MH, Kamb A, and Wiseman R (1994) *BRCA1* mutations in primary breast and ovarian carcinomas. Science 266: 120-122.

Grade K, Jandrig B, and Scherneck S (1996) *BRCA1* mutation update and analysis. J Cancer Res Clin Oncol 122:702-706.

Herrinton LJ, Stanford JL, Schwartz SM, and Weiss NS (1994) Ovarian cancer incidence among Asian migrants to the United States and their descendants. J Natl Cancer Inst 86:1336-1339.

Houlston RS, Collins A, Slack J, Campbell S, Collins WP, Whitehead MI, and Morton NE (1991) Genetic epidemiology of ovarian cancer: segregation and analysis. Ann Hum Genet 55:291-299.

Inoue R, Fukutomi T, Ushijima T, Matsumoto Y, Sugimura T, and Nagao M (1995) Germline mutation of *BRCA1* in Japanese breast cancer families. Cancer Research 55:3521-3524.

Katagiri T, Emi M, Ito I, Kobayashi K, Yoshimoto M, Iwase T, Kasumi F, Miki Y, Skolnick MH, and Nakamura Y (1996) Mutations in the *BRCA1* gene in Japanese breast cancer patients. Human Mutation 7:334-339.

Lynch HT, Lynch JF and Conway TA (1993) Hereditary ovarian cancer. In: Rubin SC, Sutton SP, eds. Ovarian cancer. New York: McGraw-Hill, pp 189-217.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, and Skolnick MH (1994) A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. Science 266:66-71.

Monteiro ANA, August A, and Hanafusa H (1996) Evidence for a transcriptional activation function of *BRCA1* C-terminal region. Proc Natl Acad Sci USA 93:13595-13599.

Sambrook J, Fritsch EF, and Maniatis T (1989) Isolation of high-molecular-weight DNA from mammalian cells. New York: Cold Spring Harbor Laboratory Press, pp 9.14-9.23.

Simard J, Tonin P, Durocher F, Morgan K, Rommens J, Gingras S, Samson C, Leblanc J-F, Bélanger C, Dion F, Liu Q, Skolnick M, Goldgar D, Shattuck-Eidens D, Labrie F, and Narod SA (1994) Common origins of *BRCA1* mutations in Canadian breast and ovarian cancer families. Nature Genet 8:392-398.

Sher C, Sharabani-Gargir L, Shobat M (1996) Letter to the editor, N Engl J Med 334:1199.

Shattuck-Eidens D, McClure M, Simard J, Labrie F, Narod S, Couch F, Hoskins K, Weber B, Castilla L, Erdos M, Brody L, Friedman L, Ostermeyer E, Szabo C, King M-C, Jhanwar S, Offit K, Norton L, Gilewski T, Lubin M, Osborne M, Black D, Boyd M, Steel M, Ingles S, Haile R, Lindblom A, Olsson H, Borg A,

Bishop DT, Solomon E, Radice P, Spatti G, Gayther S, Ponder B, Warren W, Stratton M, Liu Q, Fujimura F, Lewis C, Skolnick MH, and Goldgar DE (1995) A collaborative survey of 80 mutations in the *BRCA1* breast and ovarian cancer susceptibility gene. JAMA 273:535-541.

Steichen-Gersdorf E, Gallion HH, Ford D, Girodet C, Easton DF, DiCioccio RA, Evans G, Ponder MA, Pye C, Mazoyer S, Noguchi T, Karengueven F, Sobol H, Hardouin A, Bignon Y-J, Piver MS, Smith SA, and Ponder BAJ (1994) Familial site-specific ovarian cancer is linked to *BRCA1* on 17q12-21. Am J Hum Genet 55:870-875.

Struewing JP, Abeliovich D, Peretz T, Avishai N, Kaback MM, Collins FS, and Brody LC (1995) The carrier frequency of the *BRCA1* 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. Nature Genet 11:198-200.

Szabo CI, Wagner LA, Francisco LV, Roach JC, Argonza R, King M-C, and Ostrander EA (1996) Human, canine and murine *BRCA1* genes sequence comparison among species. Hum Mol Genet 5:1289-1298.

Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL, Yang M-C W, Hwang L-Y, Bowcock AM, and Baer R (1996) Identification of a RING protein that can interact *in vivo* with the *BRCA1* gene product. Nature Genet 14:430-440.

TABLE 1. JAPANESE OVARIAN CANCER PATIENT CHARACTERISTICS

Patient	Age of onset	Ovarian Tumor Pathology	Family History
1	61	Unknown (also breast cancer)	Mother-ovarian (age 68); 8 of mother's 9 siblings -various cancers
2	44	Mucinous	none
3	33	Mucinous	none
4	19	Serous	none
5	50	Clear Cell (also breast cancer)	none
6	22	Mucinous	none
7	22	Yolk Sac	none
8	19	Dysgerminoma	none
9	44	Endometroid	none
10	43	Clear Cell	none
11	42	Mucinous	none
12	55	Unknown	Sister-ovarian; Brother-rectal
13	32	Yolk Sac	none
14	48	Malignant teratoma	none
15	56	Clear Cell	none
16	40	Endometroid	none
17	45	Serous	Mother-ovarian
18	>45	Unknown	none
19	44	Unknown	none
20	>45	Unknown	none
21	45	Unknown	none

FIGURE 1. *BRCA1* FRAMESHIFT MUTATION IDENTIFIED IN PATIENT 1 BY SSCP

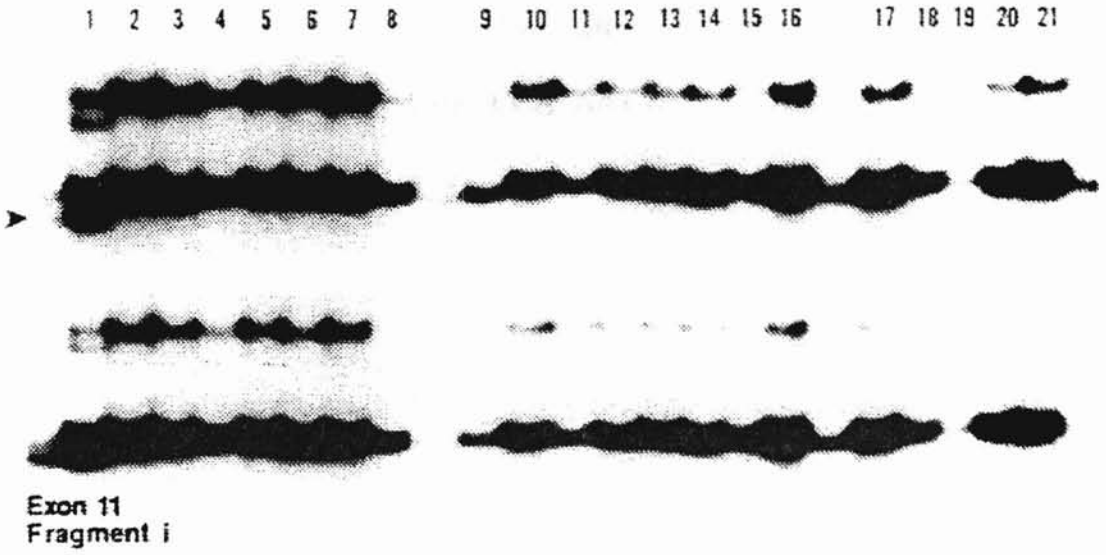


FIGURE 2. SEQUENCE CHANGE IN EXON 11, FRAGMENT i DUE TO FRAMESHIFT MUTATION, 797delAA

Patient 1:	ACA	GCC	AAA	TAA
	Thr	Ala	Lys	Stop
Control:	ACA	GAA	CCA	AAT
	Thr	Glu	Pro	Asn

CHAPTER III

LACK OF GERMLINE MUTATIONS INDICATES *Rb* GENE IS NOT INVOLVED IN HEREDITARY PREDISPOSITION TO OVARIAN CANCER IN JAPANESE WOMEN

ABSTRACT

Background: In the United States approximately 1.4% of women will develop ovarian cancer during their lifetime. In contrast, the cumulative incidence of ovarian cancer in Japan is one-fifth to one-third that of the United States.

Methods: We analyzed 21 Japanese women diagnosed with ovarian cancer for germline mutations in the retinoblastoma (*Rb*) gene using single-strand conformation polymorphism (SSCP) analysis. The women ranged in age from 19 to 61 and were randomly selected without regard for a family history of ovarian cancer.

Results: Results of the SSCP analysis of the samples yielded no mutations in the exon regions but one sequence alteration in an intronic region.

Conclusions: Allelic loss of the *Rb* gene has been reported in up to 52% of ovarian tumors, however, the existence of germline mutations in the *Rb* gene leading to ovarian cancer predisposition has not been investigated. The lack of germline mutations in the sample Japanese population provides strong evidence that mutations in the *Rb* gene do not play a part in the hereditary predisposition to ovarian cancer.

INTRODUCTION

Ovarian cancer is the leading gynecological malignancy in the United States. Clinical prognosis is generally poor once the cancer is discovered[1] because the inaccessibility of the ovary makes early detection difficult[2]. Approximately 5-10% of ovarian cancers diagnosed in American women result from hereditary predisposition with the remaining 90-95% believed to be of sporadic origin[3-5]. Several tumor suppressor genes have been positively linked to hereditary ovarian cancer, including *BRCA1* and *BRCA2*[6-9]. The retinoblastoma (*Rb*) tumor suppressor gene has been reported to exhibit allelic loss in 30-52% of ovarian tumors[10-12]. Nevertheless, a link to hereditary predisposition of ovarian cancer through germline mutations in the *Rb* gene has yet to be investigated.

The *Rb* gene is mapped to human chromosome 13 in the 13q14.2 region. The *Rb* gene contains 27 exons which are processed to produce an mRNA 4700+ basepairs in length[13-15]. The mRNA is translated into a nuclear phosphoprotein approximately 110 kDa in size[16]. The protein is hypophosphorylated as the cell enters G1, becomes phosphorylated late in G1 and remains hyperphosphorylated through S, G2 and most of M, then reverts back to a hypophosphorylated state at the M-G1 transition[17]. Phosphorylation of the protein at the G1-S transition is believed to be controlled by at least three different cyclin-dependent kinases[18]. Cell growth takes place during the hyperphosphorylation of the *Rb* protein. Growth suppression, on the other hand, occurs during the G1 phase of the cell cycle when the protein is hypophosphorylated[18]. While hypophosphorylated, the *Rb* protein has been shown to bind and inhibit transcription factor E2F[17]. Recent studies also report *Rb* protein interactions with a number of other proteins during the G1 phase. The exact mechanism by which *Rb* regulates gene

expression and cellular growth however remains to be elucidated.

While the hereditary predisposition to ovarian cancer in Japan is equivalent to the 5-10% found in American women[19], the overall incidence of ovarian cancer in Japan is one-third to one-fifth that of the United States[20]. Japanese populations lack many of the dietary risks, such as a diet high in saturated fats and frequent red meat consumption[21], commonly attributed to ovarian cancer in the United States. This dietary difference has been used as one explanation for the lower ovarian cancer incidence rates in Japan[20].

Mutations in the *Rb* tumor suppressor gene have an effect very early in a carrier's life. Approximately 90% of the individuals who inherit a defective allele will lose the second allele to a somatic mutation and develop retinoblastoma before age three. In the breast and ovarian cancer susceptibility genes, *BRCA1* and *BRCA2*, on the other hand, loss of the second allele appears to occur later in life. In all cases, this second hit is not yet fully understood. Age dependent penetrance is observed in women with inherited mutations in either *BRCA1* or *BRCA2,* with the risk of ovarian cancer increasing from 23% at age 50 to 63% by the age of 70 in the case of mutations to *BRCA1*[22]. The presence of mutations with variable penetrance would suggest a mechanism responsible for age variation in disease onset. Specifically, missense mutations may occur in less critical areas of the gene that may not immediately effect protein function in contrast to frameshift and missense mutations that lead to protein truncation. In addition, Gayther and coworkers have proposed that there exists a genotype-phenotype correlation in both *BRCA1* and *BRCA2* between the location of mutations within each gene and the onset of either breast or ovarian carcinoma[23].

Should such an age bias or genotype-phenotype correlation exist in the *Rb* gene,

the effect of germline mutations may not be limited to retinoblastoma. As a preliminary test of this hypothesis, we have examined a set of ovarian cancer patients for inherited mutations in the *Rb* gene. Should weakly penetrant alleles or selectively located mutations allow individuals to bypass retinoblastoma as a child, it is possible that the mutant *Rb* tumor suppressor gene could lead to cancer in other tissues later in life. Examination of such tumors might provide insight into the mechanisms by which second hits occur. Since few individuals are expected to develop cancers as a result of this pathway, the best mechanism by which to identify such occurrences is through genomic examination of individuals with various forms of cancer for which genetic causes, i.e. *BRCA1* mutations, have yet to be determined. Ovarian cancer is a potential candidate for association with *Rb* in light of the 30-52% incidence of allelic loss in ovarian carcinomas[10-12].

In this study, we used single-strand conformation polymorphism (SSCP) analysis to screen all exons, including intron-exon boundaries of the *Rb* gene, in genomic DNA from 21 Japanese women diagnosed with several forms of ovarian cancer.

MATERIALS AND METHODS

Materials. Buffy coats from 21 Japanese women ranging in age from 19 to 61 and diagnosed with various forms of ovarian cancer were obtained from the Tokyo Medical College, Tokyo, Japan (Table 1).

DNA Extraction. Genomic DNA was extracted from the buffy coats and purified by phenol-chloroform extraction[24].

PCR-SSCP Analysis. PCR amplification of the genomic DNA was performed using primers developed in our lab (Table 2). Primers were developed in the intronic regions flanking each exon. Due to its 1.8kb size, exon 27 was screened using nine sets of overlapping primers. Primers for exons 1 and 21 were used as published by Hogg and coworkers and Liu and coworkers[25, 26]. PCR reactions were carried out in 25 µl volumes containing 50 ng genomic DNA, 10X PCR Buffer (Boehringer-Mannheim, Indianapolis, IN), 200 µM each of dATP, dGTP and dTTP, 20 µM dCTP, 0.5 µCi [α-^{32}P] dCTP [(3000 Ci/mmol); DuPont NEN, Boston, MA], 1 µM of each primer and 1.25 U of *Taq* polymerase (Boehringer-Mannheim, Indianapolis, IN). Amplification was accomplished with 35 cycles of 94°C, 45 s; T_M, 30 s; 72°C, 30s; with a 7 min extension at 72°C following the final cycle. A 4.5 µl aliquot of the PCR product was diluted in 25 µl of denaturing loading buffer (95% formamide, 10 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF), heated at 99°C for 2 min then immediately cooled on ice. Aliquots of 4.5 µl were then loaded onto a 0.5X Mutation Detection Enhancement gel (FMC Bioproducts, Rockland, ME) and electrophoresed at room temperature for 14 hours at 6 W constant power. Following electrophoresis, gels were transferred to Whatman filter paper and exposed to film for 24 hours at -80°C.

Sequencing. The aberrant band and a normal control were eluted from the gel and reamplified with the appropriate primer pair. PCR products were purified for sequencing with a QIAquick Spin PCR Purification kit (Qiagen, Santa Clarita, CA). Purified products were bidirectionally sequenced using a model 373A automated fluorescence-based cycle sequencer (Applied Biosystems, Foster City, CA).

RESULTS

We have screened 21 Japanese women diagnosed with several forms of ovarian cancer using SSCP analysis over all 27 exons in the *Rb* gene including intron-exon boundaries. Variant banding was discovered in patient six following PCR amplification with primer pair 22 (Figure 1). DNA sequence analysis yielded no sequence changes in the exon or the portion of the 3' intron contained within the region amplified by the primer pair. Therefore we assume that the base change fell within the intron region 5' of the exon. This particular region has a tandem repeat of 24 T residues that has made identifying the exact base change difficult. Moreover, the tandem repeat may actually be the site of the alteration by leading to DNA polymerase slippage during transcription. The potential biological significance of this change is not clear however, since it is located outside of the splicing-donor and acceptor sites.

The patient was diagnosed with mucinous ovarian cancer at age 22. Diagnosis of any form of ovarian cancer prior to age 45, particularly in someone this young, is considered a strong indicator of hereditary ovarian cancer. Unfortunately, no family history or tissue samples from family members are available for further screening.

DISCUSSION

Rb gene mutations have been identified in a variety of diverse human cancers including retinoblastoma[13-15, 27], osteosarcomas[13], small cell lung carcinoma[28], breast cancer [29, 30], bladder cancer[31, 32], and prostate cancer[33]. These mutations are primarily small deletions and point mutations located between exons 12 and 23, which encode the binding regions potentially involved in the suppression of cell growth[33]. The widespread incidence of *Rb* gene mutations in such diverse cancers illustrates the prime importance of the gene in cancer progression.

Only two mutations have been reported in ovarian tumor DNA to date. Takano and coworkers report a C to A transition at codon 621 leading to the substitution of an arginine for a serine in a woman with advanced clear cell adenocarcinoma of the ovary[34]. However, this mutation was also found in normal tissue and therefore was disregarded as a polymorphism unrelated to the cancer. Liu and coworkers describe the deletion of exon 21 in its entirety in a woman displaying loss of one allele and duplication of the remaining one[35]. Exon 21 is believed to encode a portion of SV-40T antigen binding region important in the cell growth regulatory function of the *Rb* gene[33].

However, the majority of *Rb* gene studies in ovarian cancer have involved screening of tumor DNA using immunohistochemical techniques which do not reveal molecular abnormalities including gene mutations or defects to phosphorylation sites[36]. Takano and coworkers report an increase from 7% to 50% incidence of allelic loss at the *Rb* locus from early stage to advanced stage tumors[34]. Furthermore, Kim and coworkers found allelic loss of the *Rb* locus associated with tumors having invasive or metastic ability[37]. These results have led to the theory that loss of the gene's function through

somatic mutations may be important in the progression of ovarian cancer rather than the initiation[34]. This theory has also been proposed by Kubota and coworkers with regard to the progression of prostate tumors[33].

Our results suggest that germline mutations in the *Rb* gene are not present in ovarian carcinomas and therefore do not predispose women to hereditary ovarian cancer. Thus, future study of the *Rb* gene should focus on the role that somatic mutations to the gene have in the progression of ovarian cancer.

REFERENCES

1. Tsao S-W, Mok C-H, Oike K, Muto M, Goodman HM, Sheets EE, et al. Involvement of p53 gene in the allelic deletion of chromosome 17p in human ovarian tumors. Anticancer Res 1991;11:1975-1982.

2. Gallion HH, Pieretti M, DePriest PD, and van Nagell Jr. JR. The molecular basis of ovarian cancer. Cancer Supplement 1995;76:1992-1997.

3. Houlston RS, Collins A, Slack J, Campbell S, Collins WP, Whitehead MI, et al. Genetic epidemiology of ovarian cancer: segregation analysis. Ann Hum Genet 1991;55:291-299.

4. Bewtra C, Watson P, Conway T, Read-Hippee C, and Lynch HT. Hereditary ovarian cancer: a clinicopathological study. Int J Gynecol Pathol 1992;11:180-187.

5. Lynch HT, Lynch JF, and Conway TA. Hereditary ovarian cancer. In: Rubin SC, Sutton SP. Ovarian cancer. New York: McGraw-Hill, 1993: 189-217.

6. Narod SA, Fuenteun J, Lynch HT, Watson P, Conway T, Lynch J, et al. Familial breast-ovarian cancer locus on chromosome 17q12-q23. Lancet 1991;338:82-83.

7. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. Science 1994;266:66-71.

8. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, et al. Identification of the breast cancer susceptibility gene *BRCA2*. Nature 1995;378:789-792.

9. Lancaster JM, Wooster R, Mangion J, Phelan CM, Cochran C, Gumbs C, et al. *BRCA2* mutations in primary breast and ovarian cancers. Nature Genet 1996;13:238-240.

10. Li S, Schwartz PE, Lee W-H, and Yang-Feng TL. Allele loss at the retinoblastoma locus in human ovarian cancer. J Natl Cancer Inst 1991;83:637-640.

11. Sato T, Saito H, Morita R, Koi S, Lee JH, and Nakamura Y. Allelotype of human ovarian cancer. Cancer Research 1991;51:5118-5122.

12. Yang-Feng T, Li S, Han H, and Schwartz PE. Frequent loss of heterozygosity on chromosomes Xp and 13q in human ovarian cancer. Int J Cancer 1992;52:575-580.

13. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Alber DM, et al. A human DNA segment with properties of the gene that predisposes to

retinoblastoma and osteosarcoma. Nature 1986;323:643-646.

14. Fung Y-KT, Murphree AL, T'Ang A, Qian J, Hinrichs SH, and Benedict WF. Structural evidence for the authenticity of the human retinoblastoma gene. Science 1987;236:1657-1661.

15. Lee WH, Bookstein R, Hong F, Young L-J, Sheu J-Y, and Lee EY-HP. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. Science 1987;235:1394-1399.

16. Lee EY-HP. Tumor suppressor genes and their alteration in breast cancer. Cancer biology 1995;6:119-125

17. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995;81:323-330.

18. Taya Y. RB kinases and RB-binding proteins: new points of view. Trends in Biochemical Sciences 1997;22:14-17.

19. Inoue R, Fukutomi T, Ushijima T, Matsumoto Y, Sugimura T, and Nagao M. Germline mutation of *BRCA1* in Japanese breast cancer families. Cancer Research 1995;55:3521-3524.

20. Herrinton LJ, Stanford JL, Schwartz SM, and Weiss NS. Ovarian cancer incidence among Asian migrants to the United States and their descendants. J Natl Cancer Inst 1994;86:1336-1339

21. Cramer DW, Welch WR, Hutchison GB, Willett W, and Scully RE.: Dietary animal fat in relation to ovarian cancer risk. Obstet Gynecol 1984;63:833-838.

22. Hamann U, Becher H, Zimmermann T, Pella K, Bastert G, and Chang-Claude J. German family study on hereditary breast-ovarian cancer. J Med Genet 1996;33:633-635.

23. Gayther SA, Mangion J, Russell P, Seal S, Barfoot R, Ponder BAJ, et al. Variation of risks of breast and ovarian cancer associated with different germline mutations of the *BRCA2* gene. Nature Genet 1997;15:103-105.

24. Sambrook J, Fritsch EF, and Maniatis T. Isolation of high-molecular-weight DNA from mammalian cells. New York: Cold Spring Harbor Laboratory Press, 1989:9.14-9.23.

25. Hogg A, Onadim Z, Baird PN, and Cowell JK. Detection of heterozygous mutations in the *RB1* gene in retinoblastoma patients using single-strand conformation polymorphism analysis and polymerase chain reaction sequencing. Oncogene 1992;7:1445-1451.

26. Liu Z, Song Y, Bia B, and Cowell JK. Germline mutations in the *RB1* gene in patients with hereditary retinoblastoma. Genes, Chrom & Cancer 1995;14:277-284.

27. McGee TL, Yandell DW, and Dryja TP. Structure and partial genomic sequence of the human retinoblastoma susceptibility gene. Gene 1989;80:119-128.

28. Mori N, Yokota J, Akiyama T, Sameshima Y, Okamoto A, Mizoguchi H, et al. Variable mutations of the *Rb* gene in small-cell lung carcinoma. Oncogene 1990;5:1713-1717.

29. T'Ang A, Varley JM, Chakraborty S, Murphree AL, and Fung YKT. Structural rearrangement of the retinoblastoma gene in human breast carcinoma. Science 1988;242:263-266.

30. Varlay JM, Armour J, Swallow JE, Jeffreys AJ, Ponder BAJ, T'Ang A, et al. The retinoblastoma gene is frequently altered leading to loss of expression in primary breast tumors. Oncogene 1989;4:725-729.

31. Ishikawa J, Xu HJ, Hu SX, Yandell DW, Maeda S, Kamidono S, et al. Inactivation of the retinoblastoma gene in human bladder and renal cell carcinomas. Cancer Research 1991;51:5736-5743.

32. Cordon-Cardo C, Wartinger D, Petrylak D, Dalbagni G, Fair WR, Fuks Z, et al. Altered expression of the retinoblastoma gene product: prognostic indicator in bladder cancer. J Natl Cancer Inst 1992;84:1251-1256.

33. Kubota Y, Fukinami K, Uemura H, Dobashi Y, Miyamoto H, Iwasaki Y, et al. Retinoblastoma gene mutations in primary human prostate cancer. The Prostate 1995;27:314-320.

34. Takano H, Okamoto A, Terashima Y, and Yokota J. High incidence of allelic loss at the RB gene locus in advanced human ovarian cancer. Intl J Oncol 1995;6:129-135.

35. Liu Y, Heyman M, Wang Y, Falkmer U, Hising C, Székely L, et al. Molecular analysis of the retinoblastoma gene in primary ovarian cancer cells. Int J Cancer 1994;58:663-667.

36. Shimizu E, Coxon A, Otterson G, Steinberg SM, Kratzke RA, Kim YW, et al. *Rb* protein status and clinical correlation from 171 cell lines representing lung cancer, extrapulmonary small cell carcinoma, and mesothelioma. Oncogene 1994;9:2441-2448.

37. Kim TM, Benedict WF, Xu H-J, Hu S-X, Gosewehr J, Velicescu M, et al. Loss of heterozygosity on chromosome 13 is common only in the biologically more

aggressive subtypes of ovarian epithelial tumors and is associated with normal retinoblastoma gene expression. Cancer Research 1994;54:605-609.

TABLE 1. JAPANESE OVARIAN CANCER PATIENT CHARACTERISTICS

Patient	Age of onset	Ovarian Tumor Pathology	Family History
1	61	Unknown (also breast cancer)	Mother-ovarian (age 68); 8 of mother's 9 siblings -various cancers
2	44	Mucinous	none
3	33	Mucinous	none
4	19	Serous	none
5	50	Clear Cell (also breast cancer)	none
6	22	Mucinous	none
7	22	Yolk Sac	none
8	19	Dysgerminoma	none
9	44	Endometroid	none
10	43	Clear Cell	none
11	42	Mucinous	none
12	55	Unknown	Sister-ovarian; Brother-rectal
13	32	Yolk Sac	none
14	48	Malignant teratoma	none
15	56	Clear Cell	none
16	40	Endometroid	none
17	45	Serous	Mother-ovarian
18	>45	Unknown	none
19	44	Unknown	none
20	>45	Unknown	none
21	45	Unknown	none

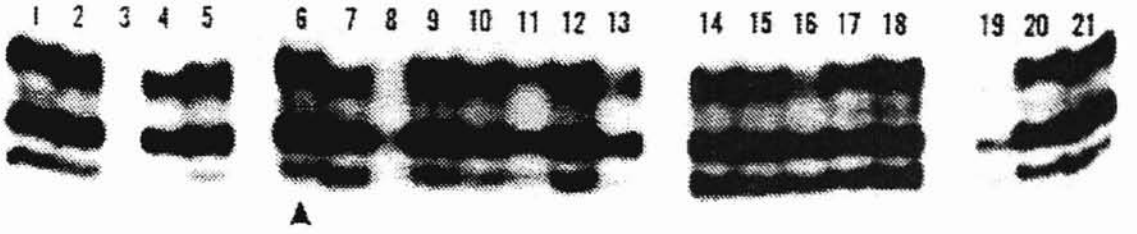
TABLE 2. *Rb* GENOMIC DNA PRIMER PAIRS

Exon	Forward Primer (5' -3')	Reverse Primer (5'-3')	Tm °C	Size
1 p*	GATCCCAAAAGGCCAGCAAGTGTCT	TCAACGTCCCCTGAGAAAAACCGGA	62	570
1**	AGCTCGCTGGCTCCCGCCGCGGAAA	TCCCGCGTGAGGGACGCGCTCTGCT	62	200
2	GTGTTATGTGCAAACTATTG	GGTAAATTTCCTCTGGG	58	358
3	GCCATCAGAAGGATGTGTTAC	CATTTCCTTTTATGGCAGAGG	56	312
4	CCTTCCAAAGGATATAGTAGTG	CCCAGAATCTAATTGTGAACAA	52	329
5	TGAATAAAGCATGAGAAAA	CGATCAAACTAACCCTAAC	50	209
6	TGCACAAAAAGAAACACC	CAGAGAATGAGGGAGGAG	61	278
7	CATGCTGATAGTGATTGTTGAATG	GCCTTAGAACCATGTTTGGTAC	58	314
8	TGGATGTACAATTGTTCTTATC	ATATTGTTAGGGAGAACTTAC	47	252
9	TTGACACCTCTAACTTACCCT	ACAATTATCCTCCCTCCACA	60	235
10	GAAATCTGTGCCTCTGTGTG	CTAAAGGTCACTAAGCTAAAGA	60	277
11	GCATAAAGCACAAATT	TGGCCTTCAATATATAT	51	261
12	TAGAGACAAGTGGGAG	TACATGTTAGATAGGAGAT	49	321
13	ACAGTATCCTCGACATTGA	TACACAGGCAGCAGGGA	60	299
14	TGCCTATAATCCCAGCCTCT	TGATCTTGATGCCTTGACCTC	68	340
15	TTCACCCCAGCCTGGCA	ACAACCTCAAGAGCGCACG	61	302
16	GAAGAAGAACGATTATCC	TCCTTCTCCTTAACCTC	55	293
17	TCCAAAAAAAATACCTAGCTC	GTAGATTTGTTAAGAAACACCTC	51	350
18	TTTTGATATGTACCTGGG	CTATTCCCTACAGTTTCTT	55	270
19	CTGTGATTCTTAGCCAACTT	ACATGATTTGAACCCAGTC	51	262
20	AGAGTGGTAGAAAAGAGGT	ACAAGTAAGTAGGGAGGAG	56	326
21*	GACTTTCAAACTGAGCTCAGTATGG	ACAAATACCTGCTTATTACAGGGAT	58	518
22	AATATGTGCTTCTTACCAGTC	GTGGACCCATTACATTAGAT	50	299
23	TTTGCAGTATGCTTCCACCA	AATAATCCCCCTCTCATTCTTT	57	328
24	GTATTTATGCTCATCTCTG	CAATATGCCTGGATG	52	222
25	TGCTAACTATGAAACACTG	TGCTGAGACTCTGGATT	53.1	268
26	GCATAAAGTAAGTCATCG	GACCTTCATTTTACATCA	44	336

Exon	Forward Primer (5' -3')	Reverse Primer (5'-3')	Tm °C	Size
27a	CACTTGCCAACTTACCC	CTGCACATTTTATATCCACA	53	271
27b	GATGTGACTGTATAACTTTCCC	TATCAGGACTCCCACTCTAG	53	263
27c	AAGTTGTAGCAGATTGTTTCCTC	GCATTTGGAAGATAGATAGGACAC	68	226
27d	GCCTGTCTGACTACTTTGCC	GGTTATACTTTGCTTCCAGC	61.5	324
27e	GGAATCTGATATACTGTGTGC	CAGGACCCAGAAACATTAG	51	324
27f	GACCCTAACACAGTATATCCCA	GCCTAACCCATAATGACCCT	55.4	346
27g	TCTCAAAATTATTCTGCCCTC	GCTGAAGCTACCTTAAATATCC	55.4	374
27h	TCCCCTCCCCTACACCTAAAG	ATCTCTGAAGTTCCTAAAATTCTGGC	62	389
27i	AGGGCTTACTATTTCTGGG	AGGAATGGATGAGATACTAGG	52.5	332

The names of the primer pairs used to screen the Rb gene by SSCP analysis are listed in the first column. Tm indicates the annealing temperature for each PCR primer pair. The size of the product is given in basepairs. Primer pairs annotated by * are as reported by Hogg and coworkers (1992), and ** are as reported by Liu and coworkers (1995).

FIGURE 1. POTENTIAL *Rb* SEQUENCE CHANGE IDENTIFIED IN PATIENT 6 BY SSCP



CHAPTER IV

CONCLUSION

This project identified two sequence alterations, a frameshift mutation in the *BRCA1* gene and a potential insertion or repeat in an intronic region of the *Rb* gene. Due to its location in an intronic region, the biological significance of this *Rb* sequence change is unclear. On the other hand, the *BRCA1* mutation, 797delAA, may represent an ethnically isolated mutation, similar to the *BRCA1* mutation 185delAG in Ashkenazi Jews, that could be used for early detection screening.

From these studies it is relatively clear that the *Rb* gene plays little or no role in the hereditary predisposition to ovarian cancer in Japanese women. However, further study of the *BRCA1* gene and in particular, the penetrance of the 797delAA mutation, is recommended. Moreover, due to the low incidence of germline mutations in the *BRCA1* and *Rb* genes in the sample population, future studies of the Japanese should focus on other tumor suppressor genes such as the *BRCA2* that may be responsible for hereditary ovarian cancer in the population.

As more mutations are identified, researchers have begun to debate the many ethical issues that surround genetic screening and the participating families. However, in light of the low survival rate following ovarian cancer, prescreening individuals may help further the fight against this disease and greatly reduce the risk in women with a family history of ovarian cancer.

BIBLIOGRAPHY

Bishop JM (1991) Molecular themes in oncogenesis. Cell 64:235-248.

Bookstein R, Shew J-Y, Chen P-L, Scully P, and Lee W-H (1990) Suppression of tumorigenicity of human prostate carcinoma cells by replacing a mutated *Rb* gene. Science 247:712-715.

Easton DF, Bishop DT, Ford D, Crockfrord GP, and the Breast Cancer Linkage Consortium (1993) Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. Am J Hum Genet 52:678-701.

Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Alber DM, and Dryja TP (1986) A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 323:643-646.

Fung Y-KT, Murphree AL, T'Ang A, Qian J, Hinrichs SH, and Benedict WF (1987) Structural evidence for the authenticity of the human retinoblastoma gene. Science 236:1657-1661.

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, Eddington K, McClure M, Frye C, Weaver-Feldhaus J, Ding W, Gholami Z, Söderkvist P, Terry L, Jhanwar S, Berchuck A, Iglehart JD, Marks J, Ballinger DG, Barrett JC, Skolnick MH, Kamb A, and Wiseman R (1994) *BRCA1* mutations in primary breast and ovarian carcinomas. Science 266:120-122.

Gallion HH, Pieretti M, DePriest PD, and van Nagell Jr. JR (1995) The molecular basis of ovarian cancer. Cancer Supplement 76:1992-1997.

Herrinton LJ, Stanford JL, Schwartz SM, and Weiss NS (1994) Ovarian cancer incidence among Asian migrants to the United States and their descendants. J Natl Cancer Inst 86:1336-1339.

Horowitz JM, Park S-H, Bogenmann E, Cheng J-C, Yandell DW, Kaye FJ, Minna JD, Dryja TP, and Weinberg RA (1990) Frequent inactiviation of the retinoblastoma anti-oncogene is restricted to a subset of human tumor cells. Proc Natl Acad Sci USA 87:2775-2779.

Lee WH, Bookstein R, Hong F, Young LJ, Sheu JY, and Lee EYHP (1987) Human retinoblastoma susceptibility gene: cloning, identification, and sequence. Science 235:1394-1399.

Lee W-H, Bookstein, RE, and Lee EY-HP (1990) Molecular biology of the human retinoblastoma gene. *In:* G. Klein (ed.), Tumor Suppressor Genes, pp169-200.

New York: Marcel Dekker, Inc., 1990.

Li S, Schwartz PE, Lee W-H, and Yang-Feng TL (1991) Allele loss at the retinoblastoma locus in human ovarian cancer. J Natl Cancer Inst 83:637-640.

Steichen-Gersdorf E, Gallion HH, Ford D, Girodet C, Easton DF, DiCioccio RA, Evans G, Ponder MA, Pye C, Mazoyer S, Noguchi T, Karengueven F, Sobol H, Hardouin A, Bignon Y-J, Piver MS, Smith SA, and Ponder BAJ (1994) Familial site-specific ovarian cancer is linked to *BRCA1* on 17q12-21. Am J Hum Genet 55:870-875.

Tsao S-W, Mok C-H, Oike K, Muto M, Goodman HM, Sheets EE, Berkowitz RS, Knapp RC, and Lau CC (1991) Involvement of *p53* gene in the allelic deletion of chromosome 17p in human ovarian tumors. Anticancer Res 11:1975-1982.

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