AD_____

Award Number: DAMD17-00-1-0675

TITLE: Effects of Altered BRCA1 and p53 on Breast Cancer Prognosis in African-American Women

PRINCIPAL INVESTIGATOR: Dr. Meena Jhanwar-Uniyal

CONTRACTING ORGANIZATION: American Health Foundation
Valhalla, NY 10595

REPORT DATE: October 2003

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2003	Final (11 Sep 2000 - 11 Sep 2003)

4. TITLE AND SUBTITLE

Effects of Altered BRCA1 and p53 on Breast Cancer Prognosis in African-American Women

5. FUNDING NUMBERS

DAMD17-00-1-0675

6. AUTHOR(S)

Dr. Meena Jhanwar-Uniyal

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

American Health Foundation
Valhalla, NY 10595

E-Mail: mjhanwar@ifcp.us

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

Original contains color plates: ALL DTIC reproductions will be in black and white

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Breast Cancer (BC) is the second leading cause of death among women in the United States. Although the incidence of breast cancer is higher in American White (AW) women, mortality in African American (AA) is considerably higher. These differences are perhaps due to histological and socioeconomic factors. Mutations of the tumor suppressor gene p53 are among the most common genetic defects in cancer cells, and in several studies alterations in p53 in breast cancer have been associated with a poor prognosis. Individual carrying mutations in p53 or inactivation of BRCA1 genes are predisposed to a variety of cancers, both tumor suppressor genes have been implicated in establishing genome stability by participating in DNA damage pathways. There have been discrete p53 mutations in AA cohort, which were different WA cohorts. Mutations in BRCA1 gene accounts for about 50% of inherited breast cancer cases, but somatic mutations of BRCA1 gene are absent in sporadic cancers. Inactivation of BRCA1 occurs via the hypermethylation of the promoter region of the BRCA1 gene in sporadic cancers. We have compared the the mutation of p53 gene and inactivation of BRAC1 gene in AA and AW population. We found that: 1)Higher p53 overexpression, representative of presence of mutant p53 protein, was observed in AAs then in AWs woman; 2) The number of p53 mutations were more in AA as compared to AWs; 3)Hypermethylation of promoter of BRCA1 gene was seen in cases where p53 was muted irrespective of race. This study, when complete will establish a causal variation in AAs as compared to WAs.

14. SUBJECT TERMS

Breast Cancer

15. NUMBER OF PAGES

32

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Abstract: Breast Cancer (BC) is the second leading cause of death among women in the United States. While the incidence of breast cancer is higher among American White (AW) women, than among African American (AA) women, mortality is considerably higher in the latter These differences are likely due to both genetic and socioeconomic factors. Mutations of the tumor suppressor gene p53 are among the most common genetic defects in cancer cells, and several studies have identified alterations of p53 in breast cancer as indication of a poor prognosis. Individuals carrying mutations in p53 or inactivation of BRCA1 genes are predisposed to a variety of cancers; both tumor suppressor genes have been implicated in establishing genome stability by participating in DNA damage repair pathways. p53 mutations in the AA cohort,have been found to be distinctly different from those in WA cohorts. Mutations in the BRCA1 gene appears in about 50% of inherited breast cancer cases, but somatic mutations of the BRCA1 gene are absent in sporadic cancers. Inactivation of BRCA1 occurs via the hypermethylation of its promoter region in sporadic cancers. We have compared the status of abnormal p53 and inactivation of the BRCA1 gene in AA and AW women. We found: 1) higher p53 overexpression, characteristic of the presence of a mutant p53 protein, in AA than in AW women; 2) the number of p53 mutations were greater in AA than in AW women; 3) an inverse relationship ($r = -0.73$) between premenopausal age and status of p53 was evident only in AA women and not in AW; 4) hypermethylation of the promoter of the BRCA1 gene was seen in cases where p53 was muted irrespective of race. This study points to a variation in the tumor suppressor p53 that may underly the differences in cancer mortality between AA and AW women. In addition, this study shows that loss of BRCA1, with or without p53 abnormalities, is not an independent prognostic marker in AA women, but rather a biological phenomenon associated with the global hypermethylation pattern.

INTRODUCTION

Breast Cancer (BC) is the second leading cause of death from cancer among women in the United States. Although the incidence of breast cancer is 13% higher in American White (AW) women than in African Americans (AA), the latter have 28% higher mortality from breast cancer (Harris et al., 2003). These differences are perhaps due to both genetic and socioeconomic factors. Mutations of the tumor suppressor gene p53 are among the most common genetic defects in cancer cells. In several studies, alterations in p53 in breast cancer have been associated with a poor prognosis. Overexpression of p53 protein occurs with similar frequency in breast cancers in AA as well as in AW women (Rose and Royak-Schaler, 2001). Studies have also suggested that AAs have a different spectrum of p53 mutations compared to WAs, yet the specific mutational differences between these studies are somewhat conflicting (Blaszyk et al., 1994; Shiao et al., 1995). In addition, AAs with p53 mutations had definitely much poorer prognosis than did AWs (Shiao et al., 1995). The available reports in the literature, thus, provide several potential biological resons for the poorer prognosis for breast cancer in AA women. Although, germline alterations in the BRCA1 gene are responsible for 50% of familial breast cancer (Friedman et al., 1994; Futreal et al., 1994), the mutation in the BRCA1 gene is not found to be mutated in sporadic breast cancer (Marajver et al., 1995; Berchuck et al., 1998). Recent studies have demonstrated that inactivation of BRCA1 in sporadic cancer can occur due to hypermethylation of its promoter region. (Esteller et al., 2000; Hedenfalk et al., 2001). This may symbolize a mechanism by which the BRCA1 gene gets inactivated in some sporadic cancers. Furthermore, loss of the tumor suppressor gene BRCA1 in sporadic forms of breast cancer may point to a novel mechanism with regard to its role in tumor initiation. Despite this fact, there have been no published studies determining whether breast cancers arising in AA women have an increased frequency of defects in either the p53 or BRCA1 gene or both, and whether there are specific types of defects associated with the two genes that distinguish these tumor groups. Mutations in p53 and BRCA1 have been studied; however, the possible relationship of p53 and BRCA1 hypermethylation has not been defined.

The main objective of this research project was to determine whether the alterations in p53 and BRCA1 genes that are involved in tumor development and progression are distinctly different in the AA population as compared to AW. We proposed to analyze the mutations of the tumor suppressor gene p53 that are among the most common genetic defects in cancers, and we planned to study the mutational spectrum of p53 in both population. Furthermore, we planned to establish that in addition to abnormal p53, inactivation of the BRCA1 gene via hypermethylation of its promoter, may contribute specifically to breast cancer in AA.

Hypothesis/Rationale/Purpose

Overexpression of p53 protein has been observed at similar frequency in breast tumors from AW and AA women. Limited published data suggest that tumors from the AA group may exhibit a different spectrum of p53 mutations (Blaszyk et al., 1994; Shiao et al., 1995). In addition, BRCA1 gene inactivation may go along with p53 mutation and may contribute to greater virility of the cancer development, which is more aggressive in AA women. Therefore, we assessed sporadic breast cancers from AA and AW women for the following:

1) Overexpression of p53 protein and/or sequestration of p53 protein in the cytoplasm by immunohistochemical (IHC) techniques using p53-specific antibodies.
2) Mutational analysis of p53, employing standard molecular biological techniques using DNA extracted from microdissected tumor tissues.
3) Inactivation of BRCA1 gene due to hypermethylation of BRCA1 promoter in chemically modified DNA, and then using methylation-specific PCR

Thus, in this project we tested the whether breast cancers from AA women, showed a higher frequency of p53 defects in individual tumors than is observed in breast tumors from White women, and whether the changes in p53 are associated with hypermethylation of BRCA1.

Methods

Tissue blocks were obtained from 94 women who have been diagnosed with breast cancer at Crozer-Chester Medical Center, located near Philadelphia, PA, and the Medical College of Pennsylvania Hahnemann University. In addition, we had obtained tumor samples from New-York Presbyterian Hospital in New York City.

Immunohistochemical Analysis: Formalin-fixed, paraffin-embedded archival surgical tissue blocks were identified as corresponding to the appropriate patient charts. The Pathology Departments at MCP and Crozer identified the tissue blocks by slide review, and this along with the pathology chart was forwarded to us. Deparaffinized and rehydrated sections were retrieved in a citrate-buffered solution via heat-induced epitope retrieval for 6 min by microwave. Endogenous peroxidase activity was blocked by incubating with 3% hydrogen peroxide for 15 min. Nonspecific protein binding was blocked using serum-free protein block (DAKO) for 7 min. A standard avidin-biotin complex procedure was used (ABC, Vector Labs). For identification of tumors showing over-expression of p53 protein, the mouse monoclonal antibody PAb-1801 (Lab Vision) was employed and graded whereby we modified the method of Elledge et al., (1994) using a scale of 0-6, in which positivity is for p53 was consider if score was 3 or more. In this scale, intensity was graded from 0-3 and the proportion of positive cells was 0 < 10%, 1 = 10-32%, 2 = 33-66%, 3 > 66%.

DNA Extraction from Formalin Fixed Tissue: Genomic DNA was extracted from paraffin fixed breast tissue sections following a paraffin removal procedure using xylene-ethanol in standardized procedures (Shambrook and Russell, 2001). Aliquots of the purified supernatant were taken directly out of the sample extraction tube and used for PEP reactions; the remaining sample was stored at -20°C.

PCR-SSCP analysis and direct DNA sequencing for p53 mutations: The genomic DNA (50-150 ng) was amplified by PCR using oligonucleotide primers designed for TP53 gene from published sequences (Orita et al., 1989; Jhanwar-Uniyal and Gulati, 1998). PCR-SSCP analysis of Exons 4, 5,6,7,8 and 9 of the p53 gene, with nucleotide length of 139 to 330 bp, was performed using a published technique (51). These regions contained domains of p53 highly conserved among species, and they are also the site of frequent mutations in breast cancer. Briefly, 50 ng of genomic DNA was amplified with 0.4 umol/L of forward and reverse primers, dNTPs (2.5 umol/L), 10 mM Tris (pH 8.8)-$MgCl_2$ buffer and 0.2 units of *Taq* polymerase (Perkin Elmer-Cetus, USA) in a final volume of 25 :l ul. Conditions for PCR were as described in the literature (Orita et al., 1989; Jhanwar-Uniyal and Gulati, 1998) and amplification was carried out in an automated DNA

Thermal Cycler (Eppendorf). An aliquot of each was diluted with 0.1 % sodium dodecyl sulfate (SDS) and 10 mmol/L EDTA, and further diluted 1:1 with sequencing stop solution (95% formamide, 20 mmol/L EDTA. 0.05% bromophenol blue, and 0.05% xylene cyanol FF). Samples were heated at 95^{0}C for 5 min, chilled on ice, and immediately loaded onto a 6% polyacrylamide (acrylamide: N, N'-bisacrylamide =49:1) in Tris/EDTA/borate buffer, stained with ethidium bromide, and photographed.

Methylation of BRCA1 gene:

Modification of genomic DNA:

Genomic DNA was modified with bisulfite to convert the cytosine nucleotide to uracil (Esteller et al., 2000). In the reaction, all cytosines are converted to uracil, but those that are methylated (5-methylcytosine) are resistant to this modification. This altered DNA can then be amplified and sequenced to provide detailed information about the region of the methylation of CpG sities for specific genes. DNA (1 ug) was denatured with NaOH for 10 min at 37^{0}C followed by bisulfite treatment and incubation at 50^{0}C for 16 hrs after which DNA was purified, treated with NaOH, and then precipitated with ethanol.

BRCA1 promoter methylation:

We have used a promoter specific to the BRCA1 gene and specifically designed for the methylated and unmethylated sequence. Modified DNA was amplified with BRCA1 promoter specific primers. PCR conditions were as follows: 96^{0}C for 5 min. then 35 cycles of 96^{0}C for 20 sec., 60^{0}C for 20 sec, 72^{0}C for 90 sec; and finally 5 min at 72^{0}C. Controls without DNA were run for each set of PCRs. The amplified PCR product was electrophorised, stained with ethidium bromide and directly visualized under UV illumination.

Results

Study population:

Breast carcinoma samples from 34 AA women and 60 WA women from the two hospitals in Pennsylvania, namely Crozer and MCP. Figure1 represents the number of breast cancer cases at age of diagnosis in AW and AA cohorts. The median age of AA was 62 years (range 30-91) and that for AW was 65 years (range:36-75).

	Race (n)		**Age at Diagnosis (years)**	
	African American (AA)	**American White (AW)**	**African American (AA)**	**American White (AW)**
MCP	30	11	30-91	43-91
Crozer	4	49	51-74	36-75
Total	**34**	**60**	**30-91** (Median age 62)	**36-91** (Median age 65)

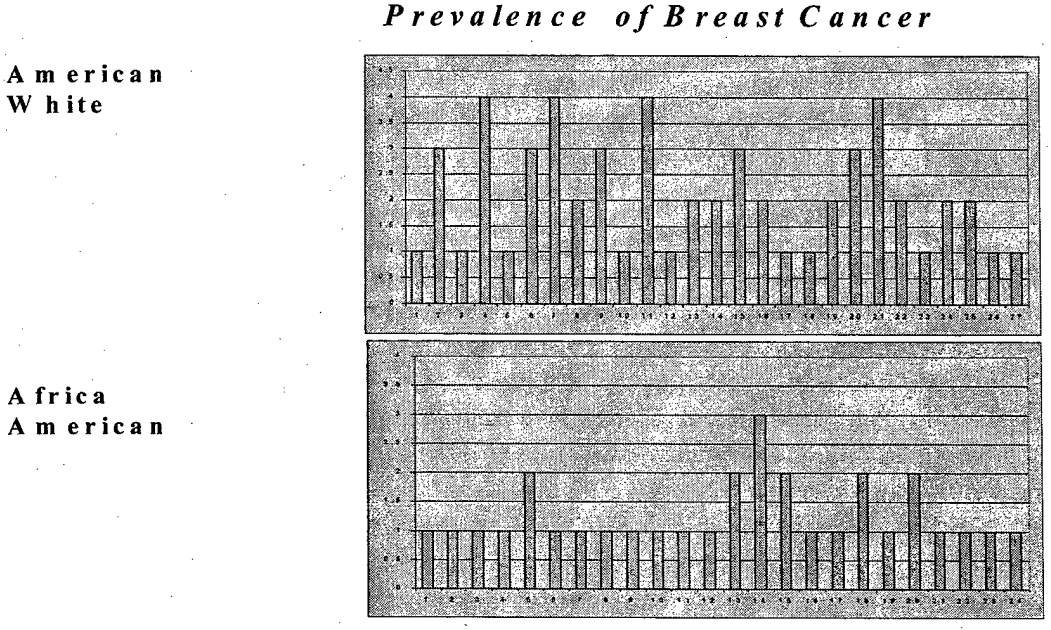


Figure.1: Age at diagnosis (X axis) versus number of patients (Y axis). As shown in Figure 1, the earlist age at diagnosis was 30 among AA and 36 among the AW. There were 7 breast cancer patients under age 55 years among the 34 for AA (20%) and 22 among the 60 AW (35%).

Table 1: Distribution of p53 Scores:

Over-expression of p53 protein was determined by employing immunohistochemistry using the mouse monoclonal antibody PAb-1801, that detects both wild-type and mutant protein. Since normal p53 has a short half-life and is generally undetectable, the expressed levels of p53 are considered to be mutant p53. The levels of p53 are graded by using the method of Elledge et al., (1994) with slight modification. In this, using a scale of 0-6, in which positivity score was 3 or higher. In this scale, intensity was graded as 0-3 and the proportion of positive cells was 0 < 10%, 1 = 10-32%, 2 = 33-66%, 3 > 66%. At the completion of grading both numbers, the proportion of cells expressing p53 and the intensity of expression are combined.

P53 Grading	0	1	2	3	4	5	6
African American (AA)*	7	10	5	2	3	6	1
White American (AW)	22	16	5	6	3	1	4

* Only n=57 samples were analyzed for AW

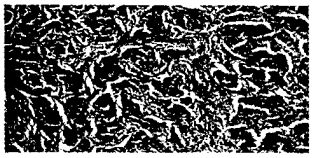
Table 2: Expression of mutant p53 in African-American and American-White Women: positive and negative scores of P53:

p53 Negative (Score 0-3)		p53 Positive (Score 4-6)	
AA	AW*	AA	AW*
24 (70%)	49 (86%)	10 (29%)	8 (14%)

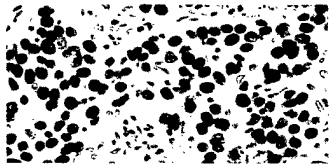
*Only n=57 samples were analyzed for AW

Accumulation of mutant p53 protein resulted in a higher immunohistochemical signal to the cells in the malignant breast epithelium. These data are presented in Tables 1 and 2. P53 positivity (overexpression and greater intensity), which confers a poor prognosis, is notably more prevalent in AW as compared to AA. Of all samples analyzed, 29% of thoseoriginating from AAs displayedp53-positive staining, while only 14% o those from AW displayed the high score of p53. Moreover, 86% of AW showed low p53 staining while 70% of AA showed low staining. It is interesting to note that a 0 score was seen in 20% of all AA, while 39% of AW breast samples displayed zero scores. A strong p53-positive staining indicates the presence of mutant p53, while absence of p53 means that wild-type p53 is present.

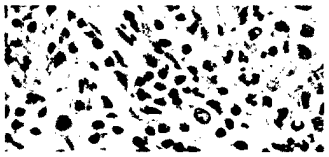
Figure 2:



H& E staining in paraffin-enbedded human breast carcinoma



Immunohistochemical staining of p53 in paraffin-embedded human breast carcinoma using p53 antibody 1801, showing intense nuclear staining of p53

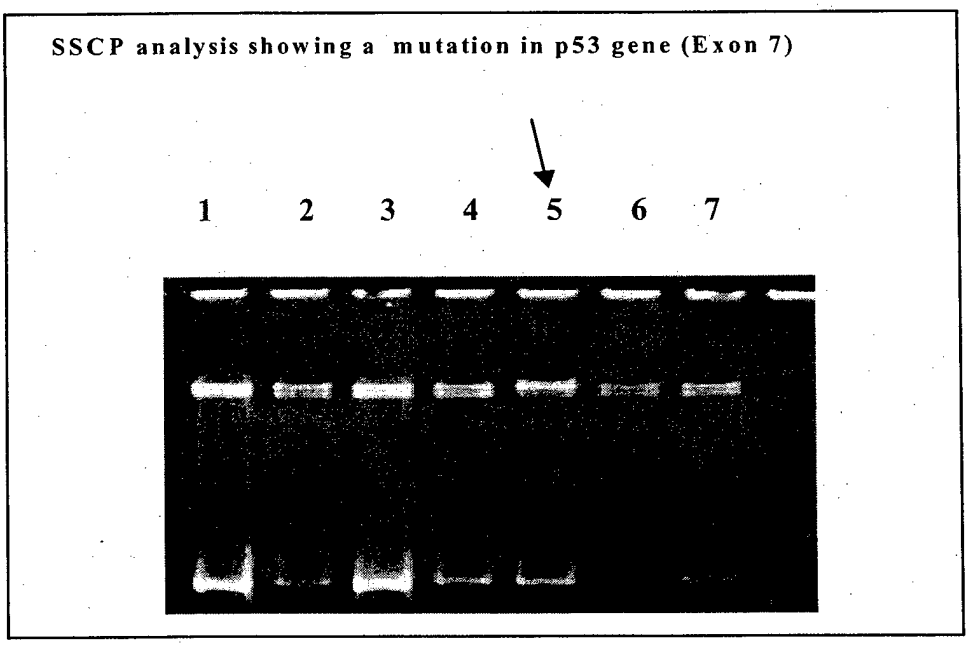


Immunohistochemical staining of p53 in paraffin-embedded human breast carcinoma using p53 antibody 1801, showing absence of p53 staining.

p53 Gene mutation analysis: Mutational analysis of the p53 gene was carried out in samples that displayed high scores of p53 immunostaining (Scores 4-6). Some samples that showed p53-negative staining but were ER and PR negative were also studied for p53 mutation. PCR-SSCP analysis of these samples demonstrated a strong link between high scores in p53

immunohistochemical analysis and p53 mutation. So far we have done p53 gene analyses only on Exon 5-9, also known as the hot-spot zone of the gene. Samples from 2 AA subjects, that displayed a high score of p53 immonohistochemistry, had mutations of p53 in Exon 7 (Figure 3). Both these subjects had infiltrating invasive ductal carcinoma. One of these two subjects was diagnosed with breast cancer at the relatively young age of 30 and had negative ER and PR status. One other sample from an AA subject with medullary carcinoma displayed high p53 IHC and showed a mutation of the p53 gene in Exon 5. Thirty percent of the samples displaying high IHC for p53 protein, from AA origin, showed mutation in the p53 gene (Exon 5-9 studied so far). We detected no mutation in Exon 5-9 in six samples that displayed high p53 IHC. However, we detected two p53 mutations in an AW-derived sample. In addition, we observed a p53 mutation in Exon 5 from DNA derived from an AW, this case had shown a border line ER and PR, and had a high proliferative index.

Figure 3:



Determining the mutations in Exons 2-4 and 10-11 of p53 in breast cancer samples that display high scores for p53 levels using IHC but failed to demonstrate mutation in the hot-spots zone (Exon 5-9), will be important in establishing the role of p53 in breast cancer in AA women.

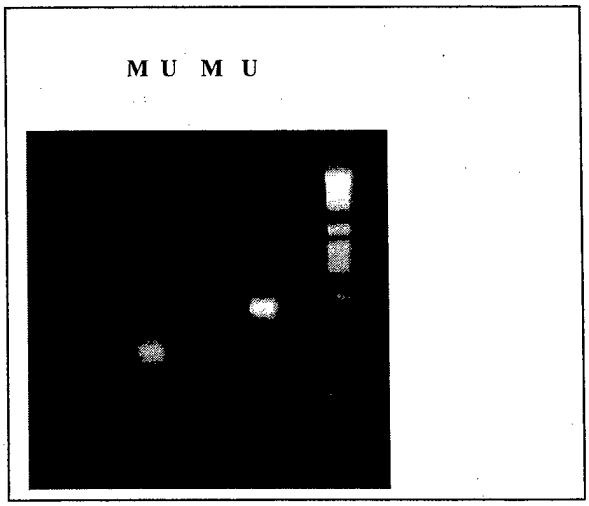
Methylation of BRCA1 Promoter:

Our results to date show hypermethylation of BRCA1 promoter (Figure 4) in samples that display p53 mutation. Three samples, from AA women had high p53 expression and two of these three had p53 mutation in Exon 7 (one is still being studied for Exons 2-4,and 10,11). Notably, two of these subjects had onset of breast cancer at a relatively very young age (30 and 33 years). We had also observed hypermethylation of BRCA1 in a sample from AW women; two of them showed ER and PR negative status. An example of a BRCA1-hypermethylated sample is shown

in Figure 4. As shown in this figure, bisulfite-modified DNA was amplified with two sets of primers (methylation-specific and unmethylation-specific), first sample (from left) shows presence of methylated (M) band and absence of unmethylated (U) and second sample showed no methylated band but shows a unmethylated allele. This epigenetic mechanism of gene inactivation of the BRCA1 gene is observed in sporadic cancer and may explain an inactivation of a tumor suppressor gene besides mutation or loss of heterozygosity (LOH).

M=Methylated; U=Unmethylated

Figure 4



Discussion:

We have confirmed histopathological diagnosis in H&E-stained sections from the tumor blocks. Immunohistochemical determination of p53 is completed and graded. Expression of abnormal p53 in this study was greater in AA than in WA women. An inverse correlation exists between the premenopausal age and levels of abnormal p53 (r= -0.73) only in AA women, which indicates that the lower the age the higher the p53 levels. Mutational analysis of Exon 5-9 of p53 gene shows that number of p53 mutation were higher in AA than AW women.. Mutational analysis of other Exons (2-4 and 10 and 11) of the p53 gene is currently underway. Hypermethylation of the promoter region of the BRCA1 gene was seen predominantly in cases with mutated p53 and negative status of ER and progesterone receptors. To date, our data suggest that a racial disparity exists in-terms of genetic factors which may contribute to poorer prognosis of breast cancer in AA women.

KEY RESEARCH ACCOMPLISHMENTS

- Median age at onset of breast cancer was 62 years in AW and 65 in AA.
- Although onset of breast cancer was at a much younger age in AA, the incidence of breast cancer in AW was generally greater at age below 55.
- Expression of abnormal p53 in this study was greater in AA than in WA women.

- Expression of abnormal p53 was always higher in samples with loss of estrogen and progesterone receptor positivity.

- The number of p53 mutations was greater in the AA than in the AW women.

- An inverse correlation exists between the premenopausal age and levels of abnormal p53 ($r = -0.73; p<0.05$) only in AA women, which indicates that the lower the age the higher the abnormal p53 levels. No such correlation was seen in AW women ($r = -0.09$; NS).

- A strong association was seen between presence of abnormal p53 and hypermethylation of the BRCA1 promoter.

REPORTABLE OUTCOMES

The outcome of this study strongly suggests that p53 abnormality in AA women, beginning during their premenopausal years, may contribute to a more virulent form of breast cancer in AA women. An inverse relationship between age and abnormal p53 in AA women indicates that AA women have an increased frequency of defects in p53 gene and these defects may contribute to the poor outcome of disease. However, inactivation of BRCA1 in both AA and AW was associated with a biological phenomenon where a common mechanism for silencing gene expression occurs via hypermethylation of the promoter region-associated CpG island. A similar mechanism is involved in the loss of estrogen receptor (ER) positivity, which is particularly common in premenopausal AA women, and has also been associated with BRCA1 inactivation and p53 overexpression or p53 gene mutation. In fact, we found that a subset of breast cancer patients with specific histological type, such as, Medullary carcinoma, from both AA and AW patients, had ER negative status with overexpression of p53 and hypermethylation of the promoter region of BRCA1 gene.

CONCLUSIONS

The outcome of this study is very important, and the results are very impressive, although they require further study to fully explain the variations in genetics that relate to breast cancer in AA women. Furthermore, the finding of this study will help design unique diagnostic and treatment strategies in this specific group. The identification of altered BRCA1, with p53 abnormalities, that are present in excess in breast cancer of AA women provides a rationale for a future prospective clinical study. This will be designed with sufficient statistical power to determine whether the loss of BRCA1 protein, with or without altered p53, is an independent prognostic biomarker. The outcome of this study is to provide a novel means of developing targeted therapeutic agents.

REFERENCE

1. Berchuck, A., Heron, K.A., Carney, M.E., Lancaster, J.M., Fraser, E.G., Vinson, V.L., Deffenbaugh, A.M., Miron, A., Marks, J.R., Futreal, P.A., and Frank, T.S. (1998)Clinical Cancer Res. 4:2433- 2437.

2. Bianco, T., Chenevix-Trench, G., Walsh, D., Cooper, J.E., Dobrovic, A. (2000) Tumour-specific distribution of BRCA1 promoter region methylation supports a pathogenetic role in breast and ovarian cancer. *Carcinogenesis* 212:147-151.

3. Blaszyk, H., Vaughn, C.B., Hartmann, A., McGovern, R.M., Schroeder, J.J., Cunningham, J., Schaid, D., Sommer, S.S., and Kovach, J.S. (1994) Novel pattern of p53 gene mutations in an American black cohort with high mortality from breast cancer. *Lancet*, 343:1195-1197.

4. Elledge, R.M., Clark, G.M., Fuqua, S.A.W., Yu, Y-Y., and Allred D.C. (1994) p53 Protein accumulation detected by five different antibodies: relationship to prognosis and heat shock protein 70 in breast cancer. *Cancer Res.*, 54:3752-3757.

5. Esteller, M., Silva, J.M., Dominguez, G., Bonilla, F., Matias-Guiu, X., Lerma, E., Bussaglia, E., Prat., J. Harkes, I.C., Repasky, E.A., Gabrieslon, E., Schutte, M., Baylin, St. B. Herman, J.G. (2000) Promoter Hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. *J. Natl. Cancer Inst.* 92:594-491.

6. Friedman, L.S., Ostermeyer, E.A., Szabo, C.I., Dowd, P. , Lynch, E.D., Rowell, S.E., and King, M.C. (1994) Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nat. Genet.*54:6374-6382.

7. Futreal, P.A., Liu, Q., Shattuck-Eidens, D., Cochran, C., Harshman, K., Tavtigian, S., Bennett, L.M., Haugen-Strano, A., Swensen, J., Miki, Y. et al. (1994) BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 266"120-122.

8. Harris, DM, Miller, JE and Davis, DM (2003) Racial differences in breast cancer screening, knowledge and complience. J. Natl. Med. Assoc. 95980:693-701.

9. Hedenfalk, I., Duggan, D., Ghen, Y., Radmacher, M., Bittner, M., Simon, R., Meltzer, P., Gusterson, B., Esteller, M., Kallioniemi, O-P., Wilfond, B., Borg, A. and Trent, J. (2001) Gene-Expression profiles in hereditary breast cancer. *New Engl. J. Med.* 344:539-548.

10. Jhanwar-Uniyal, M, and Gulati, S. (1998) p53 gene mutation in the bone marrow of a patient with Diffuse Mixed Cell Type Lymphoma at Diagnosis predicting eventual progression to large cell lymphoma. *Leukemia and Lymphoma*, 29:415-421.

11. Merajver, S.D., Pham, T.M., Caduff, R.F., Chen, M., Poy, E.L., Cooney, K.A., Weber, B.L., Collins, F. Johnston, C. and Frank, T.S. (1995) Somatic mutations in the BRCA1 gene in sporadic ovarian tumours. *Nat. Genet.* 9:439-443.

12. Orita, M., Suzuki, Y., Seyiya, T., and Hayashi, K. (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using polymerase chain reaction. *Genomics*, 5: 874-878.

13. Rose, D.P. and Royak-Schaler, R. (2001) Tumor biology and prognosis in black breast cancer patients: A review. *Cancer Detect. Prev.*, 25:16-31.

14. Shambrook, J. and Russell, D.W. (Eds.) (2001) Molecular *Cloning: A laboratory Manual*, Vol.1, 3rd Edition, Cold Spring Harbor Laboratory press, Cold Spring Harbor, NY.

15. Shiao, Y-H., Chen, V.W., Scheer, W.D., Wu, X.C., and Correa, P. (1995) Racial disparity in the association of p53 gene alterations with breast cancer survival. *Cancer Res.*, 55:1485-1490.

Appendices:

1) Based on our findings, we have submitted an abstract to Annual Meeting of American Association for Cancer Research (AACR;2003).

2) Submitted Abstract (2004)

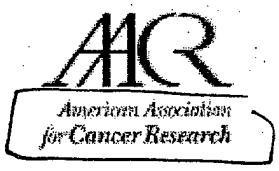
3) Two Articles where this grant is acknowledged.

4) Manuscript in preparation.

#4937 Involvement of p53 and BRCA1 genes in breast cancer in African-American and white women. Meena Jhanwar-Uniyal, Gina Day Stephenson, Renee Royak-Schaler, Chung-Xiou Wang, Mohanrao Achary, Anthony P. Albino, and John Whysner. *Institute for Cancer Prevention (American Health Foundation-Cancer Center), Valhalla, NY and Albert Einstein College Of Medicine and Montefiore Medical Center, Bronx, NY.*

The p53 and BRCA1 tumor suppressor genes play a key role in establishing genome stability. Inactivation or mutation of p53 is seen in variety of cancers including breast cancers. There have been discrete p53 mutations in tumors African-Americans (AA) that were different from those observed in White A cans (WA). Germline mutations in BRCA1 have been reported in hereditary cancer, but somatic mutations of BRCA1 gene are absent in sporadic ca Recent studies have shown that inactivation of BRCA1 occurs via the hyper ylation of the promoter region of the BRCA1 gene. The purpose of this stud to clarify the role of two susceptibility genes as determinants or potential mo of outcome differences in African-American and White women diagnosed breast cancer. AA breast cancer patients more frequently have clinical and p logical features of advanced disease and reduced survival than their white co parts. Well-characterized tumor tissues from 94 breast cancer patients (34 A 60 WA) diagnosed at two Philadelphia hospitals were screened for mutations and BRCA1 inactivation using various methods including immunohistochem DNA-modification followed by methylation-specific PCR, PCR-SSCP an and a direct DNA sequencing. Our results show that: 1) the number of p53 m cases were lower in AAs as compared to WAs, 2) p53 overexpression of m protein was more commonly observed in AAs than WAs, and also the num p53 mutations were greater in AAs compared to WAs, 3) hypermethylati promoter of BRCA1 gene occurred in cases where p53 was mutated with co rent negative estrogen receptor (ER)/progesterone receptor (PR) status in bo AA and WA tumors. No BRCA1 inactivation was detected among the p without p53 mutations or in those with p53 mutations and ER/PR positive tu We hypothesize that loss of expression of ER and PR proteins leads to ge instability that may result from the inactivation of p53 via mutation and BRC hypermethylation. Data from this study suggest that variation in these g susceptibility factors may have prognostic significance in AA and WA breast patients. (Supported by NCI CA 17613, DAMD-17-99-1-9055 (M.A.), DA 17-00-1-0675 (M.J-U.))

Print this Page for Your Records Close Window



Control/Tracking Number : 04-AB-5779-AACR
Activity : Abstract Submission
Current Date/Time : 11/7/2003 7:46:28 PM

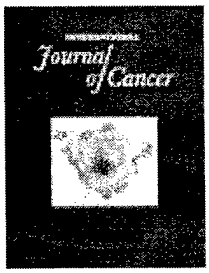
Metastasis to Brain: Gene Expression Profiling

David H. Harter, Anthony P. Gulati, Deborah L. Benzil, Gregory Khitrov, Myron R. Melamed, Raj Murali, Meena Jhanwar-Uniyal. New York Medical College, Valhalla, NY, Institute For Cancer Prevention, Valhalla, NY, The Rockefeller University, New York, NY

The main model of metastasis maintains that most primary tumors have low metastatic potential, however rare cells within the primary tumors acquire metastatic capacity through somatic mutation (Fidler, Nature, Reviews Cancer, 3; 2003). The most common brain metastatic phenotype has the ability to migrate from the primary tumor, survive in blood, pass through the blood brain barrier, invade distant tissue, and form blood vessels to establish itself as a metastatic tumor. Most of the studies of metastasis have focused on gene expression profiling of metastatic tumors to organs such as lymph node, liver and bone. Characteristic genetic changes underlying the metastatic progression of malignant adenocarcinoma to the brain, however, is not fully understood. The goal of this study was to explore specific gene alterations occurring in brain metastases. Microarray analysis was performed in tissues of metastatic tumors excised from the brain using a 22k-gene chip. The primary site of the tumors was the lung. Data analysis was performed using IOBION Genetraffic software, with 2-fold up or down gene expression cutoff. Selected microarray results were verified by RT-PCR and Western-blot analysis. Among the 22,000 genes examined, 125 genes were consistently upregulated or downregulated in these metastatic tumors. Upregulated genes were considerably more numerous than those that were down-regulated. Examination of cell adhesion- and migration-related genes revealed up-regulation of actin, bigycan, myosin light chain kinase, integrin beta2 and 6, integrin alpha V, fibronectin, Rho GTPase-activating protein and Laminin beta 4. Increased expression of TGF-β and its related genes was evident. Up-regulation of oncogenes, such as the platelet-derived growth factor beta Met proto-oncogene (hepatocyte growth factor) and RAB2, was also seen. The angiogenic mediator vascular endothelial growth factor (VEGF) expression was upregulated in the metastatic tumor. Most of the DNA repair genes were downregulated, however, three prime repair Exonuclease 2 (TREX2), which is involved in DNA replication, repair and recombination, was markedly upregulated. Among the down-regulated genes, the neuroprotective heat shock protein 70 (Hsp70) was noticeably down-regulated in metastatic tumors. The results of this study demonstrate that those genes that are involved in adhesion, motility and angiogenesis are upregulated in metastatic tumors, thus making metastasis-prone cells more susceptible to migration, homing, angiogenesis and tumor growth. (Supported by NCI CA 17613 and DAMD17-00-1-0675).

Author Disclosure Block: D.H. Harter, None; **A.P. Gulati**, None; **D.L. Benzil**, None; **G. Khitrov**, None; **M.R. Melamed**, None; **R. Murali**, None; **M. Jhanwar-Uniyal**, None.

Category and Subclass (Complete): CB13-04 Expression profiling of tumor progression and metastasis
Keyword (Complete): Metastases ; Gene expression profiling ; Brain tumors ; Microarrays



International Journal of Cancer

Early View (Articles online in advance of print)

Published Online: 4 Dec 2003

Cancer Genetics

Expression profile of genes associated with antimetastatic gene: *nm23*-Mediated metastasis inhibition in breast carcinoma cells

Hui Zhao [1], Meena Jhanwar-Uniyal [2], Prasun K. Datta [3], Srishailam Yemul [4], Lap Ho [4], Gregory Khitrov [5], Ilya Kupershmidt [6], Giulio M. Pasinetti [4], Tarun Ray [7], Raghbir S. Athwal [8], Mohanrao P. Achary [1,*,†]

[1]Metastasis Laboratory, Department of Oncology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY, USA

[2]Institute for Cancer Prevention, American Health Foundation-Cancer Center, Valhalla, NY, USA

[3]Department of Nephrology, University of Medicine and Dentistry of New Jersey, New Brunswick, NJ, USA

[4]Department of Psychiatry, Mount Sinai Medical Center, New York, NY, USA

[5]Genearray Center, Rockefeller University, New York, NY, USA

[6]Silicon Genetics, Redwood City, CA, USA

[7]Department of Medicine, Temple University School of Medicine, Philadelphia, PA, USA

[8]Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA, USA

email: Mohanrao P. Achary (achary@temple.edu)

*Correspondence to Mohanrao P. Achary, Medical Research Building, Room 706, Department of Radiation Oncology, Temple University School of Medicine, Philadelphia, PA 19140

†Fax: +215-886-8760

Funded by:

- U.S. Army; Grant Number: DAMD17-99-1-9055, DAMD-17-00-1-0675
- Klingers' Research Foundation, New York

Keywords

mammary carcinoma cell lines • nm23 • metastasis • cDNA microarrays • gene expression

Abstract

Metastases of various malignancies have been shown to be inversely related to the abundance of nm23 protein expression. However, the downstream pathways involved in nm23-mediated suppression of metastasis have not been elucidated. In the present

investigation, we used cDNA microarrays to identify novel genes and functional pathways in nm23-mediated spontaneous breast metastasis. Microarray experiments were performed in a pair of cell lines, namely, C-100 (only vector transfected; highly metastatic) and H1-177 (nm23 transfected; low metastatic), derived from human mammary carcinoma cell line MDA-MB-435. The cDNA microarray analysis using GeneSpring software revealed significant as well as consistent alterations in the expression (up- and downregulation) of 2,158 genes in a total of 18,889 genes between high and low metastatic cells. Some of these genes were grouped into 6 functional categories, namely, invasion and metastasis, apoptosis and senescence, signal transduction molecules and transcription factors, cell cycle and repair, adhesion, and angiogenesis to extrapolate an association between these genes and different functional pathways involved in nm23-regulated metastasis. The results suggest that nm23 gene plays a major role in metastasis and its mechanism of action of metastasis suppression may involve downregulation of genes associated with cell adhesion, motility (integrins α2, -8, -9, -L and -V, collagen type VIII α1, fibronectin 1, catenin, *TGF-β2*, *FGF7*, *MMP14* and *16*, *ErbB2*) and possibly certain tumor/metastasis suppressors (2 members of *SWI/SNF*-related matrix-associated proteins 2 and 5 and *PTEN*). © 2003 Wiley-Liss, Inc.

Received: 16 April 2003; Revised: 16 July 2003; Accepted: 11 September 2003

Digital Object Identifier (DOI)

10.1002/ijc.11676 About DOI

Related Articles

- Find other articles like this in Wiley InterScience
- Find articles in Wiley InterScience written by any of the authors

Wiley InterScience is a member of CrossRef.

[Frontiers in Bioscience 8, s1107-1117, September 1, 2003]

BRCA1 IN CANCER, CELL CYCLE AND GENOMIC STABILITY

Meena Jhanwar-Uniyal

Institute for Cancer Prevention, American Health Foundation Cancer Prevention Center, Valhalla, New York

TABLE OF CONTENTS

1. ABSTRACT

The BRCA1 gene was isolated in 1994; germline mutations of this gene are known to confer susceptibility to breast and ovarian cancer in high-risk families. Since its discovery, several mutations have been identified in this gene; these are scattered throughout the gene, and include insertion and deletion frameshifts, base substitutions, and inferred regulatory mutations. It role in the pathogenesis of breast cancer, which accounts for almost 95%, although unproven to date, cannot be ruled out. The functional inactivation of both copies of this gene in sporadic tumor cells does not follow the traditional mode: the loss of function in BRCA1 is not accompanied by underlying mutation of the gene in tumor cells with loss of heterozygosity for the BRCA1 gene. Several studies now suggest that an alternate mechanism of inactivation, involving promoter hypermethylation that results in reduced expression of the gene, may be common to a significant proportion of sporadic breast and ovarian cancers. BRCA1 as a tumor suppressor plays an important role in maintaining genomic stability. BRCA1 has the ability to interact with numerous proteins and to form complexes that are involved in recognizing and subsequently repairing DNA. BRCA1 contains several functional domains that directly or indirectly interact with a variety of proteins via protein-protein interaction; these include tumor suppressors (BRCA2, p53, Rb and ATM), oncogenes (c-Myc, casein kinase II and E2F), DNA damage repair proteins (RAD50 and RAD51), cell cycle regulators (cyclins and cyclin dependent kinases), transcriptional activators and repressors (RNA polymerase II, RHA, histone deacetylase complex and CtIP), DNA damage-sensing complex and mismatch repair proteins (BRCA1- Associated Surveillance Complex; BASC) and signal transducer and activator of transcription (STAT) among others Formation of foci containing BRCA1 by inherited mutations, or epigenetic mechanisms (promoter methylation) in sporadic cancers leads to a loss of DNA repair ability, disrupts the potential to form complexes with other proteins that are crucial for DNA repair pathways. Thus, BRCA1 plays a significant role in maintaining genomic stability and serves as a tumor suppressor in breast cancer tumorigenesis.

2. INTRODUCTION

2.1. Mutation spectrum of BRCA1-associated tumors

The American Cancer Society estimated that a total of 203,500 new cases of breast cancer (out of 647,400 estimated cancers at all sites) would occur among US women in the year 2002 and assesses the probability that one in eight American women would develop breast cancer during their lifespan (American Cancer Society Facts and Figures, 2002). Taken together, cancers of the breast and ovaries constitute almost one fourth of all cancer-related mortality in this country. BRCA-1 and BRCA-2 genes (1,2,3) are known to be associated with early onset familial breast and ovarian cancer. Patients with a strong hereditary component account for only 5% of all breast cancers occurring in the Unites States (4,5); nevertheless, identification of genes responsible for hereditary cancers is important, as such genes have been shown to play a critical role in the much more common form of "sporadic" tumors (6) in a variety of cancers. The role of the BRCA1 gene in sporadic breast cancer, however is not well defined, as mutations of these two genes in tumors with loss of heterozygosity (LOH) for BRCA1 and 2 are very rare (6). As reviewed by Szabo and King (1995) (4), BRCA1 and BRCA2 combined contribute to only 6-10% of breast and ovarian cancer regardless of the family history. In addition, approximately 30% of high-risk families do not exhibit mutations in either BRCA1 and BRCA2 genes. Such observations are consistent with the fact that there may be other genes that may predispose individuals to breast cancer. A limited number of recurring mutations (BRCA1 185delAG, 5382insC; BRCA2 617delT) in the BRCA 1 and 2 genes account for a substantial fraction of the breast cancer burden in the Jewish population (4,7).

Molecular studies on hereditary cancer syndromes, such as retinoblastoma, adenomatous polyposis

coli, etc., have demonstrated that at least two genetic events are necessary for the development of tumors. One of these events must be mutation in the tissue-specific cancer-predisposing gene, whereas second event results in inactivation of the corresponding normal allele, which is normally accomplished by LOH.

A wealth of information is now available regarding the extent and type of germline mutations detected in various populations in the world. These studies clearly demonstrated that mutation in the BRCA1 gene are not localized to a specific exon.

The first breast cancer susceptibility gene discovered BRCA1, spans approximately 100 kb on the long arm of chromosome 17 (17q21.3) from which a 7.8-kb mRNA is transcribed that encodes a protein of 1863 amino acids (8), and consists of 24 exons, including a very large exon 11(8). The tumor suppressor gene BRCA1 was cloned through its linkage to inherited breast cancer (8).

BRCA1 is well conserved among species. Approximately 50% of inherited breast cancer cases are the result of germ-line mutation in the BRCA1 gene, and virtually all families have a history of both ovarian and breast cancer carry mutation in the gene (6,9)

Hereditary breast cancers account for 10-15% of all breast cancer cases whereby ~50% of these are associated with the susceptibility genes BRCA1 and BRCA2. Mutations in the BRCA1 gene are rare in sporadic breast cancer. LOH and mutation in BRCA1 has been observed in ovarian cancers (10). Loss of the wild-type allele is often seen in the tumor of a carrier with germline BRCA1 mutation, qualifying BRCA1 as a tumor suppressor gene. Inheritance of a mutated copy of the BRCA1/2 genes increases the lifetime risk of breast cancer 5-to 8-fold and that of ovarian cancer 20-to 40- fold. Genetically-predisposed individuals typically present with cancer at an earlier age, i.e., >50% of BRCA1 mutant carriers develop cancer by age 50. Members of breast cancer-prone families often seek genetic counseling to assess their relative risk for cancer development. High-risk patients are then evaluated by identifying germline mutations in the BRCA1 (and/or the BRCA2) gene. Most of these individuals carry a nucleotide sequence alteration in the BRCA1 gene (~80%) that results in a frameshift or missense mutation, whereas a subset of patients (~10%) possess chromosomal rearrangements affecting the gene. A third subset, ~10% of high-risk patients, lack discernable mutations in either BRCA1 or BRCA2, despite a calculated high probability for mutation on the basis of family history. Recent studies show that methylation-dependent epigenetic silencing of BRCA1 can contribute to the development of breast cancer that is indistinguishable from that of patients with BRCA1 mutation.

2.2. Mutation spectrum and phenotypes of BRCA1-associated tumors

BRCA1-associated tumors are largely ductal type, histological grade III, and show significant lymphocytic infiltration (11). BRCA1-associated tumors display distinct histological and biological features indicating that these tumors are under distinct genetic control. A strong correlation is seen between the position of a BRCA1 mutation and the ratio of breast to ovarian cancer incidence within the families (12). In particular, mutations located upstream of exon 13 are more likely to give a high proportion of ovarian and breast cancer while mutations at the 3' end of this exon give an opposite phenotype. Furthermore, BRCA1 mutations at the 5' or 3'-ends of the coding region correlate with highly proliferative tumors (13).This may suggest that inactivation of a functional domain of the BRCA1 protein, such as the RING finger or BRCT domain, results in a more severe phenotype than mutation occurring somewhere else. Since the discovery of the BRCA1 gene in 1994 {8), about 113,705 cases have been reported in OMIM (Online Mendelian Inheritence in Man). Unique coding region mutations have been identified as listed in the Human Gene Mutation data base (www.nhgri.nih.gov/intramural.research). Almost all kinds of mutations seen in the BRCA1 gene, include inversion, deletion, insertion, missense/nonsense and aberrant splicing. Most of these caused frameshift of the coding region leading to a truncated BRCA1 protein. The relatively low number of point mutations found in BRCA1 suggest that a significant portion of the encoded protein has to be inactivated to confer susceptibility to breast cancer. Most of the tumor-associated point mutations are found in conserved domains such as the RING finger and the BRCT domain. No obvious signs of clustering or mutational hotspots have been seen in BRCA1, but certain sequences and nucleotides of BRCA1 may be more susceptible to mutations than others (14). Certain polymorphisms and unclassified variants also have been reported. Most mutations generate a premature stop codon, which results in the production of a truncated protein. Many BRCA1 mutations occur in repeated motifs, including strings of homonucleotides, short direct repeats, and inverted repeats. A good example is the mutation 185delAG, prevalent among Ashkenazi Jews, which precedes a second AG pair (TCTTAGAGTGTC) (15). The allelic frequency of this mutation (185delAG) in the Ashkenazi Jew population in USA is about 1% (16). However, among Ashkenazi Jews in Australia the prevalence of this breast carcinoma-predisposing allele is even greater (17). This mutation as well as BRCA1 5382incC are considered to be founder mutations, associated with the migration of carriers with this mutation. The length of the Ashkenazi Jewish founder mutation, 185delAG, has been estimated to be 760 years old (18). Similarly, among African-Americans, three recurrent BRCA1 mutations, namely 943ins10, 1832del5, and 5296del4, have been described and it has been considered that these are likely to arise from a common ancestory (reviewed by Olopade *et al.*, 2003) (19). Similarly, many recurrent mutations have been described in many populations. These mutations may occur due to slippage and misalignment in DNA replication. The physiological and biological alterations caused by this mutation can lead to breast cancer. The individual heterozygote for germ-line mutation in BRCA15382incC developed breast cancer at a relatively early age. A breast tumor cell line (HCC1937; ATCC) that is homozygous for BRCA1 5382insC mutation (20), has been used to

decipher the physiological role of BRCA1 in DNA repair pathways. These aspects are discussed later in this chapter. There have been cases of breast cancer-prone families where rearrangements involving 3.4 and 11.5 Kb of the BRCA1 gene resulted in loss of amino acid in the C-terminus of the gene, which is involved in DNA repair pathways (21;22). Since then, many more novel ethnic mutations have been described.

Recent studies have suggested that inherited BRCA1 mutations are able to promote an oncogenic event, perhaps by masking the functions of the remaining wild-type BRCA1 allele (23).Mutations in BRCA1 gene sporadic cases are absent or rare. Nonetheless, BRCA1 has been shown to play a role in the development of breast cancer in sporadic breast cancers. In case of early onset of breast cancer without a family history, germline mutation of the BRCA1 gene has also been reported (6). Sporadic breast cancers account for about 90% of all cases, with hereditary breast cancer accounting for the balance. A great many studies have examined the molecular pathogenesis of sporadic and hereditary breast cancer, but very few have examined the epigenetic contributions to this process. The possible contribution of methylation-dependent epigenetic regulation of BRCA1 in sporadic and hereditary breast cancer remains to be determined. BRCA1-associated tumors are generally estrogen Receptor (ER-) negative (24,25). Some studies have shown that loss of ER positivity in tumors is due to methylation of ER, specifically of ER-alpha. ER-alpha-negative cancers arising in BRCA1 mutation carriers were more extensively methylated than ER-alpha negative cancers from women without a BRCA1 mutation (26).

2.3. Methylation in the promoter region of BRCA1

In many sporadic breast cancers methylation of the BRCA1 promoter has been reported. In non-hereditary breast cancers, which account for 90% of cases, the involvement of BRCA1 has not been so clearly elucidated. A great variety of genetic mutations was found in BRCA1-linked families. Inactivation of a tumor suppressor gene by loss of one allele, linked to mutation of the remaining allele, is a common mechanism. BRCA1, in some ways, puts itself on the brink of ignoring the two hit model proposed by Knudson that is generally followed by other tumor suppressor genes; because no sporadic mutations was seen in BRCA1, an alternative mechanism to genetic mutations, namely hypermethylation of the BRCA1 promoter resulting in a loss of expression, has been shown. Methylation of DNA represents a significant epigenetic alteration in humans. It occurs most frequently on the 5'-methylcytosine residue of 5'-CpG-3' dinucleotides. De novo enzymatic methylation of 5'-CpG islands can lead to the inactivation of the gene. CpG sites are generally clustered into islands, called "CpG islands", usually cover 0.2 to sevaral KB and are found in the promoter region of the genes (27). Enzymatic methylation of 5'-CpG islands can lead to the inactivation of the contiguous gene. DNA methylation inhibits transcription by interfering with transcription initiation. This repression can arise by several means.

In human cancer, this epigenetic (non-genetic) event has been shown to be a powerful mechanism by which tumor suppressor gene activity is inhibited. Local promoter hypermethylation in human cancer is often part of global genomic hypermethylation. DNA methylation inhibits transcription by interfering with transcription initiation. Thus the potential mechanism is a reduction of binding affinity of sequence-specific transcription factors. The BRCA1 gene is regulated by two promoters. Two distinct transcripts differing by the alternative use of the first exon have been described (28). BRCA1 promoter lacks TATA , but has several CAAT boxes, GC boxes and PEA3 binding sites, and a CREB binding site (29,30). Sequence homology searches reveal that a classic estrogen response element (ERE) sequence was not present in either BRCA1-α or BRCA1-β promoter regions (31). An alternative ERE was observed in BRCA1 β promoter (30) which is believed to be responsive to estrogen stimulation via the classical ER pathway to stimulate transcription. Estrogen may regulate BRCA1 α promoter via some complex or indirect mechanism(s) since BRCA1-α lacks a conventional ERE. A putative AP1 site is present in the BRCA1 promoter (28,32). Several CpG sites exist in BRCA1 promoter; the 5' CpG island of BRCA1 encompasses about 2 Kb. The region of the BRCA1 promoter shares the first exon of another gene, NBR2, and its bi-directional promoter with exon 1a and 1b of BRCA1 (28,33). Hypermethylation of BRCA1 promoter has been detected in about 11-31% of breast cancer cases and about 5-15% of ovarian cancer cancer. Several methods have been used to identify methylation, namely Southern analysis with methylation-specific PCR (MSP) and sodium bisulfite, followed by PCR. Normal tissues or cell lines did not show BRCA1 methylation; it has been observed exclusively in malignant breast and ovarian tissues. BRCA1 hypermethylation varies according to histological subtypes and is common in mucinous and medullary subtypes (34).. Interestingly, these histological sub-types are also highly represented in inherited BRCA1 mutant (35). These cancers display a distinct phenotype, such as loss of ER and PR positivity, and often have mutated p53 (25). Hypermethylation was more frequent in high-grade breast cancer (24,36). There is a strong correlation between promoter hypermethylation and decrease in gene expression and protein expression (37). On the other hand, in one study, 37 tumors that showed a reduction in BRCA1 expression gave no evidence of hypermethylation (38). This indicates, that a mechanism other than methylation is also responsible for suppression of BRCA1 expression. Methylation specifically inhibited the binding of the CREB protein to the CRE site within the 5' regulatory region of the BRCA1 promoter (39). The CRE site appears to play a constitutive role in BRCA1 expression. One possible mechanism by which methylation could abrogate gene expression is by impairing the interaction of transcription factors with DNA binding sites. In this regard the putative CREB binding motif present in α promoter of BRCA1 has been shown to be sensitive to methylation (37). The 5' SmaI site was found to be in close proximity to a Sp1 binding motif (28) and it has been suggested that the Sp1 element plays a role in protecting CpG islands from de novo methylation (40). A strong correlation has been observed between loss of ER positivity and BRCA1 methylation (41,42). In a study of 96 sporadic breast cancers, 10 out of 11 BRCA1 methylated cases were ER and PR negative (ER- and PR-). On the

other hand, another 31 of 96 cases , that were ER- and PR- failed to show methylation of BRCA1. In our study, we found all ER- and PR- cases to be BRCA1 methylated. It is interesting that the ER CpG island has been found to be methylated in 25% of ER- breast cancers (43), and the same percentage of ER- cases was found to be hypermethylated in the BRCA1 promoter region (41). An abnormal methylation was detected in approximately ten percent of sporadic breast tumors, it indicated that this mechanism alone cannot account for the reduction in BRCA1 mRNA levels observed in the majority of sporadic invasive breast cancer cases (44).

It would be intriguing to see whether a strong corretaion exists between loss of ER positivity and BRCA1 hypermethylation. Reduced BRCA1 expression levels in sporadic cancer are strongly correlated with negative ER status (45). Furthermore, an increased DNA methyltransferase activity in ER- cell lines compared to ER+ cell lines, suggests that loss of ER positivity is due to increased methylation. This may mean that methylation of the BRCA1 promoter in ER- tumours may also be secondary to increased DNA methyltransferase activity. BRCA1 expression was low or completely lost in invasive carcinoma, however an opposite, namely higher expression was seen in non-invasive breast cancers (45). The highest levels were observed in samples from non-comedo ductal carcinoma *in situ*, a premalignant breast lesion with a finite, but relatively low rate of progression to invasion. Determination of hypermethylation of BRCA1 may play an important role in early detection of tumors. It has been observed recently that one case of sporadic breast cancer, misclassified for a BRCA1 mutation, turned out to be hypermethylated BRCA1 (46). These investigators used microarray analysis to identify genes associated with BRCA1 hereditary tumors that could contribute to BRCA1 positivity of breast tumors. Direct interaction of BRCA1 with known DNA methyltransferase, or demethylase complex has not been demonstrated. However, BRCA1 has been shown to be physically associated with a component of the histone deacetylase complex (47). It is possible that histone acetylation and CpG methylation may be inter related epigenetic processes. It is also feasible that methylation reflects gene activation rather than being the cause of it. Thus, restoring gene expression by treating cells with the demethylating agent 5' aza-deoxycytosine could restore BRCA1 expression. Such studies remain to be explored.

2.4. Physiological role of BRCA1

It is a phospho-nuclear protein, generally residing in the nucleus; however, BRCA1 may "shuttle" between nucleus and cytoplasm (48) . BRCA1 consists of the N-terminal Really Interesting New Gene (RING) finger domain and a C-terminal acidic domain termed BRCA1 C terminus (BRCT) (8;49). BRCA1 has both nuclear localization signals (NLSs), nuclear export signals (NES), and 2 C-terminal BRCT domains of about 100 residues (50). BRCT domains are a common protein-protein interaction motif involved in DNA damage response and repair. BRCA1 may participate in mammalian heat shock response pathways (51). BRCA1 has been shown to localize in the mitotic centrosome, where it interacts with γ-tubulin (Liu *et al.*, 2002), suggesting its role in mitosis. BRCA1 is shown to be associated with a hyperphosphorylated form of retinoblastoma protein (pRb), an interaction that is crucial for BRCA1-induced growth arrest in the G1/S phase of the cell cycle (53). pRB interacts with E2F to inhibit cell proliferation and it is possible that BRCA1 keeps RB in the hyperphosphorylated state to achieve growth arrest. Furthermore, the BRCA1-RB complex interacts with a histone-deacetylase complex (47) to suppress the transcription of E2F-responsive genes, perhaps to inhibit cell growth.

The BRCT domain is found in various proteins, including 53BP1, RAD9, RAD4, crb2 and RAP1(54). The recently described MDC1 (mediator of DNA damage checkpoint protein 1) also possesses a BRCT domain (55). The NH2-terminal RING domain of BRCA1 mediates association with protein BARD1, which is similar in structure to BRCA1 (56) and BAP1that binds to the wild-type BRCA1-RING finger, but not to germline mutants of the BRCA1-RING finger found in breast cancer kindreds (57; 58). The BRCA1 and BARD1 complex was shown to exhibit ubiquitin ligase activity, which is lost by mutations in the BRCA1 gene in the RING finger region (59,60,61). In fact, recent studies have indicated that BARD1 plays a critical role in preventing nuclear export of BRCA1 by masking NES (48). BRCA1-BARD is the first example of a RING-dependent Ubiquitin Ligase that depends on the heterodimer to exhibit ubiquitin activity.

BRCA1 has a protein interaction domain for p53 (62), and BRCA1 expression is modulated by p53 (63). Levels of BRCA1 are down-regulated in response to p53 induction by DNA damage in cells that undergo either growth arrest or apoptosis (63). It has been suggested that, once phosphorylated, BRCA1 acts synergistically with p53 to cause cell cycle arrest after DNA damage; it then is degraded in a p53-dependent manner, when its is no longer required. BRCA1 initially participates in the accumulation of p53 protein but later p53 acts to reduce BRCA1 expression, perhaps via a feedback loop (64,65).

2.5. BRCA1 in Cell Cycle Control and DNA Repair

BRCA1, also called the caretaker of the genome (66), is involved in maintaining genome stability by virtue of its important role in cell cycle control, DNA double strand break repair, and transcription-coupled repair (reviewed by Deng and Brodie, 2000(50)). The BRCA1 RING domain has a direct link with Ub ligase, and mutations in these regions of BRCA1 have been shown to predispose to cancer perhaps by altering the Ub ligase activity (61,67). The BRCA1 protein and its interacting proteins are shown in Figure 1.

The BRCA1 protein shows no homology to any known protein and it is expressed widely (8). The functional motifs in the BRCA1 protein that have been described include a RING finger domain, a carboxy-terminal domain called BRCT, binding sites for tumor suppressor p53 and DNA repair protein RAD51, a human homolog of RecA. BRCA1 physically interacts with the proto-oncogene, c-Myc, and it may function as a tumor

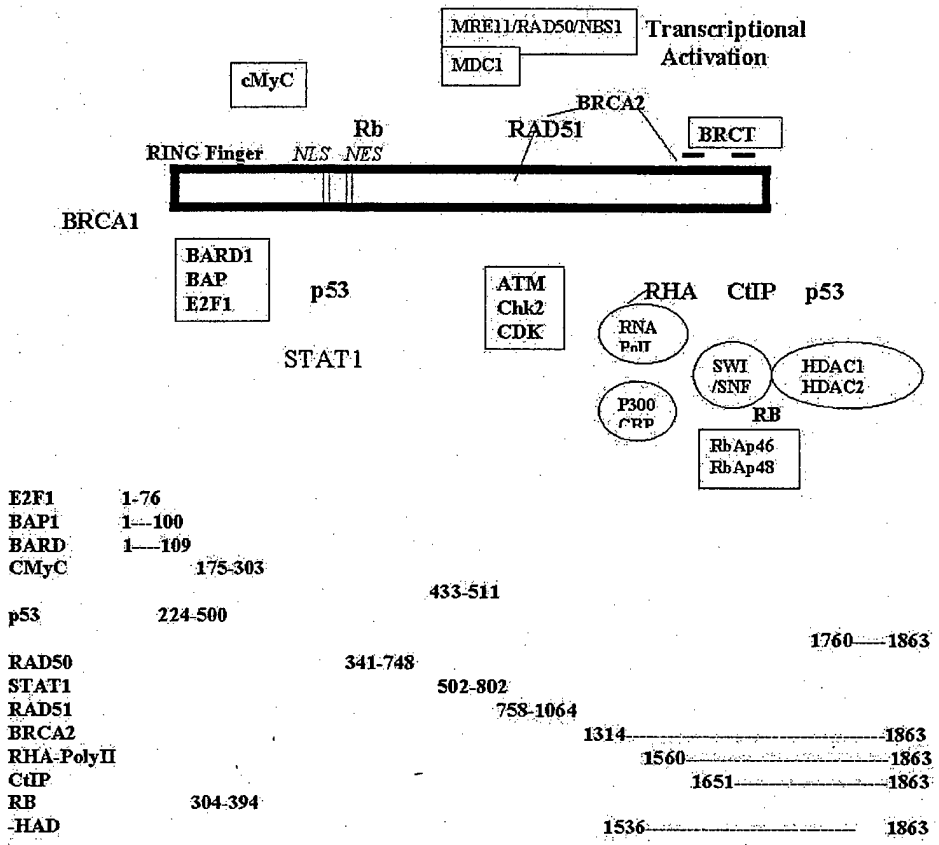


Figure1. Diagrammatic representation of the BRCA1 protein. The interacting proteins are shown. (See text for detail also see Ref. 50, 77,78).

suppressor by regulating the activity of c-Myc (68). The region of BRCA1 between amino acids 502 and 802 interacts with the C-terminal transcriptional activation domain of the signal and activator of transcription 1(STAT1) and this interaction contributes to its IFN-γ activation (69). Each domain interacts with specific protein(s) pivotal for distinct functions in cellular processes. Thus, BRCA1 may directly control various assigned functions and also influence these functions by modulating their interaction with other proteins.

Evidence of a BRCA1/BARD1 interaction stems from immonofluorescence localization studies which show that both proteins co-localize to S-phase nuclear dots or foci (70). Neither BRCA1 nor BARD1form nuclear foci in G1; however, they come together just before the S phase. Unlike BARD1, which shows constant levels throughout the cell cycle, the expression of BRCA1 is generally absent or low during G1, but it peaks before the S-phase. BARD1 and RAD51 localize to PCNA nodules following treatment with hydroxyurea or UV (71,72).

Double strand breaks (DSB) are considered to be highly dangerous lesions in cells, such breaks can be generated by various genotoxic agents, from exogenous and endogenous sources. Exogenous sources include ionizing radiation (IR), radiomimetic agents, and chemotherapeutic agents. Endogenous agents are generated by mechanical stress and reactive oxygen species. In addition, endogenous topoisomerase, DNA cleavage, replication, meiosis, and fragile site formation can also generate DSB. Two distinct mechanisms have been established for the repair of DSB: I) Homologous recombination (HR) uses a sister chromosome as a template for repair; II) non-homologous end-joining (NHEJ), rejoins two broken ends of DNA directly.

One of the clues linking BRCA1 to DNA repair was its association with Rad51, the primary RecA homolog in eukaryotic cells (70,71,73,). RAD51 shares significant homology with bacterial RecA, which has been shown to mediate the pairing and ATP-dependent exchange of DNA strands in recombination (74). RAD51 interacts with the C-terminal region of the BRCA1 protein, between amino acid 758 and 1064. The BRCA1 protein co-localizes with Rad51 in nuclear dots during the S-phase and in response to DNA damage, suggesting that it may also be involved in homologous recombination and recombinational repair. BRCA1 null mice suffer embryonic lethality and are very similar in phenotype to mice lacking Rad51 or BRCA2

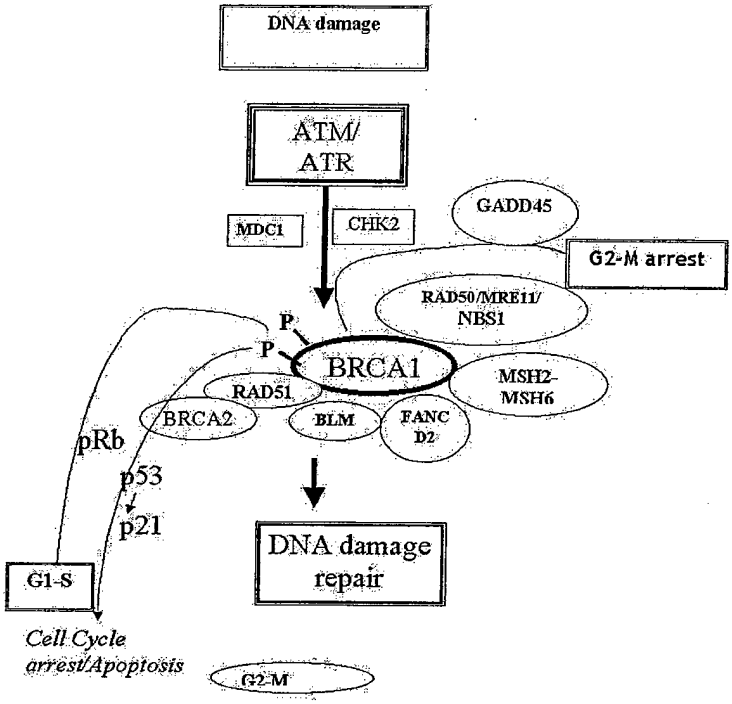


Figure 2. A model showing interaction of BRCA1 with other proteins in response to DNA damage. In this case, BRCA1 is phosphorylated by ATM/ATR. BRCA1forms complexes to repair Double Strand Breaks (DSB) by homologous recombination. BRCA1 also has a role in cell cycle arrest at G2-M checkpoint, possibly via upregulation of GADD45. The BRCA1-associated genome surveillance complex (BASC)-containing RAD50/MRE11/NBS1, DNA mismatch repair proteins, DNA helicase BLM (Bloom's syndrome protein) and others are in sensing and repair of abnormal DNA. BRCA1 may interact with p53 pathways to initiate cell cycle arreats/ apoptosis, if necessary, or interacts with Rb to control G1-S stage of cell cycle (Ref. 50, 77,79).

genes. These embryos display cellular proliferation defects, are sensitive to ionizing radiation, and exhibit high levels of chromosomal abnormalities; the latter can be partially rescued by p53 mutation.

BRCA1 is associated with Rad50 as a part of the Mre11/Rad50/Nbs1 (nibrin) complex (MRN) (75,76), which is involved in both nonhomologous end joining (NHEJ) and homologous recombination in yeast and vertebrate cells (74,77,78,79). The MRN complex localizes to the sites of DBS *in vivo* and plays a critical role in DNA metabolism, including DSB repair, meiotic recombination, and telomere maintenance. Cells deficient in Mre11 or Nbs1 continue DNA replication following X-ray damage, and thus display defective checkpoint signaling during the S-phase. In response to double strand breaks BRCA1 is phosphorylated by ATM, a kinase that phosphorylates the multiple protein complex (75,80,81,). ATM also phosphorylates Nbs1 in response to DNA damage (75,82,83). The BRCA1 foci that appear after ionizing radiation, are co-localized with the subset of foci formed by the MRN complex, re-stating a role for BRCA1 in the cellular response to DSB. In addition, BRCA1 is also involved in another kind of DNA repair, namely base excision repair (BER) (84). Embryonic stem cells of BRCA1-deficient mice exhibit defects in transcription-coupled repair, which are generally the result of accumulation of oxidized bases following insult by ionizing radiation. BRCA1 may manifest its role in BER through its association with mismatch repair enzyme (85) or by transcription via its interaction with RNA polymerase II holoenzyme. During the S-phase of the mitotic cell cycle, BRCA1 colocalizes with RAD51 in subnuclear structures, Scully *et al.* (1997)(70,71), known as "BRCA1 nuclear dots" that are succulent in nature. However, in response to DNA damage these dots appear to disperse (70,71). BRCA1 interacts with BRCA2, which directly binds with RAD51 (77,79). Recently it has been shown that BRCA1 co-purified with several proteins that are associated with the DNA damage machinery, including mismatch repair enzyme, MSH2, MSH6, MLH1, ATM, BLM, as well as MRN protein complex, in a single complex referred as the BRCA1-Associated Surveillance Complex (BASC) (75). BASC contains at least 15 subunits and is involved first in sensing damaged DNA and then in repairing it. All of these BRCA1-associated proteins may have BRCA1-dependent as well as independent functions and BRCA1 may regulate such proteins for a specialized repair. Figure 2 illustrates that BRCA1 forms various complex(es) with DNA-repair proteins to participate in repair of damaged

DNA or accelerate cell cycle arrest. Also, DNA replication factor C, itself a protein complex that recruits PCNA onto DNA polymerase δ, was found to be a component of BASC. Many proteins in BASC are tumor suppressors, indicating that loss of integrity of BASC may be a central mechanism in tumor development. The MSH mismatch proteins are involved in repair of mismatched DNA lesions is due to spontaneous errors during DNA replication or during repair of DSBs. It is possible that a MSH2-MSH6 complex may signal regulation of down-stream events, such as apoptosis or cell cycle arrest via BRCA1. In this manner, MSH2-MSH6 as a multi-protein complex interacts with the repair machinery and modulates cell cycle checkpoints and apoptosis decisions. The DNA helicase gene Bloom-syndrome (BLM) may be involved in sensing abnormal double strand DNA structures formed during replicatio). R/M/N protein complex is involved in DNA repair at stalled replication forks (87). Both ATR and ATM are components of BASC, which can phosphorylate numerous proteins of the BASC complex (89,90). BRCA1 can participate in DNA repair in many ways. One possibility is that BRCA1 acts as a scaffold protein. BRCA1 may also exert local activities at DSB sites via its interaction with enzymes that alter chromatin and DNA structures. BRCA1 interacts with SWI/SNF and other proteins that remodel chromatin with regulators of histone acetylation/deacetylation (reviewed by 77,78,79). BRCA1 also interacts with DNA helicases, including the RecQ homolog encoded by Bloom's syndrome gene, BLM and the helicase BACH1 (88). Perhaps these interactions are required for the accessibility of the repair machinery. One response of BRCA1 to DNA damage is to monoubiquitinate histones (H2A and H2B) in conjunction with BARD1; in this way chromatin remodeling takes place that could allow the DNA repair machinery to gain access to the damaged DNA.

The two kinases, CHK1 and CHK2 are responsible for the maintenance of the G2-M DNA damage checkpoint. CHK2 phosphorylates BRCA1 in response to DNA damage induced by IR. Chk1 and Chk2 are classic serine-threonine kinases that are required for cell cycle arrest in response to DNA damage. As downstream kinases, they are phosphorylated by an ATM/ATR-dependent process and then Chk2 phosphorylates BRCA1 (87,89). BRCA1 phosphorylates CHK1 to control G2-M transition. Reciprocal co-immunoprecipitation of BRCA1 and CHK1 has been shown in HeLa and MCF7 cells. In BRCA1 mutant HCC1937 cells, where BRCA1 is expressed, colocalization of BRCA1 and CHK1 is maintained even after gamma-radiation (90). It has been proposed that, in response to DNA damage, BRCA1 controls cell cycle progression to mitosis via CHK1, which regulates Cdc2 kinase, Cdc25C and WEE1 (91). Some studies suggest that BRCA1 forms a complex with the transcriptional co-repressor complex CtP and CtBP through its BRCT domain, and during the DNA damage response, this complex gets dissociated from BRCA1, which, in turn, activates GADD45 and p21.

Specific sites in BRCA1 are responsive to DNA damage repair stimuli. BRCA1 becomes phosphorylated in response to treatment of cells with a variety of DNA damaging agents, such as, UV, IR, adriamycine, hydroxyurea, mitomycin C, and hydrogen peroxide. Multiple phosphorylation sites at the serine (S) residue, including S1330, S1423, S1466, S1466, S1524 and S1542, have been detected by mass spectrometry analysis of recombinant BRCA1 peptides, phosphorylated *in vivo* in an ATM-dependent manner (89). These phosphorylations may lead to a change in BRCA1 subnuclear localization. Among specific BRCA1 phosphorylation sites that have been responsive to DNA damage. For example, in MCF-7 cells, IR-and UV-induced phosphorylation of BRCA1 at Ser-988/-1524 and Ser-988, respectively, was seen during the S-phase (92); however, in the G2/M phase, IR and UV treatment induced phosphorylation of Ser-988/ser-1423 and Ser-1423, respectively (92). In HCC1937 cells, with specific BRCA1 mutation where the functional C-terminal BRCT domain is lost, phosphorylation of Ser-1423 and –1524 was not induced. It is possible that allosteric change of the BRCA1 structure due to phosphorylation, may affect its interaction with other proteins involved in DNA damage repair pathways (93).

3. CONCLUSIONS

Thus, in summary, it is evident from the discussion presented above that BRCA1 serves as one of the important tumor suppressor genes in the etiology of the breast cancer, particularly in high risk families. The wide spectrum of mutations observed in the gene in various populations of the world, with a few exceptions, is not specific to any particular population and mutations are scattered throughout the coding region of the gene. While BRCA1 follows Knudson's "two hit" hypothesis, in familial early on set cancers, the mode of its inactivation, in the much more common in sporadic cancers, is poorly understood; the promoter hypermethylation as a mechanism of inactivation is prevalent only in less than half of the cases. A significant amount of work, therefore, is needed to elucidate the role of BRCA1 in sporadic cancers. Its physiological role in DNA damage sensing and repair sheds light on its function as a caretaker to maintain the genomic stability. Inactivation of BRCA1 confer on cells, new genetic abnormalities; this, in turn, leads to tumorigenesis. Future studies will focus on elucidating the mechanisms of BRCA1 in the multistep process of tumorigenesis relating to sporadic cancers, and eventually means to prevent cancers.

4. ACKNOWLEDGMENTS

This chapter is dedicated in loving memory to my Parents, Late Professor G.P. Uniyal and Mrs.Kamla Bahuguna Uniyal. Special thanks are extended to Mrs. Ilse Hoffmann for her editorial assistance. This work was supported by NCI CA 17613 and DAMD17-00-0675, and the TOW Foundation, for which author is deeply grateful.

5. REFERENCES

1. Li,C.I., B.O Anderson, ,J.R., Dalingand & R.E. Moe: Trends in incidence rates of invasive lobular and ductal breast carcinoma. *JAMA* 289, 1421-1424 (2003a)

2. Li,C.I., J.R. Daling & K.E. Malone: Incidence of invasive breast cancer by hormone receptor status from 1992 to 1998. J. Clin. Oncol. 21, 28-34 (2003b).
3. Li,C.I., K.E. Malone & J.R. Daling: Differences in breast cancer stage, treatment, and survival by race and ethnicity. *Arch. Intern. Med.* 163, 49-56 (2003c)
4. Szabo,C.I. & M.C. King: Inherited breast and ovarian cancer. *Hum. Mol. Genet.* 4 Spec No, 1811-1817(1995)
5. Welcsh,P.L., M.K. Lee, R.M.Gonzalez-Hernandez,D.J. Black,M. Mahadevappa,E.M.Swisher,J.A.Warrington & M.C. King: BRCA1 transcriptionally regulates genes involved in breast tumorigenesis. *Proc. Natl. Acad. Sci. U. S. A* 99, 7560-7565(2002)
6.Futreal,P.A.,Q.Liu,D.Shattuck-Eidens, C. Cochran, K. Harshman,S. Tavtigian, L.M .Bennett, A. Haugen-Strano, J.Swensen, &Y., Miki, *et al.*, BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 266, 120-122(1994)
7. Welcsh,P.L. & M.C. King: BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. 22. *Hum. Mol. Genet.* 10, 705-713(2001)
8.Miki,Y.,J.Swensen, D.Shattuck-Eidens, P.A. Futreal,K. Harshman,S.Tavtigian,Q.Liu,C.Cochran,L.M.Bennett, W. Ding, *et al.*: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266, 66-71(1994)
9. Ramus,S.J., P.D.Pharoah,P. Harrington,C. Pye,B. Werness,L. Bobrow,A. Ayhan, D. Wells, A. Fishman,M.Gore, R.A.DiCioccio,M.S. Piver, A.S.Whittemore,B.A. Ponder & S.A.,Gayther: BRCA1/2 mutation status influences somatic genetic progression in inherited and sporadic epithelial ovarian cancer cases. *Cancer Res.* 63, 417-423(2003)
10. Merajver,S.D., T.S.Frank,J. Xu, T.M.Pham, K.A.Calzone,P.Bennett-Baker, J.Chamberlain,J. Boyd,J.E. Garber,F.S., Collins, *et al.*,: Germline BRCA1 mutations and loss of the wild-type allele in tumors from families with early onset breast and ovarian cancer. *Clin. Cancer Res.* 1, 539-544(1995)
11. Johannsson,O.T., I.Idvall,C. Anderson,A. Borg,R.B. Barkardottir,V.Egilsson & H.,Olsson: Tumour biological features of BRCA1-induced breast and ovarian cancer. *Eur. J. Cancer* 33, 362-371(1997)
12. Gayther,S.A., P. Harrington, P. Russell,G. Kharkevich, R.F. Garkavtseva, & B.A.,Ponder: Frequently occurring germ-line mutations of the BRCA1 gene in ovarian cancer families from Russia. *Am. J. Hum. Genet.* 60, 1239-1242(1997)
13. Sobol,H., D.Stoppa-Lyonnet,B.Bressac-de-Paillerets, J.P.Peyrat, F.Kerangueven, N.Janin, T.Noguchi, F. Eisinger,J.M. Guinebretiere,J. Jacquemier,&,D., Birnbaum: Truncation at conserved terminal regions of BRCA1 protein is associated with highly proliferating hereditary breast cancers. *Cancer Res.* 56, 3216-3219(1996).
14. Rodenhiser,D.I., J.D.Andrews,D.N.Mancini,J.H.Jung,& S.M., Singh: Homonucleotide tracts, short repeats and CpG/CpNpG motifs are frequent sites for heterogeneous mutations in the neurofibromatosis type 1 (NF1) tumour-suppressor gene. *Mutat. Res.* 373, 185-195(1997)
15. Struewing,J.P., D. Abeliovich,T. Peretz,N. Avishai,M.M.Kaback,F.S. Collins & L.C.,Brody: The carrier frequency of the BRCA1 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. *Nat. Genet.* 11, 198-200 (1995)
16. Offit,K., T. Gilewski,P. McGuire,A.Schluger,H. Hampel,K. Brown,J.Swensen,S. Neuhausen,M.Skolnick,L. Norton & D. Goldgar Germline BRCA1 185delAG mutations in Jewish women with breast cancer. *Lancet* 347, 1643-1645(1996)
17. Bahar,A.Y.,P.J. Taylor,L. Andrews,A. Proos,L. Burnett,K.Tucker,M.Friedlander & M.F.Buckley: The frequency of founder mutations in the BRCA1, BRCA2, and APC genes in Australian Ashkenazi Jews: implications for the generality of U.S. population data. *Cancer* 92, 440-445. (2001)
18. Neuhausen,S.L., S.Mazoyer,L.Friedman,M.Stratton,K. Offit,A.Caligo,G.Tomlinson,L.Cannon-Albright, T.Bishop, D.Kelsell,E.Solomon,B.Weber,F. Couch,J. Struewing, P.Tonin,F. Durocher, S.Narod, M.H.Skolnick, G.Lenoir, O.Serova, B.Ponder,D.Stoppa-Lyonnet, D. Easton, M.C.King & D.E. Goldgar: Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. *Am. J. Hum. Genet.* 58, 271-280(1996)
19. Olopade,O.I., J.D. Fackenthal,G.Dunston,M.A.Tainsky, F. Collins& D.E. Whitfield-Broome: Breast cancer genetics in African Americans. *Cancer* 97, 236-245(2003)
20. Gazdar,A.F., V. Kurvari,A.Virmani,L. Gollahon, M.Sakaguchi,M. Westerfield, D. Kodagoda, V.Stasny, H.T. Cunningham,I.I. Wistuba, G. Tomlinson, V. Tonk, R.Ashfaq, A.M. Leitch, J.D.Minna & J.W. Shay: Characterization of paired tumor and non-tumor cell lines established from patients with breast cancer. *Int. J. Cancer* 78, 766-774(1998)
21. Rohlfs,E.M., C.H.Chung,Q. Yang,C. Skrzynia,W.W. Grody,M.L. Graham & L.M. Silverman In-frame deletions of BRCA1 may define critical functional domains. Hum. Genet. 107, 385-390 (2000)
22. Ruffner,H., C.A.Joazeiro,D. Hemmati,T.Hunter & I.M. Verma:Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proc. Natl. Acad. Sci. U. S. A* 98, 5134-5139(2001)
23. Fan,S., R. Yuan,Y.X. Ma,Q. Meng,I.D.Goldberg & E.M. Rosen:Mutant BRCA1 genes antagonize phenotype of wild-type BRCA1. *Oncogene* 20, 8215-8235(2001)
24. Catteau,A., W.H. Harris,C.F.Xu & E. Solomon: Methylation of the BRCA1 promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics. *Oncogene* 18, 1957-1965(1999)
25. Jhanwar-Uniyal, M., G. D Stephenson, R. Royak-Schaler, C.-X Wang, M. Achary, A.P. Albino & J. Whysner: Involvement of p53 and BRCA1 genes in Breast Cancer in African-American and White Woman. *Proc.American Association for Cancer Research* 44, 1132-1133 (2003)
26. Archey,W.B., K.A. McEachern,M. Robson, K. Offit,K., S.A.Vaziri, G. Casey,A.Borg & B.A. Arrick: Increased CpG methylation of the estrogen receptor gene in BRCA1-linked estrogen receptor-negative breast cancers. *Oncogene* 21, 7034-7041(2002)
27. Bird,A. DNA methylation de novo. *Science* 286, 2287-2288(1999)

28. Xu,C.F., M.A.Brown, J.A.Chambers,B. Griffiths,H. Nicolai & E. Solomon: Distinct transcription start sites generate two forms of BRCA1 mRNAHum. Mol. Genet. 4, 2259-2264(1995)
29. Xu,C.F., M.A.Brown,H. Nicolai,J.A. Chambers,B.L. Griffiths & E. Solomon: Isolation and characterisation of the NBR2 gene which lies head to head with the human BRCA1 gene. *Hum. Mol. Genet.* 6, 1057-1062(1997a)
30. Xu,C.F., J.A.Chambers & E. Solomon: Complex regulation of the BRCA1 gene. *J. Biol. Chem.* 272, 20994-20997(1997b)
31. Klein-Hitpass,L., M. Schorpp,U. Wagner & G.U., Ryffel:An estrogen-responsive element derived from the 5' flanking region of the Xenopus vitellogenin A2 gene functions in transfected human cells. *Cell* 46, 1053-1061(1986).
32. Brown,M.A., C.F.Xu,H. Nicolai,B. Griffiths,J.A. Chambers,D. Black & E. Solomon: The 5' end of the BRCA1 gene lies within a duplicated region of human chromosome 17q21. *Oncogene* 12, 2507-2513(1996)
33. Thakur, S. & C.M.,Croce Positive regulation of the BRCA1 promoter. *J. Biol. Chem.* 274, 8837-8843 (1999)
34. Esteller,M., J.M. Silva,G. Dominguez,F. Bonilla,X. Matias-Guiu,E. Lerma, E. Bussaglia,J. Prat,I.C, Harkes,E.A. Repasky,E. Gabrielson,M. Schutte,S.B. Baylin & J.G. Herman: Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. *J. Natl. Cancer Inst.* 92, 564-569(2000)
35. Bertucci,F., F. Eisinger,R. Tagett,H. Sobol & D.Birnbaum: Re: Gene expression profiles of BRCA1-linked, BRCA2-linked, and sporadic ovarian cancers. *J. Natl. Cancer Inst.* 94, 1506-1507(2002)
36. Miyamoto,K., T. Fukutomi,K. Asada,K. Wakazono,H. Tsuda,T. Asahara, T.Sugimura & T. Ushijima: Promoter hypermethylation and post-transcriptional mechanisms for reduced BRCA1 immunoreactivity in sporadic human breast cancers. *Jpn. J. Clin. Oncol.* 32, 79-84(2002)
37. Mancini,D.N., D.I.Rodenhiser,P.J. Ainsworth,F.P. O'Malley,S.M. Singh,W. Xing & T.K. Archer: CpG methylation within the 5' regulatory region of the BRCA1 gene is tumor specific and includes a putative CREB binding site. *Oncogene* 16, 1161-1169(1998)
38. Magdinier,F., S.Ribieras,G.M. Lenoir,L. Frappart & R. Dante: Down-regulation of BRCA1 in human sporadic breast cancer; analysis of DNA methylation patterns of the putative promoter region. *Oncogene* 17, 3169-3176(1998)
39. DiNardo,D.N., D.T.Butcher,D.P. Robinson,T.K. Archer & D.I.Rodenhiser: Functional analysis of CpG methylation in the BRCA1 promoter region. *Oncogene* 20, 5331-5340(2001)
40. MacLeod,M.C: A possible role in chemical carcinogenesis for epigenetic, heritable changes in gene expression. Mol. Carcinog. 15, 241-250(1996)
41. Catteau,A., C.F. Xu,M.A.Brown,S. Hodgson,J. Greenman,C.G. Mathew, A.M. Dunning & E. Solomon: Identification of a C/G polymorphism in the promoter region of the BRCA1 gene and its use as a marker for rapid detection of promoter deletions. Br. J. Cancer 79, 759-763(1999b)
42. Jhanwar-Uniyal, M., S.A.Hoda, A.Raziuddin, D.M. Knowles, H.-F.Kung,S.C. Gulati, & R. Raziuddin: BRCA1 promoter-specific DNA binding protein activity in estrogen dependent breast tumor tissue is mediated by NF-kB nuclear factor. *Proc.American Association for Cancer Research* 38, 562-563(1997)
43. Ferguson,A.T., R.G.Lapidus,S.B. Baylin & N.E.Davidson: Demethylation of the estrogen receptor gene in estrogen receptor-negative breast cancer cells can reactivate estrogen receptor gene expression. *Cancer Res.* 55, 2279-2283(1995)
44. Sourvinos,G. & D.A. Spandidos: Decreased BRCA1 expression levels may arrest the cell cycle through activation of p53 checkpoint in human sporadic breast tumors. *Biochem. Biophys. Res. Commun.* 245, 75-80(1998)
45. Thompson,M.E., R.A. Jensen,P.S. Obermiller,D.L. Page & J.T.Holt: Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. *Nat. Genet.* 9, 444-450(1995)
46. Hedenfalk,I., D. Duggan,Y. Chen,M. Radmacher,M. Bittner,R. Simon,P. Meltzer, B. Gusterson, M. Esteller, O.P. Kallioniemi,B. Wilfond,A. Borg & J. Trent: Gene-expression profiles in hereditary breast cancer. *N. Engl. J. Med.* 344, 539-548 (1995)
47. Yarden,R.I. & L.C.Brody: BRCA1 interacts with components of the histone deacetylase complex. *Proc. Natl. Acad. Sci. U. S. A* 96, 4983-4988(1999)
48. Fabbro,M. & B.R.Henderson: Regulation of tumor suppressors by nuclear-cytoplasmic shuttling. *Exp. Cell Res.* 282, 59-69(2003)
49. Koonin,E.V., S.F.Altschul & P. Bork: BRCA1 protein products ... Functional motifs.. *Nat. Genet.* 13, 266-268(1996)
50. Deng,C.X. & S.G. Brodie: Roles of BRCA1 and its interacting proteins. *Bioessays* 22, 728-737(2000)
51. Xian,M.Y., S. Fan,J. Xiong,R.Q. Yuan,Q. Meng,M. Gao,I.D. Goldberg,S.A. Fuqua,R.G. Pestell & E.M.Rosen: Role of BRCA1 in heat shock response. *Oncogene* 22, 10-27(2003)
52. Liu,Y., D.M.Virshup, R.L. White &L.C. Hsu: Regulation of BRCA1 phosphorylation by interaction with protein phosphatase 1alpha. *Cancer Res.* 62, 6357-6361(2002)
53. Aprelikova,O.N., B.S.Fang,E.G. Meissner,S. Cotter,M. Campbell,A. Kuthiala, M.Bessho,R.A. Jensen, & E.T. Liu: BRCA1-associated growth arrest is RB-dependent. Proc. Natl. Acad. Sci. U. S. A 96, 11866-11871(1999)
54. Callebaut,I. & J.P.Mornon: From BRCA1 to RAP1: a widespread BRCT module closely associated with DNA repair. *FEBS Lett.* 400, 25-30(1997)
55. Stewart,G.S., B.Wang,C.R. Bignell,A.M. Taylor & S.J.Elledge: MDC1 is a mediator of the mammalian DNA damage checkpoint. Nature 421, 961-966(2003)
56. Wu,L.J.C., Z.W. Wang,J.T. Tsan,M.A. Spillman,A. Phung,X.L. Xu,M.C.W. Yang, L.Y. Hwang,A.M. Bowcock & R. Baer: Identification of a RING protein that can interact *in vivo* with the BRCA1 gene product. *Nature Genetics* 14, 430-440(1996)
57. Jensen,D.E., M.Proctor,S.T. Marquis,H.P. Gardner,S.I. Ha,L.A. Chodosh, A.M. Ishov,N. Tommerup,H. Vissing,Y. Sekido,J. Minna,A. Borodovsky,D.C. Schultz, K.D.Wilkinson,G.G. Maul,N. Barlev,S.L. Berger,G.C. Prendergast & F.J. Rauscher III: BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and

enhances BRCA1-mediated cell growth suppression. *Oncogene* 16, 1097-1112(1998)
58. Jensen,D.E. & F.J. Rauscher, III:Defining biochemical functions for the BRCA1 tumor suppressor protein: analysis of the BRCA1 binding protein BAP1. *Cancer Lett.* 143 Suppl 1, S13-S17(1999)
59. Chen,A., F.E.Kleiman,J.L. Manley,T. Ouchi & Z.Q. Pan: Autoubiquitination of the BRCA1*BARD1 RING ubiquitin ligase. *J. Biol. Chem.* 277, 22085-22092(2002)
60. Lorick,K.L., J.P.Jensen,S. Fang,A.M. Ong,S. Hatakeyama, & A.M. Weissman: RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc. Natl. Acad. Sci. U. S. A* 96, 11364-11369(1999)
61. Ruffner,H., W.Jiang,A.G. Craig,T. Hunter & I.M. Verma: BRCA1 is phosphorylated at serine 1497 *in vivo* at a cyclin-dependent kinase 2 phosphorylation site. *Mol. Cell Biol.* 19, 4843-4854(1999)
62. Moynahan,M.E.:The cancer connection: BRCA1 and BRCA2 tumor suppression in mice and humans. *Oncogene* 21, 8994-9007(2002)
63. Arizti,P., L.Fang,I. Park,Y. Yin,E. Solomon,T. Ouchi,S.A. Aaronson & S.W. Lee: Tumor suppressor p53 is required to modulate BRCA1 expression. *Mol. Cell Biol.* 20, 7450-7459(2000)
64. MacLachlan,T.K., B.C.Dash,D.T. Dicker & W.S. El Deiry: Repression of BRCA1 through a feedback loop involving p53. *J. Biol. Chem.* 275, 31869-31875(2000)
65. MacLachlan,T.K., R.Takimoto & W.S. El Deiry: BRCA1 directs a selective p53-dependent transcriptional response towards growth arrest and DNA repair targets. *Mol. Cell Biol.* 22, 4280-4292(2002)
66. Kinzler,K.W. & B. Vogelstein: Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* 386, 761, 763(1997)
67. Xia,Y., G.M. Pao,H.W. Chen,I.M. Verma & T. Hunter: Enhancement of BRCA1 E3 Ubiquitin Ligase Activity through Direct Interaction with the BARD1 Protein. *J. Biol. Chem.* 278, 5255-5263(2003)
68. Wang,Q., H. Zhang,K. Kajino & M.I. Greene: BRCA1 binds c-Myc and inhibits its transcriptional and transforming activity in cells. *Oncogene* 17, 1939-1948(1998)
69. Ouchi,T., S.W.Lee,M. Ouchi,S.A. Aaronson & C.M. Horvath: Collaboration of signal transducer and activator of transcription 1 (STAT1) and BRCA1 in differential regulation of IFN-gamma target genes. *Proc. Natl. Acad. Sci. U. S. A* 97, 5208-5213(2000)
70. Scully,R., J.J.Chen, R.L.Ochs,K. Keegan,M. Hoekstra,J. Feunteun & D.M. Livingston: Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. *Cell* 90, 425-435(1997)
71. Scully,R., J.Chen,A. Plug,Y. Xiao,D. Weaver,J. Feunteun,T. Ashley & D.M. Livingston: Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* 88, 265-275(1997)
72. Scully,R. & D.M. Livingston (2000) In search of the tumour-suppressor functions of BRCA1 and BRCA2. Nature 408, 429-432.
73. Chen,J., D.P. Silver,D. Walpita,S.B. Cantor,A.F. Gazdar,G. Tomlinson,F.J. Couch,B.L. Weber,T. Ashley,D.M. Livingston & R.Scully: Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Mol. Cell* 2, 317-328(1998)
74. Ivanov,E.L. & J.E. Haber: DNA repair: RAD alert. *Curr. Biol.* 7, R492-R495(1997)
75. Wang,Y., D. Cortez, P.Yazdi,N. Neff, S.J. Elledge & J. Qin: BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev.* 14, 927-939(2000)
76. Zhong,Q., C.F.Chen,S. Li,Y. Chen,C.C. Wang,J. Xiao,P.L. Chen,Z.D. Sharp & W.H.Lee: Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. *Science* 285, 747-750(1999)
77. Venkitaraman,A.R.: Breast cancer genes and DNA repair. *Science* 286, 1100-1102(1999)
78. Venkitaraman,A.R.:Functions of BRCA1 and BRCA2 in the biological response to DNA damage. *J. Cell Sci.* 114, 3591-3598(2001)
79. Venkitaraman,A.R.: Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* 108, 171-182(2002)
80. Cortez,D., Y. Wang,J. Qin & S.J.Elledge: Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. *Science* 286, 1162-1166(1999)
81. Tibbetts,R.S., D. Cortez,K.M. Brumbaugh,R. Scully,D. Livingston,S.J. Elledge & R.T.Abraham: Functional interactions between BRCA1 and the checkpoint kinase ATR during genotoxic stress. Genes Dev. 14, 2989-3002(2000)
82. Elledge,S.J. & A. Amon: The BRCA1 suppressor hypothesis: an explanation for the tissue-specific tumor development in BRCA1 patients. *Cancer Cell* 1, 129-132(2002)
83. Paull,T.T., D.Cortez,B. Bowers,S.J. Elledge & M. Gellert: Direct DNA binding by Brca1. *Proc. Natl. Acad. Sci. U. S. A* 98, 6086-6091(2001)
84. Gowen,L.C., A.V. Avrutskaya,A.M. Latour,B.H. Koller & S.A.Leadon: BRCA1 required for transcription-coupled repair of oxidative DNA damage. *Science* 281, 1009-1012 (1998)
85. Bernstein,C.,H. Bernstein,C.M. Payne & H.Garewal: DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. *Mutat. Res.* 511, 145-178(2002)
86. Thomspson L.H.& D.Schild: Recombinational DNA repair and human disease. *Mutat. Res* 509, 49-78(2002)
87. Khanna,K.K. & S.P.Jackson: DNA double-strand breaks: signaling, repair and the cancer connection. *Nat. Genet.* 27, 247-254(2001)
88. Cantor,S.B., D.W.Bell,S. Ganesan,E.M. Kass,R. Drapkin,S. Grossman,D.C. Wahrer,D.C. Sgroi,W.S. Lane,D.A. Haber & D.M.Livingston: BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* 105, 149-160(2001)
89. Gatei,M., K. Sloper,C. Sorensen,R. Syljuasen,J. Falck,K. Hobson,K. Savage, J. Lukas,B.B. Zhou,J. Bartek & K.K.Khanna: ATM and NBS1 dependent phosphorylation of CHK1 on S317 in response to IR. *J. Biol. Chem.* (2003) *In Press.*
90. Yarden,R.I., S. Pardo-Reoyo,M. Sgagias,K.H. Cowan & L.C. Brody: BRCA1 regulates the G2/M checkpoint by

activating Chk1 kinase upon DNA damage. *Nat. Genet.* 30, 285-289(2002)
91. Oe,T., N. Nakajo,Y.Katsuragi, K. Okazaki & N. Sagata: Cytoplasmic occurrence of the Chk1/Cdc25 pathway and regulation of Chk1 in Xenopus oocytes. *Dev. Biol.* 229, 250-261(2001)
92. Okada,S. & T., Ouchi: Cell Cycle Differences in DNA Damage-induced BRCA1 Phosphorylation Affect Its Subcellular Localization. *J. Biol. Chem.* 278, 2015-2020 (2003)
93. Williams,R.S. & J.N.,Glover: Structural Consequences of a Cancer-causing BRCA1-BRCT Missense Mutation. J. Biol. Chem. 278, 2630-2635(2003)

Key Words: BRCA1 Breast Cancer Mutations Methylation Review DNA repair Cell cycle

Send correspondence to: Dr. Meena Jhanwar-Uniyal, Institute for Cancer Prevention, American Health Foundation Cancer Prevention Center, One Dana Road, Valhalla, New York 10595, Tel: 914-789-7135, Fax: 914-592-6317, E-mail: mjhanwar@ifcp.us

Involvement of p53 and BRCA1 genes in Breast Cancer in African-American and White Women

Chung-Xiou Wang, Sayed Hoda, William Thelmo, Mo Tika, Renee Royak-Schaler, Gina Day Stephenson, Mohanrao Achary, Anthony P. Albino, John Whysner and Meena Jhanwar-Uniyal

Institute for Cancer Prevention (American Health Foundation-Cancer Center), Valhalla, NY; Albert Einstein College Of Medicine and Montefiore Medical Center, Bronx, NY.

Key words: p53, BRCA1, Breast Cancer, African American
Running Title: Role of p53 and BRCA1 in development of breast cancer in African American and AmericanWhite women.

Corresponding Author:
Meena Jhanwar-Uniyal
Institute for Cancer Prevention
American Health Foundation,
One Dana Road,
Valhalla, New York 10595.
Tel. 1-914-789-7135.
FAX. 1-914-592-6317
E-mail: mjhanwar@ifcp.us