J. Toxicol. Pub. Health
Vol. 14, No. 2, pp. 123-127, 1998

BRCA1 Protein Was Not Expressed in a Normal Human Breast Epithelial Cell Type With Stem Cell and Luminal Characteristics

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(Received May 4, 1998)
(Accepted May 30, 1998)

ABSTRACT *: BRCA1 is a tumor suppresser gene in familial cases of breast cancer. It has been controversial whether the subcellular localization of BRCA1 is located in nuclei or cytoplasm in normal human breast cells. We found that a p220 protein was expressed in Type II Normal human breast epithelial cells (NHBEC) but not in Type I NHBEC in Western blot analysis using the 17F8 (3A2) antibody. Immunostaining using the same antibody revealed positive staining in nuclei, cytoplasm and perinuclei of Type II cells and negative staining in Type I NHBEC. The p220 protein, however, was expressed in SV40 immortalized Type I NHBEC and tumorigenic cells derived from them after x-ray and neu oncogene treatment. The subcelluar localization was mostly cytoplasmic and punctate in the nuclei. The breast carcinoma cell lines, MCF-7 and T47D, also expressed the p220 protein. Using RT-PCR, we observed the expression of BRCA1 mRNA in both Type I and Type II NHBEC. This result indicated that there might be mechanisms involved in post-translational or translational regulation of BRCA1 gene. It is speculated that the absence of BRCA1 protein expression in Type I NHBEC might play a role in their susceptibility to neoplastic transformation.*

Key Words *: Normal Human Breast Epithelial Cells(NHBEC), BRCA 1, Breast Cancer*

I. INTRODUCTION

The breast and ovarian cancer susceptible gene, BRCA1 is a phosphoprotein which functions as a tumor suppresser gene (Holt *et al.*, 1996). When one copy of BRCA1 is inactivated in the germline, affected individuals are predisposed to developing breast, ovarian, other malignant tumors (Trikkonen *et al.*, 1997 and Montagna *et al.*, 1996). Initial studies have identified BRCA1 proteins differing in size and in subcellular location. Chen *et al.* (1995) showed that BRCA1 was a 220 Kd protein and was aberrantly localized to the cytoplasm of cells or tumors derived from breast and ovarian cancers, whereas BRCA1 was found in the nucleus in non-tumorigenic breast or ovarian cells or tissues (Chen *et al.*, 1995). However, Scully *et al.* (1997) found that BRCA1 was localized primarily to the nucleus in tumor-derived cell lines. Thus, the subcellular localization of BRCA1 is still controversial. We have recently isolated and charac-

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CELL LINES USED IN THIS STUDY
- *Type I and Type II* normal human breast epithelial cell (NHBEC).
- *M13SV1* - A SV40 immortalized Type I HBEC line.
- *M13SV1R2* - A weakly tumorigenic cell line derived from.
- *M13SV1R2N1, M13SV1R2N8* - highly tumorigenic cell lines derived from M13SV1R2 after transfection with neu oncogene.
- *M15SV1* - A SV40 immortalized Type I HBEC line.
- *M15SV30* - A SV40 immortalized Type II HBEC line.
- *MCF-7, T47D* - breast carcinoma cell lines.
- *HBL100* - a milk cell-derived human breast epithelial cell line.

ABBREVIATIONS
NHBEC, Normal Human Breast Epithelial Cells.
BRCA1, Breast Cancer Gene 1.
NGS, Normal Goat Serum.

terized two types of normal human breast epithelial cells (NHBEC) derived from reduction mammoplasty (Kao *et al.*, 1995). Type I NHBEC expressed a variant estrogen receptor and had luminal and stem cell characteristics (i.e., the ability to differentiate into other cell type and to form budding/ductal structure in Matrigel matrix) (Kang *et al.*, 1997; Kang *et al.*, 1988; Kang *et al.*, 1988). Type II NHBEC were estrogen receptor-negative and showed basal cell phenotypes (Kang *et al.*, 1997). We also established immortal and tumorigenic cell lines from these cells (Kang et al., 1988). In this study, we investigated the expression and subcellular localization of BRCA1 in these cell lines.

II. MATERIALS AND METHODS

1. Immunofluorescent staining of BRCA1 protein

Immnunofluorescent staining to detect BRCA1 protein was done as previously described (Kang *et al.*, 1996). Briefly, cells were fixed by 4% paraformaldehyde and then blocked with 10% normal goat serum(NGS). The cells were incubated overnight at 4°C with a primary antibody against BRCA 1 from Dr. Wen-Hwa Lee (17F8(3A2), diluted 1 : 200 in PBS containing 0.1% bovine serum albumin and 1% NGS), followed by incubation with a second anti-mouse antibody conjugated with rhodamine (Jackson Immunoresearch Lab, West Grove, PA; diluted 1 : 100 in PBS). The cells were examined and photographed using the Ultima laser confocal scanning microscope (Meridian Instruments, Okemos, MI).

2. Western Blotting

Proteins were extracted for Western blot analysis in 100 mm dishes by treatment with 20% SDS lysis solution containing several kinds of protease and phosphatase inhibitors. Proteins were separated on 12.5% SDS polyacrylamide gels and transferred to PVDF membranes at 20 V for 16 hr. BRCA1 was detected by the anti-BRCA1 monoclonal antibody (17F8(3A2)) after blocking with 5% dried skim milk in PBS containing 0.1% Tween 20. This was then followed by incubation with horseradish peroxidase-conjugated with a secondary antibody and detected with the ECL chemiluminiscent detection reagent (Amersham Co., Arlington Heights, IL).

3. Reverse-Transcription Polymerization Chain Reaction

cDNA was synthesized from the isolated RNA by reverse transcription in 20 μl reaction solution containing 2.5 μM of random hexamers (Perkin Elmer, Madison, WI). The 20 μl of solution which contains the reverse transcribed cDNA were added to 30 μl of PCR reaction mixture. The primers chosen for PCR have the following sequences : 5-AACTAAG-CCAGGTGATTG-3 (up-stream primer exon 9 at position 593), and 5-CTGACATGTTTGGTTCCAGAT-3(downstream primer, exon 13 at position 4225). The BRCA1 PCR product is 352 bp. The primers used to generate the 306-base pairs of GAPDH products are 5-CGGAGTCAACGGATTTGGTCGTAT-3 and 5-AGCCTTCTCCATGGTGG TGAAC-3. Thermal cycling was performed in a GeneAMP 9600 PCR system (Perkin Elmer, Madison, WI) by using the following two steps amplification profile. The PCR products were then electrophoresed in a 2% agarose gel and stained with 0.5 μg/ml ethidium bromide.

III. RESULTS AND DISCUSSION

Type I NHBEC may not express BRCA1 protein as shown by Western blot analysis and immunostaining (Fig. 1 and Fig. 3). Type II NHBEC expressed a 220 Kd of BRCA1 protein (Fig. 1). But the expression level of BRCA1 protein in Type II HBEC was lower than that in cancer cell lines. These cells showed nuclear, perinuclear or cytoplasmic staining of the BRCA1 protein as shown in confocally scanned-image (Fig. 2). These results suggested that the aberrant expression of BRCA1 might be related to cell cycle and phosphorylation (Thomas *et al.*, 1997 and Chen *et al.*, 1996). BRCA1 protein was not expressed in Type I NHBEC, which may be involved in their highly proliferating activity and similar characteristics as cancer cells. Type I NHBEC were more susceptible to neoplastic transformation by SV40

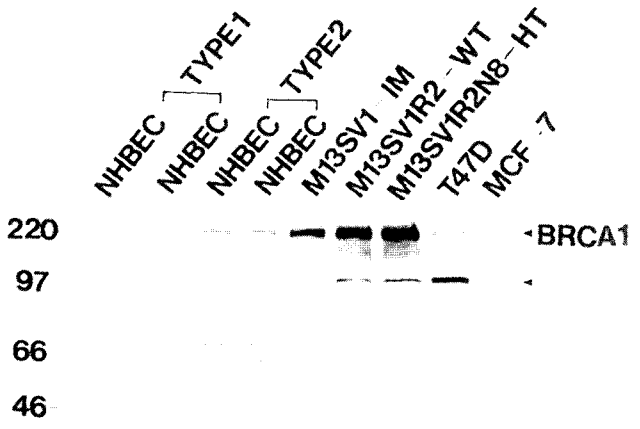

Fig. 1. Western blot analysis of the expression of BRCA1 gene using an anti-BRCA1 antibody[17F8(3A2)]. A 220 kd and a 110 kd bands were observed in Type II NHBEC, M13SV1 HBEC, M13SV1R2, M13SV1R2N8, T47D and MCF-7 cells. IM, immortal cell line derived from Type I NHBEC; WT, weakly tumorigenic cell line; HT, highly tumorigenic cell line.

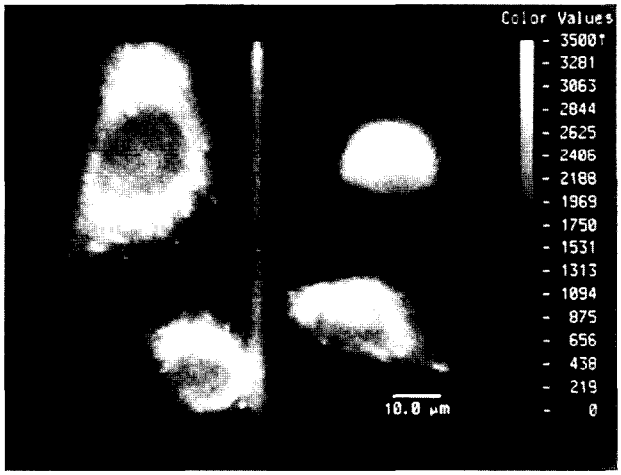

Fig. 2. Confocally scanned-image of a Type II HBEC using an anti-BRCA1 antibody [17F8(3A2)] provided by Dr. Wen-Hwa Lee. Cells showing either nuclear, perinuclear or cytoplasmic staining were observed.

large T-antigen, i.e., Type I NHBEC have the ability to grow in soft agar (AIG+), whereas Type II NHBEC were AIG-; Type I NHBEC were more frequently to become immortal (10/11) as compared to Type II NHBEC (1/10) (data not shown). Therefore, we thought that Type I NHBEC are putative human breast stem cells. We were able to observe a telomerase activity in Type I NHBEC (manuscript in preparation). This result also strongly indicated that Type I NHBEC could be a stem cell existed in human breast tissue. We also observed a band (about

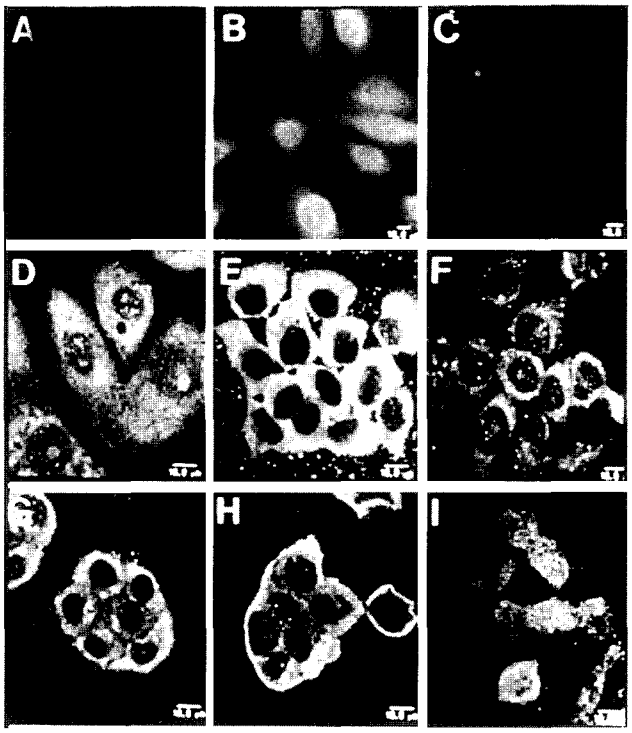

Fig. 3. Immunostaining using an anti-BRCA1 antibody [17F8(3A2)]. A, Type I NHBEC; B, Type II NHBEC; C, Control without primary antibody; D, M13SV1 immortal cell derived from Type I NHBEC; E, M15SV30 immortal cell derived from Type II NHBEC; F, M13SV1R2N8 highly tumorigenic cell; G, T47D; H, MCF-7; I, HBL 101.

110 kd) in all cell lines besides Type I NHBEC. This band might be a splicing band of BRCA1 deleted exon 11 (Thakur *et al.*, 1997). And this splicing variant might give rise to alter the subcellular localization of BRCA1 protein. These results suggest that splicing is one form of regulation of BRCA1 function. Interestingly, by RT-PCR, both Type I and Type II NHBEC expressed BRCA1 transcripts, even though Type I NHBEC did not express BRCA1 protein by Western blot analysis and immnunofluorescent staining. And we also observed two truncated transcript bands besides a 352 bp one (Fig. 4). These data strongly suggested that there might be splicing variants BRCA1 transcripts as previously described (Thakur *et al.*, 1997).

This is the first report describing the expression of BRCA1 in normal human breast epithelial cells. We established unique systems to understand human breast cancer (Kang *et al.*, 1988). Using this established cell line, we compared our cell line with other well-known cancer cell lines for the expression

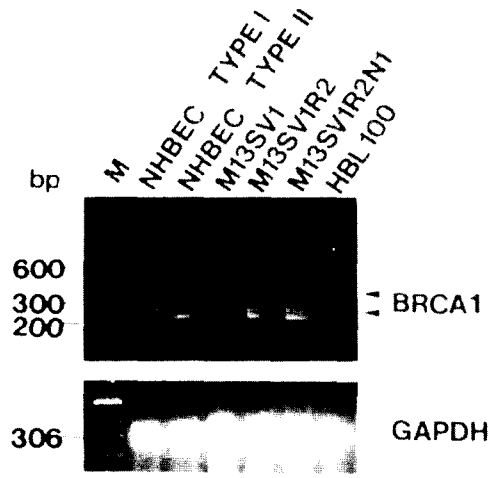

Fig. 4. Expression of BRCA1 transcripts by RT-PCR analysis. 5-AGAAACTAAGCCAGGTGATTG-3 (Up-stream primer exon 9 at position 593), 5-CTGACATGTTTGGTTC-CAGAT-3(downstream primer, exon 13 at position 4225). The product is 352 bp.

of BRCA1 protein. Like MCF-7 and T47D cells, our carcinogenic cell lines (M13SV1, M13SV1R2, M13SV1R2N1, M15SV1 and M15SV30) mainly expressed BRCA1 in the cytoplasm and minorly punctated in the nuclei (Fig. 3). We also used a monoclonal antibody from Oncogene Science Co. The results showed similar staining patterns as 17F8 (3A2) antibody. So far, the problems and controversies regarding on the subcellular localization of BRCA1 protein were that there was no good normal human breast epithelial cells isolated. Chen *et al.* (1995) used HBL 100 cells as a normal cell line. But HBL 100 cell line are not normal but immortal cells infected by SV 40 virus. Therefore, they did not use real normal cells for their experiment. As we have shown in Fig. 3, our SV40 immortalized cells expressed BRCA1 in both nuclei and cytoplasm, while HBL 100 cells expressed BRCA1 mainly in nuclei.

Therefore, it is concluded that the absence of BRCA1 protein expression in Type I NHBEC, which is considered as putative breast stem cells, might play a role in their susceptibility to neoplastic transformation, and BRCA1 in Type II HBEC is expressed in the cytoplasm, perinuclear and nuclei, as well.

ACKNOWLODGEMENT

The authors thank Dr. Wen-Hwa Lee who provided an antibody against BRCA 1 protein.

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