

Comparison of *BRCA1* Expression between Triple-Negative and Luminal Breast Tumors

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ABSTRACT

Background: Previous studies have suggested that *BRCA1* dysregulation has been shown to have a role in triple-negative phenotypic manifestation. However, differences of *BRCA1* expression, as a tumor suppressor gene, have rarely been investigated between luminal and triple-negative breast tumors. Therefore, the present study attempted to compare the *BRCA1* expression in triple-negative with luminal breast tumors and its association with the clinicopathologic characteristics of patients. **Methods:** *BRCA1* expression was evaluated by real-time PCR in 26 triple-negative and 27 luminal breast tumors. **Results:** The results revealed that there is a high frequency of *BRCA1* underexpression in both triple-negative and luminal breast tumors. The *BRCA1* underexpression was related to young age at diagnosis, lymph node involvement, and grade III tumors. **Conclusion:** The observations suggest that decreased *BRCA1* expression, regardless of tumor subtype, has a general role in breast malignancy and associated with poor prognostic features in breast tumors. **DOI: 10.22034/ibj.22.3.210**

Keywords: Breast cancer, *BRCA1*, Triple-negative breast neoplasm

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INTRODUCTION

Breast cancer is the most common cancer among women worldwide^[1]. In Iran, breast malignancy is the fifth most common cause of death with a fast-rising trend^[2]. Breast tumors show different molecular features and can be divided into at least four main molecular subtypes: luminal A and B as well as triple-negative and HER2-overexpressing tumors^[3]. Luminal tumors are estrogen receptor positive (ER+), progesterone receptor-positive (PR+), and positive or negative for human epidermal growth factor receptor 2 (HER2+ or HER2-). These tumors have a good prognosis and respond to targeted therapies such as tamoxifen. On the other hand, triple-negative tumors (ER-/PR-/HER-) show aggressive

behavior and a worse prognosis in comparison with other subtypes^[4].

BRCA1 is one of the genes that involves in breast cancer. The protein product of the *BRCA1* gene is a 220-kD nuclear phosphoprotein with 1863 amino acids and different important cellular functions. *BRCA1* protein helps to repair DNA double-strand breaks and plays a critical role in maintaining the genomic stability, cell cycle regulation, and apoptosis^[5,6]. Accordingly, *BRCA1* deficiency can activate the tumorigenesis process. The association between germline mutations of *BRCA1* and hereditary form of breast cancers is well known^[7,8].

Previous investigations have shown that the majority of *BRCA1*-mutated breast tumors (over 80%) are categorized as triple-negative subtype^[9]. Furthermore,

several studies have demonstrated that the reduced levels of *BRCA1* expression, due to promoter hypermethylation or somatic mutation, may take a part in sporadic breast cancers^[10-12]. Interestingly, the sporadic *BRCA1*-deficient breast tumors often show similar histological characteristics with the *BRCA1*-related hereditary breast cancers^[13-15]. Therefore, it seems that the dysfunctional *BRCA1* pathway has a function in the manifestation of the triple-negative phenotype in breast tumors^[16-18]. However, the comparison of *BRCA1* mRNA expression between different subtypes of breast tumors is rarely available. It is unclear that *BRCA1* down-regulation is a prominent feature of triple-negative breast tumors, or it must be noticed as a more general molecular alteration in breast cancer regardless of tumor subtype. Therefore, the aim of the present investigation was to compare *BRCA1* expression in the setting of triple-negative and luminal tumors and to study the association of *BRCA1* expression with clinicopathological features in Iranian breast cancer patients.

MATERIALS AND METHODS

Patients and tissue collection

A total of 53 surgically resected breast tumors were obtained from the Iran National Tumor Bank (INTB) of the Cancer Institute at Imam Khomeini Hospital Complex, Tehran, Iran. As calibrator samples, four normal breast tissues were acquired from women who were undergoing mastectomy. Tissues were placed in liquid nitrogen immediately after resection and stored at -80 °C for later use. None of the patients were under chemotherapy or radiotherapy before surgery. Clinicopathological features of the patients (age, tumor size, ER/PR/HER2 status based on immunohistochemistry results, axillary lymph node involvement, and grade) were collected from their medical records in INTB. Informed consents were obtained from all participants, and the study was approved by the local ethical committee at Tehran University of Medical Sciences, Iran.

RNA extraction and cDNA synthesis

Total RNA was isolated from tissues using Hybrid-R™ kit from GeneAll Biotechnology Company (Korea) according to the manufacturer's instructions. The purity and quantity of extracted RNA were checked by NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA). Hyperscript™ kit (GeneAll Biotechnology Co., Korea) was applied to synthesize first-strand cDNA.

Gene expression study

Real-time quantitative RT-PCR of the *BRCA1* gene was performed using RealQ Plus 2× Master Mix Green (Ampliqon, Denmark) following the manufacturer's instructions. The primers sequence for the *BRCA1* mRNA expression assay were: forward 5'-CCCTCAA GGAACCAGGGATG-3' and reverse 5'-GCTGCA CGCTTCTCAGTGGT-3'. *BRCA1* expression levels were normalized against *PUM1* (Pumilio RNA-binding family member 1), as a housekeeping gene. The primers for *PUM1* mRNA expression assay were: forward 5'-AGTGGGGGACTAGGCGTTAG-3' and reverse 5'-GTTTTTCATCACTGTCTGCATCC-3'.

The real-time PCR reaction mix consisted of 10 µL SYBR Green master mix, 0.5 µL of each forward and reverse primers (primer concentration: 5 pmol), 1 µL target cDNA, and 8 µL sterile water in a total volume of 20 µL. The PCR conditions were as follows: initial denaturation at 95 °C for 15 minutes, followed by 40 cycles of 95 °C for 15 seconds, and 59 °C for 60 seconds. Four normal breast tissues were used as the calibrator for obtaining relative expression between breast tumors and normal breast tissues ($2^{-\Delta\Delta CT}$ method)^[19]. As the range of *BRCA1* expression values in four normal breast tissues was 0.51 to 2.38, the values of ≥ 2.5 and ≤ 0.4 were considered overexpression and underexpression status, respectively, in breast tumors.

Statistical analysis

Statistical calculations were performed using SPSS 21 statistical software. Data were presented with mean and 95% CI for numerical data or percentage for qualitative data. Student's *t*-test was performed to analyze difference in *BRCA1* expression between luminal and triple-negative tumors. The relationship between *BRCA1* relative expression and clinicopathologic factors were assessed by *t*-test or ANOVA and alternative non-parametric tests. Differences were considered significant when $p < 0.05$ was obtained.

RESULTS

Clinicopathological characteristics

In a total of 53 samples, 26 and 27 breast cancer patients were classified as triple-negative and luminal (types A or B) subtypes, respectively. The tumors were divided into different groups according to age (<50 years: 31 tumors; ≥ 50 years: 22 tumors), size (≤ 2 cm: 3 tumors; 2-5 cm: 44 tumors; >5 cm: 6 tumors), grade (I, II: 26 tumors; III: 27 tumors), and nodal status (positive: 19 tumors; negative: 34 tumors).

Table 1. BRCA1 mRNA expression in breast tumors

BRCA1 mRNA expression status	Total (%) (n = 53)	Triple-negative tumors (%) (n = 26)	Luminal tumors (%) (n = 27)
Underexpression	67.9	73.1	63.0
Normal expression	28.3	26.9	29.6
Overexpression	3.8	0.0	7.4

Expression status of BRCA1 mRNA in breast tumors

As the expression of BRCA1 mRNA values for all four normal breast samples were between 0.51-2.38, values of ≥ 2.5 were considered as the overexpression status and those of ≤ 0.4 as underexpression status in breast tumors. The frequency of different statuses of BRCA1 mRNA expression in luminal and triple-negative tumors is indicated in Table 1.

Comparison of BRCA1 expression between triple-negative and luminal subtypes

Independent samples *t*-test showed that the means of BRCA1 mRNA relative expression were not significantly different ($p = 0.065$) between luminal and triple-negative subtypes (Fig. 1). Also, in triple-negative tumors, BRCA1 relative expression (mean mean \pm SD: 0.26 ± 0.32) showed a decreased level in comparison with luminal tumors (mean \pm SD: 1.3 ± 1.5).

BRCA1 mRNA expression and its clinicopathological significance

Independent samples *t*-test in 53 breast tumors indicated that decreased expression of BRCA1 significantly related to young age at diagnosis (< 50 years, $p = 0.028$), lymph node involvement ($p = 0.04$), and grade III ($p = 0.04$) in breast tumor samples, but it did not significantly associate with tumor size and ER/PR/HER2 status of the studied population.

DISCUSSION

The dysfunctional BRCA1 pathway is involved in the pathogenesis of both hereditary and sporadic breast cancers. The BRCA1-related hereditary breast cancers show a trend toward triple-negative phenotype^[9]. Furthermore, decreased BRCA1 expression, due to promoter hypermethylation or somatic mutations, have been reported in sporadic breast cancers, regardless of breast tumor subtypes^[11,12]. As BRCA1-deficient sporadic triple-negative tumors show the same histological characteristics as BRCA1-related hereditary breast cancers^[13,15], it has been suggested that BRCA1-deficiency has a role in inducing the triple-negative phenotype. However, the difference in BRCA1

expression levels, based on the tumor subtypes, has rarely been reported. Accordingly, in the current study, the BRCA1 mRNA expression was compared in the setting of triple-negative and luminal tumors, and clinicopathological significance of BRCA1 expression was evaluated in Iranian breast cancer patients.

The results of this study demonstrated that the BRCA1 underexpression is slightly different between triple-negative and luminal tumors (73.1% and 63%, respectively). This observation suggests that decreased BRCA1 expression is frequent not only in triple-negative but also in luminal breast cancer tumors. Consequently, BRCA1 deficiency has possibly a key role in breast malignancy process, apart from tumor subtypes. Interestingly, in our studied patients, BRCA1 overexpression was observed in two luminal tumors, which belonged to patients with older age at diagnosis (60 and 81 years) and low-grade breast tumors.

Our study revealed that BRCA1 expression is not significantly different between triple-negative and luminal tumors, though triple-negative tumors overallly show a trend to more decrease in BRCA1 expression as compared to luminal tumors ($p = 0.065$). An investigation on Japanese patients indicated that BRCA1 mRNA expression is significantly decreased in triple-negative rather than luminal tumors^[20].

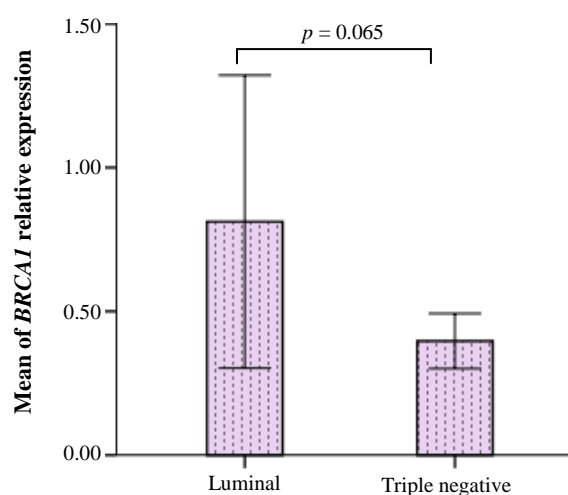


Fig. 1. Differences in the expression of BRCA1 in luminal and triple-negative tumors. Data were normalized to Pumilio RNA-binding family member 1 expression, as a housekeeping gene (error bars: 95% CI).

In this study, decreased expression of *BRCA1* significantly associated with young age at diagnosis, high grade, and lymph node-positive tumors. It seems that the decrease in *BRCA1* expression, whether due to germline mutations in hereditary breast cancers or hypermethylation in sporadic breast cancers, could increase the risk of breast cancer in women at younger ages^[21,22]. In several previous studies, it has been demonstrated that the lower level of *BRCA1* expression, as a tumor suppressor gene, was associated with poor prognostic features^[21-25]. On the other hand, some studies did not find any association between *BRCA1* mRNA expression and clinicopathological characteristics^[11,26-28], suggesting a more complex molecular association. For instance, Egawa *et al.*^[26] suggested that decreased *BRCA1* expression alone might not be enough for the development of poor prognostic features, and additional genetic alterations such as *p53* abnormality might be necessary.

In conclusion, in the present study, the decreased levels of *BRCA1* mRNA expression in the majority of triple-negative and luminal tumors compared to normal breast tissues indicates the involvement of *BRCA1* even in luminal subtype, though down-regulation of *BRCA1* expression was more remarkable in triple-negative tumors.

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CONFLICT OF INTEREST. None declared.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer* 2015; **136**(5): E359-E386.
2. Rafiemanesh H, Salehiniya H, Lotfi Z. Breast cancer in Iranian woman: Incidence by age group, morphology and trends. *Asian Pacific journal of cancer prevention* 2016; **17**(3): 1393-1397.
3. Sørli T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lønning PE, Brown PO, Børresen-Dale AL, Botstein D. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proceedings of the national academy of sciences* 2003; **100**(14): 8418- 8423.
4. Blows FM1, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J, Cheang MC, Gelmon K, Nielsen TO, Blomqvist C, Heikkilä P, Heikkinen T, Nevanlinna H, Akslen LA, Bégin LR, Foulkes WD, Couch FJ, Wang X, Cafourek V, Olson JE, Baglietto L, Giles GG, Severi G, McLean CA, Southey MC, Rakha E, Green AR, Ellis IO, Sherman ME, Lissowska J, Anderson WF, Cox A, Cross SS, Reed MW, Provenzano E, Dawson SJ, Dunning AM, Humphreys M, Easton DF, García-Closas M, Caldas C, Pharoah PD, Huntsman D. Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. *PLoS medicine* 2010; **7**(5): e1000279.
5. Paterson JW. BRCA1: a review of structure and putative functions. *Disease markers* 1998; **13**(4): 261-274.
6. Deng CX. BRCA1: cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. *Nucleic acids research* 2006; **34**(5): 1416-1426.
7. Krainer M, Silva-Arrieta S, FitzGerald MG, Shimada A, Ishioka C, Kanamaru R, MacDonald DJ, Unsal H, Finkelstein DM, Bowcock A, Isselbacher KJ, Haber DA. Differential contributions of BRCA1 and BRCA2 to early-onset breast cancer. *New England journal of medicine* 1997; **336**(20): 1416-1422.
8. Szabo CI, King M-C. Population genetics of BRCA1 and BRCA2. *American journal of human genetics* 1997; **60**(5): 10-13.
9. Lakhani SR, van de Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L, Osin P, McGuffog L, Easton D. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. *Journal of clinical oncology* 2002; **20**(9): 2310-2318.
10. Signori E, Bagni C, Papa S, Primerano B, Rinaldi M, Amaldi F, Fazio VM. A somatic mutation in the 5'UTR of BRCA1 gene in sporadic breast cancer causes down-modulation of translation efficiency. *Oncogene* 2001; **20**(33): 4596-4600.
11. Li Q, Wei W, Jiang Y, Yang H, Liu J. Promoter methylation and expression changes of BRCA1 in cancerous tissues of patients with sporadic breast cancer. *Oncology letters* 2015; **9**(4): 1807-1813.
12. Saelee P, Chaiwerawattana A, Ogawa K, Cho YM, Tiwawech D, Suktangman V. Clinicopathological significance of BRCA1 promoter hypermethylation in Thai breast cancer patients. *Asian Pacific journal of cancer prevention* 2014; **15**(24): 10585-10589.
13. Turner NC, Reis-Filho JS, Russell AM, Springall RJ, Ryder K, Steele D, Savage K, Gillett CE, Schmitt FC, Ashworth A, Tutt AN. BRCA1 dysfunction in sporadic basal-like breast cancer. *Oncogene* 2007; **26**(14): 2126-2132.
14. Joosse SA, Brandwijk KI, Mulder L, Wesseling J, Hannemann J, Nederlof PM. Genomic signature of

- BRCA1 deficiency in sporadic basal-like breast tumors. *Genes, chromosomes and cancer* 2011; **50**(2): 71-81.
15. Lips EH, Mulder L, Oonk A, van der Kolk LE, Hogervorst FB, Imholz AL, Wesseling J, Rodenhuis S, Nederlof PM. Triple-negative breast cancer: BRCAness and concordance of clinical features with BRCA1-mutation carriers. *British journal of cancer* 2013; **108**(10): 2172-2177.
 16. Turner N, Tutt A, Ashworth A. Hallmarks of BRCAness' in sporadic cancers. *Nature reviews cancer* 2004; **4**(10): 814-819.
 17. Foulkes WD, Stefansson IM, Chappuis PO, Bégin LR, Goffin JR, Wong N, Trudel M, Akslen LA. Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. *Journal of the national cancer institute* 2003; **95**(19):1482-1485.
 18. Zhang L, Fang C, Xu X, Li A, Cai Q, Long X. Androgen receptor, EGFR, and BRCA1 as biomarkers in triple-negative breast cancer: a meta-analysis. *Biomed research international* 2015; **2015**: 357485.
 19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods* 2001; **25**(4): 402-408.
 20. Toyama T, Yamashita H, Kondo N, Okuda K, Takahashi S, Sasaki H, Sugiura H, Iwase H, Fujii Y. Frequently increased epidermal growth factor receptor (EGFR) copy numbers and decreased BRCA1 mRNA expression in Japanese triple-negative breast cancers. *BMC cancer* 2008; **8**: 309.
 21. Birgisdottir V, Stefansson OA, Bodvarsdottir SK, Hilmarsdottir H, Jonasson JG, Eyfjord JE. Epigenetic silencing and deletion of the BRCA1 gene in sporadic breast cancer. *Breast cancer research* 2006; **8**(4): R38.
 22. Ben Gacem R, Hachana M, Ziadi S, Amara K, Ksia F, Mokni M, Trimeche M. Contribution of epigenetic alteration of BRCA1 and BRCA2 genes in breast carcinomas in Tunisian patients. *Cancer epidemiology* 2012; **36**(2): 190-197.
 23. Rakha EA, El-Sheikh SE, Kandil MA, El-Sayed ME, Green AR, Ellis IO. Expression of BRCA1 protein in breast cancer and its prognostic significance. *Human pathology* 2008; **39**(6): 857-865.
 24. Hedau S, Batra M, Singh UR, Bharti AC, Ray A, Das BC. Expression of BRCA1 and BRCA2 proteins and their correlation with clinical staging in breast cancer. *Journal of cancer research and therapeutics* 2015; **11**(1): 158-163.
 25. Anquer Y, Mandelbrot L, Lehy T, Salomon L, Dhainaut C, Madelenat P, Feldmann G, Walker F. Expression of BRCA1, HER-1 (EGFR) and HER-2 in sporadic breast cancer and relationships to other clinicopathological prognostic features. *Anticancer research* 2005; **25**(6C): 4535-4541.
 26. Egawa C, Miyoshi Y, Taguchi T, Tamaki Y, Noguchi S. Quantitative analysis of BRCA1 and BRCA2 mRNA expression in sporadic breast carcinomas and its relationship with clinicopathological characteristics. *Japanese journal of cancer research* 2001; **92**(6): 624-630.
 27. Matros E, Wang ZC, Lodeiro G, Miron A, Iglehart JD, Richardson AL. BRCA1 promoter methylation in sporadic breast tumors: relationship to gene expression profiles. *Breast cancer research and treatment* 2005; **91**(2): 179-186.
 28. Alkam Y, Mitomi H, Nakai K, Himuro T, Saito T, Takahashi M, Atsushi Arakawa, Yao T, Saito M. Protein expression and methylation of DNA repair genes hMLH1, hMSH2, MGMT and BRCA1 and their correlation with clinicopathological parameters and prognosis in basal-like breast cancer. *Histopathology* 2013; **63**(5): 713-725.