The Correlation of BRCA 1 Promoter Methylation and Clinicopathological Appearance in Breast Cancer

by Upik Miskad

FILE	HYLATION_AND_CLINICOPATHOLOGICAL_APPEARANCE_IN_BREAST_CANCER.PDF (162.19K)		
TIME SUBMITTED	15-JAN-2021 07:43AM (UTC+0700)	WORD COUNT	3810
SUBMISSION ID	1487774890	CHARACTER COUNT	20669

International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

The Correlation of BRCA 1 Promoter Methylation and Clinicopathological Appearance in Breast Cancer

Sony Sugiharto[a*], Muh. Nasrum Massi[b], Syariffudin Wahid[c], Andi Fachruddin Benyamin[d], Nurjati Chaerani Siregar[e], Upik Anderiani Miskad[f], William Hamdani[g], Mochammad Hatta[h], Ilhamjaya Patellongi[i], Rosdiana Natsir[j]

[a]*Postgraduate School,Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[a]*Anatomic Pathology Department, Faculty of Medicine,Tarumanagara University, Jakarta, Indonesia*
[b,h]*Microbiology Departement, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[c,f]*Anatomic Pathology Department, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[d]*Internal Medicine Department Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[e]*Anatomic Pathology Department, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia*
[g]*Surgical Oncology Department, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[i]*Physiology Department, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
[j]*Biochemistry Department, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*
Email: marias@fk.untar.ac.id. Telp:+62-8568807737 Fax +62-215663126

Abstract

Breast cancer is one of the common cancers in the worldwide. Most of breast cancer are sporadic. BRCA1 expression levels are reduced or totally loss in sporadic breast cancer. BRCA1 promoter methylation as one of the mechanisms to inactivating its function. BRCA1 promoter methylation associated with triple negative breast cancer (TNBC), poor prognosis, high grade, negativity of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2(HER2). This study aims are to examine prevalence and correlation between BRCA1 promoter methylation and clinicopathology appearance in Indonesian women breast cancer. Subject are women with primary breast cancer and their formalin-fixed paraffin -embedded (FFPE) tumor specimen retrieved.

* Corresponding author.

DNA was isolated and subjected to methylation specific PCR(MSPCR). DNA was isolated from primary tumor of 113 samples. Median age at diagnosis was 48 years (with range 28-80 years). Most of them, 67(59,3%) are include in aged categories ≤50 years. Incidence BRCA promoter methylation was found 82,3% (93 of 113.) There is significant correlation with BRCA1 promoter methylation with age<50 years old (p-value = 0.038) and Luminal B subtype (p-value = 0.033). Conclusion: BRCA1 promoter methylation in Indonesian women higher than other nations, correlate with younger patient and Luminal B subtype.

Keywords: Breast cancer; BRCA1 promoter methylation; prognosis.

1. Introduction

Breast cancer is one of the common cancers in the worldwide. Globocan 2012 reported 1.67 million new cases of breast cancer are found [1]. Breast cancer is the most common cancer in Indonesia with incidence 18.6 patient per 100,000 people [2]. Germline mutations in BRCA1 approximately 5-10% in breast cancer, and the others were sporadic breast cancer [3,4]. BRCA1 expression levels are reduced or totally loss in sporadic breast cancer. Some mechanism could decreased BRCA expression, one of them is hypermethylation of BRCA1 promoter [4, 5]. Normally BRCA1 promoter is hypomethylation, but aberrant addition of methyl groups in CpG island inhibit transcription factor that regulate the BRCA1 promoter to production of this tumor suppressor protein, and thus their loss or gain of function [4]. Many research's had done to prove role of BRCA1 methylation in development sporadic breast cancer, as predictor prognosis and therapy [6–9]. BRCA1 promoter methylation is observed in 13-59,2% of sporadic breast cancer [6, 7,10–15]. Generally BRCA1 promoter methylation associated with triple negative breast cancer (TNBC), poor prognosis, high grade, negativity of estrogen receptor(ER), progesterone receptor (PR) and human epidermal growth factor receptor 2(HER2)[9, 11, 12]. This study aims are to examine prevalence and correlation between BRCA1 promoter methylation and clinicopathology appearance in Indonesian women breast cancer.

2. Materials and Method

2.1 Collection of samples

This is an analytical study using retrospective cohort to assess the correlation between clinicopathological appearance and BRCA1 promoter methylation on women who suffered primary breast cancer. This research was conducted in HUMRC (Hassanuddin University Medical Research Center) laboratory, Makassar, South Sulawesi.

The study subject was all women suffered from primary breast cancer who underwent operation in MRCCC Siloam Hospital Semanggi, Jakarta during 2011-2015 and never got neoadjuvant chemotherapy before surgery. Inclusion criteria were the patient who have good formalin-fixed paraffin -embedded (FFPE) block, had been examined ER, PR, HER 2 and Ki67 immunohistochemistry staining, and have good medical record. Follow up had done until March 27th2018. Exclusion criteria was patient who have bilateral breast cancer. We performed clinicopathological data collection which involved age, histology type, grading, staging, ER, PR, HER2, Ki67 and metastatic. For ER and PR, if we found nuclei staining ≥1% are positive and < 1% are negative [16]. HER2

positive , if > 10% tumor cells showed score +3 (membrane staining strong and complete) and negative if score 0, +1 and +2 but no showed amplification in fluorescent insitu hybridization(FISH)[17]. Ki-67 is negative if there is <14% of nuclei staining and positive if ≥14%. Based on immunohistochemistry staining we categorized molecular subtype according St Gallen consensus [18].

2.2 BRCA1 promoter methylation

Tumor dense areas of FFPE tissue sections were manually dissected for 4 sections, each with a thickness 10 µm and genomic DNA (gDNA) was isolated by QIAamp® DNA FFPE Tissue Kit (Qiagen), and bisulfite converted using the EpiTect® Bisulfite Kit (Qiagen). Purified converted DNA was subjected to methylation-specific PCR (MSPCR) using the EpiTect® MSP Kit (Qiagen). The unmethylated template primers were (forward) TTGGTTTTTGTGGTAATGGAAAAGTGT and (reverse) CAAAAAATCTCAACAAACTCACACCA, resulting in an 86 base pair PCR product. The methylated template primers were (forward) TCGTGGTAACGGAAAAGCGC and (reverse) AAATCTCAACGAACTCACGCCG, resulting in a 75 base pair PCR product. These primers have been extensively characterized by previous group[12]. PCR conditions were as follows: 95.0°C for 10 minutes, then 35 cycles of 94.0°C for 15 seconds, 55.0°C for 30 seconds, 72.0°C for 30 seconds, and a final extension at 72.0°C for 10 minutes. PCR products were electrophoresed on a 2.5% agarose gel stained with ethidium bromide and visualized on a UVP Bioimaging system. Specificity of the reactions was confirmed using the EpiTect® Control DNA set (Qiagen) with the same primers and PCR conditions. The presence of a methylated band was recorded as "positive" for BRCA1 promoter methylation. (Figure 1)

2.3 Data analysis

Statistical analysis was performed using SPSS 22.0 software. Analysis of patient's characteristics and BRCA1 promoter methylation using Pearson chi square and Fischer exact.

2.4 Ethical Clearance

Ethical approval for this study was obtained from Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia with a number: 390 / H4.8.4.5.31 / PP36/KOMETIK / 2016.

3. Result

During the study period from March 2016 until March 2018, we collected breast cancer patient's data, and found 113 sample who met the inclusion-exclusion criteria.

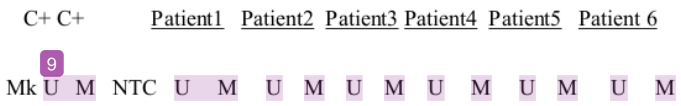
3.1 Patient Characteristic

The clinicopathology characteristic summarized in Table 1. In this study the median age at diagnosis was 48 years old (with range 28-80 years old). Most of them, 67(59,3%) are include in aged categories ≤50 years. Most of histology type are invasive ductal carcinoma 105 (92,9%). Staging was dominated by stage II, 53 (46,9%)

were followed by stage III as many as 49 (43,4%). Grading was dominated by grade III, 60 (53,1%) were followed by grade II as many as 45 cases (39,8%). From immunohistochemistry staining the majority cases showed ER positive 75 (66,4%), PR positive 68 (60,2%), HER2 negative 78 (69%) and Ki 67 positive 64(56,6%). From molecular subtype we found Luminal B 47(41,6%), Luminal A 31(27,4%), HER 2(17,7%) and TNBC (13,3%). In follow-up 40 patient (35,4%) showed metastatic local, regional or distant metastatic.

Table 1: Patient characteristic

Characteristic	Total (%)
Age	
≤50	67 (59,3%)
>50	46 (40,7%)
Histology type	
Invasive ductal carcinoma	105 (92,9%)
Invasive lobular carcinoma	3(2,7%)
Mucinous carcinoma	2(1,8%)
Papillary carcinoma	1(0,9%)
Medullary carcinoma	2(1,8%)
Stage	
I	11(9,7%)
II	53(46,9%)
III	49(43,4%)
Grade	
I	8 (7,1%)
II	45(39,8%)
III	60(53,1%)
ER	
Negative	38(33,6%)
Positive	75(66,4%)
PR	
Negative	45(39,8%)
Positive	68(60,2%)
HER2	
Negative	78(69,0%)
Positive	35(31,0%)
Ki67	
Negative	49(43,4%)
Positive	64(56,6%)
Molecular subtype	
Luminal A	31(27,4%)
Luminal B	47(41,6%)
Her 2	20(17,7%)
TNBC	15(13,3%)
Metastatis	
Negative	73(64,4%)
Positive	40(35,4%)



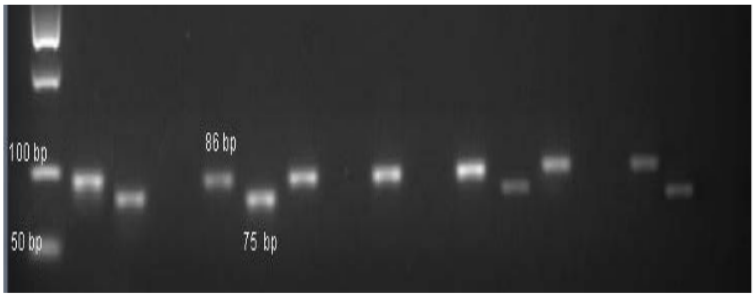


Figure 1: BRCA1 promoter methylation.

DNA methylation status of the BRCA1 promoter determined by MSPCR. U-labeled lanes represent PCR products amplified with unmethylated primers (86 bp). M-labeled lanes represent PCR products amplified with methylated primers (75 bp). Patients 1, 4, and 6 shows the presence of a PCR product in both reactions, indicating BRCA1 methylated (positive).

Patients 2, 3 dan 5 only shows unmethylated reaction(negative). Mk: Molecular weight marker used is a 100-bp ladder. C+U: positive control unmethylated. C+M: positive control methylated. NTC: no template control.

3.2 Correlation between characteristic patient and BRCA1 promoter methylation

BRCA1 promoter methylation was detected in 93 (82,3%) of the 113 samples, almost samples were positive for both unmethylated and methylated reactions. Only one sample (1,1%) were positive only for the methylated reaction.

The patient in categories aged ≤ 50 years have significant correlation with methylated BRCA1 promoter (p-value 0,038).

The significant correlation also showed by Luminal B subtype (p-value 0,033) There is no significant correlation between BRCA1 promoter methylation and histology type, staging, grading, ER, PR, HER2, Ki67 and metastatic.

3.3 Correlation between subtype molecular and BRCA1 promoter methylation in metastatic patient

From 113 patient we found metastatic in 40 patient. Most of the metastatic patient showed BRCA1 promoter methylation 35(87,5%). Most of the metastatic patient showed Luminal B subtype 18(45%).

Despite no correlation significance between molecular subtype and BRCA1 promoter methylation (p-value 0,330), we found all of HER 2 and TNBC subtype showed Methylated BRCA1 promoter, and none of them showed Unmethylated BRCA1 promoter.

Table 2: Correlation between characteristic patient and BRCA1 promoter methylation

Characteristic	Total	BRCA1 promoter methylation		P-Value
		Unmethylated	Methylated	
		N (%)	N (%)	
N	113(100%)	20 (17,7%)	93 (82,3%)	
Age at diagnosis				
≤50	67(59,3%)	16(80%)	51(55%)	0,038*
>50	46(40,7%)	4(20%)	42(45%)	
Morphol				
Invasive ductal carcinoma	105(92,9%)	20(100%)	85(91%)	0,199**
Others	8(7,1%)	0 (0%)	8(9%)	
Stage				
I	11(9,7%)	1(5%)	10(11%)	0,662*
II	53(46,9%)	9(45%)	44(47%)	
III	49(43,4%)	10(50%)	39(42%)	
de				
I	8 (7,1%)	0 (0%)	8(9%)	0,378*
II	45(39,8%)	9(45%)	36(39%)	
III	60(53,1%)	11(55%)	49(53%)	
ER				
Negative	38(33,6%)	5(25%)	33(35,5%)	0,523*
Positive	75(66,4%)	15(75%)	60(64,5%)	
PR				
Negative	45(39,8%)	5(25%)	40(43%)	0,215*
Positive	68(60,2%)	15(75%)	53(57%)	
HER2				
Negative	78(69,0%)	14(70%)	64(69%)	1,000*
Positive	35(31,0%)	6(30%)	29(31%)	
Ki67				
≤14	49(43,4%)	5(25%)	4(47,3%)	0,115*
	64(56,6%)	15(75%)	49(52,7%)	
Molecular subtype				
Luminal A	31(27,4%)	2(10%)	29(31,2%)	0,033*
Luminal B	47(41,6%)	14(70%)	33(35,5%)	
Her 2	20(17,7%)	3(15%)	17(18,3%)	
TNBC	15(13,3%)	1(5%)	14(15,0%)	
Metastasis				
Negative	73(64,4%)	15(75%)	58(62,4%)	0,210**
Positive	40(35,4%)	5(25%)	35(37,6%)	

*Pearson chi square ** Fisher exact test

Tabel 3: Correlation between subtype molecular and BRCA1 promoter methylation in metastatic patient

Subtype molecular		BRCA1 promoter methylation		p-value
		Unmethylated	Methylated	
Luminal A	9(22,5%)	1(20%)	8 (22,86%)	0,330
Luminal B	18(45%)	4(80%)	14(40%)	
HER 2	7(17,5%)	0(0%)	7(20%)	
TNBC	6(15%)	0(0%)	6(17,14%)	

4. Discussion

Breast cancer is the most cancer in the Indonesian women. Increasing incidence of breast cancer in developing country such as Indonesia is caused by improvement socioeconomic that made lifestyle changes including dietary changing, obesity, lower physical activity, alcohol, smoking, delayed childbearing, and using hormonal contraceptive [19, 20]. BRCA1 promoter hypermethylation has been implicated as one of the mechanisms of loss of gene expression and has been identified in 13-59,2% of sporadic breast cancer [6, 7,10–15]. In this study we found hypermethylation of BRCA1 is present in 82,3% (92 of 113, that is significantly higher than previously report. This result is consistent with Nindrea and his colleagues that found strong correlation between BRCA1 promoter hypermethylation and breast cancer in Asia [21]. Some factors may account for these differences. First, some research's report the incidence BRCA1 promoter hypermethylation correlated with invasive ductal carcinoma [14, 21] dan grade III [22]. Since most of our samples are invasive ductal carcinoma 82,3% (93 of 113) and high grade 53,1% (60 of 113), this finding may be comparable to that literature. Secondly, although most published studies mentioned above used MSP, the primer sequences, target regions and kind of sample (frozen tissue, FFPE block) varied from study to study [6, 7,10–15].

From this study we found significant correlation between BRCA1 promoter hypermethylation with patient age categories ≤ 50 tahun (55%). The same result also showed in Korean women [23] and African-American women[24]. In the other hand BRCA1 promoter hypermethylation correlated with older patient in Caucasian women. Globally breast cancer patient < 50 years old usually found in less develop region like Africa (36,2 years old), Asia (29,1years old), South East Asia (34,8 years old), and older in American (67,6 years old), Europe (71,1 years old) and Australia (85,8 years old)[1] This difference possibly is caused by lifestyle factors, diet pattern, or the existence of certain gene which is related to race so that the difference in age occurred. This study also observed significant correlation between BRCA1 promoter hypermethylation with Luminal B molecular subtype. Our result is different from some studies that found correlation between BRCA1 promoter hypermethylation with TNBC[7,10, 14] and Luminal A [25]. Luminal B subtype exhibit low expression of ER or PR, variable expression of, and high expression of Ki67. Luminal B correlate with younger age group, high grade and nodal metastasis [26]. Despite Luminal B patient can be treat with hormonal therapy and target therapy (Anti Her2) beside chemotherapy, they have residual risk [27] and the risk of recurrence and metastasis higher than non-luminal [28]. Patients non TNBC who had BRCA1 promoter methylation had significantly worse disease-free survival than patients with non-methylated BRCA1 promoters [11]. In this study, most of the metastatic patient showed BRCA1 promoter methylation 35(87,5%), and most of them showed Luminal B subtype 18(45%) despite not statistically significant. Until now ER, PR, HER2, Ki 67, grade, and lymph node involvement used as marker to predict prognosis [29]. Because of BRCA1 promoter methylation can correlate with any molecular subtype and poor prognosis we suggest it as a predictor marker.

5. Conclusion

Incidence BRCA1 promoter methylation in Indonesian women breast cancer is higher than the other nations. BRCA1 promoter methylation correlate with younger age (< 50 years old) and Luminal B subtype.

6. Recommendation

It is recommend to use BRCA1 promoter methylation as a predictor marker of a poor prognosis especially in Luminal B subtype.

Acknowledgments

We give our gratitude to Siloam Hospital Semanggi staffs and HUMRC staffs that have supported this study.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare

References

[1] J. Ferlay et al., "Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012," Int. J. Cancer, vol. 136, no. 5, pp. E359-386, Mar. 2015.

[2] M. Wahidin, R. Noviani, S. Hermawan, V. Andriani, A. Ardian, and H. Djarir, "Population-Based Cancer Registration in Indonesia," Asian Pac. J. Cancer Prev., vol. 13, no. 4, pp. 1709–1710, Apr. 2012.

[3] R. D. Kennedy, J. E. Quinn, P. B. Mullan, P. G. Johnston, and D. P. Harkin, "The Role of BRCA1 in the Cellular Response to Chemotherapy," JNCI J. Natl. Cancer Inst., vol. 96, no. 22, pp. 1659–1668, Nov. 2004.

[4] C. R. Mueller and C. D. Roskelley, "Regulation of BRCA1 expression and its relationship to sporadic breast cancer," Breast Cancer Res., vol. 5, no. 1, pp. 45–52, 2003.

[5] A. Catteau and J. R. Morris, "BRCA1 methylation: a significant role in tumour development?," Semin. Cancer Biol., vol. 12, no. 5, pp. 359–371, Oct. 2002.

[6] M. Esteller et al., "Promoter Hypermethylation and BRCA1 Inactivation in Sporadic Breast and Ovarian Tumors," JNCI J. Natl. Cancer Inst., vol. 92, no. 7, pp. 564–569, Apr. 2000.

[7] O. A. Stefansson et al., "CpG island hypermethylation of BRCA1 and loss of pRb as co-occurring events in basal/triple-negative breast cancer," Epigenetics, vol. 6, no. 5, pp. 638–649, May 2011.

[8] A. M. Dworkin, T. H.-M. Huang, and A. E. Toland, "Epigenetic alterations in the breast: Implications for breast cancer detection, prognosis and treatment," Semin. Cancer Biol., vol. 19, no. 3, pp. 165–171, Jun. 2009.

[9] L. Zhang and X. Long, "Association of BRCA1 promoter methylation with sporadic breast cancers:

Evidence from 40 studies," Sci. Rep., vol. 5, p. 17869, Dec. 2015.

[10] A. Bal et al., "BRCA1-methylated sporadic breast cancers are BRCA-like in showing a basal phenotype and absence of ER expression," Virchows Arch. Int. J. Pathol., vol. 461, no. 3, pp. 305–312, Sep. 2012.

[11] Y. Xu et al., "Promoter methylation of BRCA1 in triple-negative breast cancer predicts sensitivity to adjuvant chemotherapy," Ann. Oncol., vol. 24, no. 6, pp. 1498–1505, Jun. 2013.

[12] P. Sharma et al., "The prognostic value of BRCA1 promoter methylation in early stage triple negative breast cancer," J. Cancer Ther. Res., vol. 3, no. 2, pp. 1–11, Mar. 2014.

[13] E. Honrado et al., "Immunohistochemical classification of non-BRCA1/2 tumors identifies different groups that demonstrate the heterogeneity of BRCAX families," Mod. Pathol. Off. J. U. S. Can. Acad. Pathol. Inc, vol. 20, no. 12, pp. 1298–1306, Dec. 2007.

[14] N. C. Hsu, Y.-F. Huang, K. K. Yokoyama, P.-Y. Chu, F.-M. Chen, and M.-F. Hou, "Methylation of BRCA1 promoter region is associated with unfavorable prognosis in women with early-stage breast cancer," PloS One, vol. 8, no. 2, p. e56256, 2013.

[15] X. Xu et al., "BRCA1 Promoter Methylation is Associated with Increased Mortality among Women with Breast Cancer," Breast Cancer Res. Treat., vol. 115, no. 2, pp. 397–404, May 2009.

[16] M. E. H. Hammond et al., "American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer," J. Clin. Oncol., vol. 28, no. 16, pp. 2784–2795, Jun. 2010.

[17] A. C. Wolff et al., "Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update," J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol., vol. 31, no. 31, pp. 3997–4013, Nov. 2013.

[18] A. Goldhirsch, W. C. Wood, A. S. Coates, R. D. Gelber, B. Thürlimann, and H.-J. Senn, "Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011," Ann. Oncol., vol. 22, no. 8, pp. 1736–1747, Aug. 2011.

[19] P. Porter, "'Westernizing' Women's Risks? Breast Cancer in Lower-Income Countries," N. Engl. J. Med., vol. 358, no. 3, pp. 213–216, Jan. 2008.

[20] S. Solikhah, S. Promthet, N. Rakkapao, and C. P. Hurst, "Validation of an Indonesian Version of the Breast Cancer Awareness Scale (BCAS-I)," Asian Pac. J. Cancer Prev. APJCP, vol. 18, no. 2, pp. 515–

522, 2017.

[21] R. D. Nindrea, W. A. Harahap, T. Aryandono, and L. Lazuardi, "Association of BRCA1 Promoter Methylation with Breast Cancer in Asia: A Meta- Analysis," Asian Pac. J. Cancer Prev. APJCP, vol. 19, no. 4, pp. 885–889, 2018.

[22] V. Birgisdottir, O. A. Stefansson, S. K. Bodvarsdottir, H. Hilmarsdottir, J. G. Jonasson, and J. E. Eyfjord, "Epigenetic silencing and deletion of the BRCA1 gene in sporadic breast cancer," Breast Cancer Res., vol. 8, no. 4, p. R38, 2006.

[23] J. S. Lee et al., "A comparative study of Korean with Caucasian breast cancer reveals frequency of methylation in multiple genes correlates with breast cancer in young, ER, PR-negative breast cancer in Korean women," Cancer Biol. Ther., vol. 6, no. 7, pp. 1114–1120, Jul. 2007.

[24] J. Mehrotra et al., "Estrogen receptor/progesterone receptor-negative breast cancers of young African-American women have a higher frequency of methylation of multiple genes than those of Caucasian women," Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res., vol. 10, no. 6, pp. 2052–2057, Mar. 2004.

[25] M. M. Hosny, N. A. Sabek, T. B. El-Abaseri, F. M. Hassan, and S. H. Farrag, "Promoter Methylation Status of Breast Cancer Susceptibility Gene 1 and 17 Beta Hydroxysteroid Dehydrogenase Type 1 Gene in Sporadic Breast Cancer Patients," International Journal of Breast Cancer, 2016. [Online]. Available: https://www.hindawi.com/journals/ijbc/2016/9545241/. [Accessed: 27-Jul-2018].

[26] A. A. Hashmi et al., "Prognostic parameters of luminal A and luminal B intrinsic breast cancer subtypes of Pakistani patients," World J. Surg. Oncol., vol. 16, Jan. 2018.

[27] L. A. Carey, "Directed Therapy of Subtypes of Triple-Negative Breast Cancer," The Oncologist, vol. 16, no. Supplement 1, pp. 71–78, Jan. 2011.

[28] Z. Li, P. Hu, J. Tu, and N. Yu, "Luminal B breast cancer: patterns of recurrence and clinical outcome," Oncotarget, vol. 7, no. 40, pp. 65024–65033, Aug. 2016.

[29] Prihantono, P., Hatta, M., Binekada, C., Sampepajung, D., Islam AA, and Nilawati Usman, A. Ki-67 expression by immunohistochemistry and quantitative real-time polymerase chain reaction as predictor of clinical response to neoadjuvant chemotherapy in locally advanced breast cancer. J Oncol. 2017: 6209849. doi: 10.1155/2017/6209849

The Correlation of BRCA 1 Promoter Methylation and Clinicopathological Appearance in Breast Cancer

ORIGINALITY REPORT

%24	%11	%21	%7
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Hsu, Nicholas C., Ya-Fang Huang, Kazunari K. Yokoyama, Pei-Yi Chu, Fang-Ming Chen, and Ming-Feng Hou. "Methylation of BRCA1 Promoter Region Is Associated with Unfavorable Prognosis in Women with Early-Stage Breast Cancer", PLoS ONE, 2013. Publication	%3
2	Submitted to University of Hull Student Paper	%3
3	www.sci-int.com Internet Source	%1
4	R. Ben Gacem, M. Hachana, S. Ziadi, K. Amara, F. Ksia, M. Mokni, M. Trimeche. "Contribution of epigenetic alteration of BRCA1 and BRCA2 genes in breast carcinomas in Tunisian patients", Cancer Epidemiology, 2012 Publication	%1
5	www.hindawi.com Internet Source	%1

6	Robert W Mutter, Nadeem Riaz, Charlotte KY Ng, Rob Delsite et al. "Bi-allelic alterations in DNA repair genes underpin homologous recombination DNA repair defects in breast cancer", The Journal of Pathology, 2017 Publication	%1
7	Li Zhang, Xinghua Long. "Association of BRCA1 promoter methylation with sporadic breast cancers: Evidence from 40 studies", Scientific Reports, 2015 Publication	%1
8	Xinran Xu. "BRCA1 promoter methylation is associated with increased mortality among women with breast cancer", Breast Cancer Research and Treatment, 06/03/2008 Publication	%1
9	webio.hu Internet Source	%1
10	Ahn, S.J.. "Overexpression of @bPix-a in human breast cancer tissues", Cancer Letters, 20030410 Publication	%1
11	worldwidescience.org Internet Source	%1
12	X. Zhu, L. Shan, F. Wang, J. Wang, F. Wang, G. Shen, X. Liu, B. Wang, Y. Yuan, J. Ying, H.	%1

Yang. "Hypermethylation of BRCA1 gene: implication for prognostic biomarker and therapeutic target in sporadic primary triple-negative breast cancer", Breast Cancer Research and Treatment, 2015
Publication

13	Submitted to Salem State University Student Paper	%1
14	www.oncotarget.com Internet Source	%1
15	wjso.biomedcentral.com Internet Source	%1
16	T. Ignatov, H. Eggemann, S. D. Costa, A. Roessner, T. Kalinski, A. Ignatov. "BRCA1 promoter methylation is a marker of better response to platinum–taxane-based therapy in sporadic epithelial ovarian cancer", Journal of Cancer Research and Clinical Oncology, 2014 Publication	%1
17	T. Ignatov, A. Poehlmann, A. Ignatov, A. Schinlauer, S. D. Costa, A. Roessner, T. Kalinski, J. Bischoff. "BRCA1 promoter methylation is a marker of better response to anthracycline-based therapy in sporadic TNBC", Breast Cancer Research and Treatment, 2013 Publication	%1

18	An Sen Tan, Joe Poe Sheng Yeong, Chi Peng Timothy Lai, Chong Hui Clara Ong et al. "The role of Ki-67 in Asian triple negative breast cancers: a novel combinatory panel approach", Virchows Archiv, 2019 Publication	<%1
19	tessera.spandidos-publications.com Internet Source	<%1
20	Zoran Gatalica, Rebecca Feldman, Semir Vranić, David Spetzler. "Chapter 4 Immunohistochemistry-Enabled Precision Medicine", Springer Science and Business Media LLC, 2019 Publication	<%1
21	cest.gnest.org Internet Source	<%1
22	P. Sharma, W.E. Barlow, A.K. Godwin, H. Pathak et al. "Impact of homologous recombination deficiency biomarkers on outcomes in patients with triple-negative breast cancer treated with adjuvant doxorubicin and cyclophosphamide (SWOG S9313)", Annals of Oncology, 2018 Publication	<%1
23	pesquisa.bvsalud.org Internet Source	<%1

24	Methods in Molecular Biology, 2015. Publication	<%1
25	Feng-Feng Cai, Su Chen, Ming-Hong Wang, Xiao-Yan Lin et al. "Pyrosequencing quantified methylation level of BRCA1 promoter as prognostic factor for survival in breast cancer patient", Oncotarget, 2016 Publication	<%1
26	Karsli-Ceppioglu, Seher, Aslihan Dagdemir, Gaëlle Judes, Marjolaine Ngollo, Frédérique Penault-Llorca, Amaury Pajon, Yves-Jean Bignon, and Dominique Bernard-Gallon. "Epigenetic mechanisms of breast cancer: an update of the current knowledge", Epigenomics, 2014. Publication	<%1
27	www.jimmunol.org Internet Source	<%1
28	R. D. Kennedy. "The Role of BRCA1 in the Cellular Response to Chemotherapy", CancerSpectrum Knowledge Environment, 11/17/2004 Publication	<%1
29	eprints.uwe.ac.uk Internet Source	<%1
30	www.sabcs.org	

	Internet Source	<%1
31	dlisv03.media.osaka-cu.ac.jp Internet Source	<%1
32	Paula Rocha Moreira, Mariana Moreira Guimarães, Carolina Cavaliéri Gomes, Marina Gonçalves Diniz et al. "Methylation frequencies of cell-cycle associated genes in epithelial odontogenic tumours", Archives of Oral Biology, 2009 Publication	<%1
33	biolres.biomedcentral.com Internet Source	<%1
34	Parisa Paydar, Gholamreza Asadikaram, Hamid Zeynali Nejad, Hamed Akbari et al. " Epigenetic modulation of and genes, and histones H4 and H3 are associated with breast tumors ", Journal of Cellular Biochemistry, 2019 Publication	<%1
35	Otani, Yoko, Tomohiro Miyake, Naofumi Kagara, Masafumi Shimoda, Yasuto Naoi, Naomi Maruyama, Atsuhi Shimomura, Kenzo Shimazu, Seung Jin Kim, and Shinzaburo Noguchi. "BRCA1 Promoter Methylation of Normal Breast Epithelial Cells as a Possible Precursor for BRCA1 -Methylated Breast Cancer", Cancer	<%1

Science, 2014.
Publication

36	Jong, Y.J.. "Chromosomal comparative genomic hybridization abnormalities in early- and late-onset human breast cancers: correlation with disease progression and TP53 mutations", Cancer Genetics and Cytogenetics, 20040101 Publication	<%1
37	"Breast", Laboratory Investigation, 2010 Publication	<%1

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	< 5 WORDS
EXCLUDE BIBLIOGRAPHY	ON		