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Expression of PARP1 Gene in Breast Cancer Patients

Mustafa ULASLI[1], Serdar GURSES[2], Beyhan CENGIZ[3], Serdar OZTUZCU[1], Ozan BALAKAN[4], Ali SUNER[4], Bulent GOGEBAKAN[5], Mehri IGCI[1], Ahmet BALIK[6], Ahmet ARSLAN[1], Celalettin CAMCI[4]

[1] Gaziantep University Faculty of Medicine, Department of Medical Biology, Gaziantep
[2] Zirve University Emine Bahaeddin Nakıboğlu Faculty of Medicine, Department of Medical Biology, Gaziantep
[3] Gaziantep University Faculty of Medicine, Department of Physiology, Gaziantep
[4] Gaziantep University Faculty of Medicine, Department of Medical Oncology, Gaziantep
[5] Mustafa kemal University Faculty of Medicine Department of Medical Biology, Hatay
[6] Gaziantep University Faculty of Medicine, Department of General Surgery, Gaziantep, TURKEY

ABSTRACT

Poly (ADP-ribose) polymerase (PARP) family of enzymes is part of the DNA repair mechanism. One of these enzymes, PARP-1 is involved in detection of signal single-strand DNA breaks (SSBs) that leads to base excision repair (BER) mechanism. Breast cancer tumors that lack Breast cancer susceptibility gene 1 (BRCA1) and Breast cancer susceptibility gene 2 (BRCA2) are ineffective in DNA double-strand breaks (DSB) repair. Activity of the poly (ADP-ribose) polymerase (PARP) enzymes in these tumors is of interest as a lack of PARP activity leads to accumulation of SSBs that are converted to DSBs and accumulation of DSBs lead to irreparable DNA damage and cell death. Therefore inhibition of PARP in tumor cells might be effective in killing cancer tumors and activity of PARP inhibitors in selectively killing breast cancer tumors is currently being evaluated. In this study, expression of PARP-1 and cancer markers estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) were determined in a group of breast cancer patients to assess the potential for using PARP inhibitors against this form of cancer. Expression of PARP-1 was found not to correlate with the onset of breast cancer in the patients.

Keywords: Breast cancer, PARP1, Real time, qPCR

ÖZET

Meme Kanserli Hastalarda HastalardaPARP1 Gen Ekspresyonu

Poli (ADP riboz) polimeraz (PARP), DNA onarım mekanizmasında yer alan bir enzim ailesidir. Bu enzimlerden PARP1, baz kesip-çıkarma onarım (BER) mekanizmasına öncülük eden tek zincir DNA kırıklarının saptanmasında rol alır. BRCA1 ve BRCA2 genlerinden yoksun meme kanseri tümörleri, DNA çift zincir kırıklarının onarımında yetersizdirler. PARP enzimlerinin bu tümörlerdeki aktivitesi ilgi konusudur çünkü PARP aktivitesinin kaybı, tek zincir DNA kırıklarının birikimine, biriken tek zincir DNA kırıklarının çift zincir DNA kırıklarına dönüşmesine ve takiben tamir edilemeyen DNA hasarı ve hücre ölümüne yol açmaktadır. Bu yüzden PARP inhibisyonunun, kanser hücrelerini öldürmede etkili olabileceği düşünülmekte ve PARP inhibitörlerinin seçici olarak meme kanseri tümörlerini öldürmedeki etkinliği araştırılmaktadır. Bu çalışmada, PARP inhibitörlerinin meme kanserine karşı kullanılma potansiyelinin değerlendirilmesi için bir grup meme kanserli hastada, PARP-1 gen ifade düzeyi ve kanser markırlarından östrojen reseptörü (ER), progesteron reseptörü (PR) ve insan epidermal büyüme faktör reseptörü 2 (HER2)'nin gen ifade düzeyleri belirlenmiştir. Hastalarda PARP-1 gen ifade düzeyinin meme kanseri oluşumu ile ilişkili olmadığı gösterilmiştir.

Anahtar Kelimeler: Meme kanseri, PARP1, Gerçek zamanlı, qPCR

INTRODUCTION

The poly (ADP-ribose) polymerase (PARP) family consists of 17 members; PARP-1, PARP-2, PARP-3, PARP-4 (Vault-PARP), PARP-5 (Tankyrases-1 and 2), PARP-6, PARP-7 (tiPARP), PARP-8, PARP-9 (BAL1), PARP-10, PARP-11, PARP-12, PARP-13 (ZAP), PARP-14 (CoaSt6), PARP-15, and PARP-16.[1,2] PARP enzymes have different structures and play a serious role in the maintenance of DNA reliability as part of DNA repair mechanism.[3] PARP-1 is localized predominantly to nucleus and to some extent to mitochondria.[4] The nuclear enzyme PARP-1 plays important roles in a wide variety of processes in the nucleus such as; regulation of chromatin structure, transcription, and genomic integrity.[5] The main role of PARP-1 is to detect and signal SSB to the enzymatic machinery involved in the SSB repair. If any damage occurs on DNA (base pair-excised), PARP-1 is triggered by DNA breaks and cleaves nicotinamide adenine dinucleotide (NAD+) generating nicotinamide and ADP-ribose.[6] PARP-1 then adds ADP-ribose moieties (poly ADP ribosylation) to nuclear acceptor proteins adjacent to DNA breaks.[6-8] These negatively charged protein polymers recruit other proteins that are critical in base excision repair (BER)/SSBR.[9]

PARP-1 also activates transcription factors and upregulates the expression of pro-inflammatory genes. Therefore, PARP-1 plays important roles in the pathogenesis of many diseases, such as stroke, myocardial infarction, circulatory shock, diabetes, neurodegenerative disorders including Parkinson's and allergy, asthma, colitis, and other inflammatory disorders.[5,10,11]

Certain tumors were shown to have increased PARP-1 activity compared to normal cells.[12] Increased PARP-1 activity leads to low levels of Nicotinamide adenine dinucleotide (NAD+) and ATP, thus favoring a necrotic cell death rather than apoptotic. As PARP-1 is not required for survival of healthy cells, its inactivation can increase the effect of DNA-binding antitumor drugs and lead to apoptotic death of cancer cells and prevent inflammation.[13] Some PARP-1 inhibitors were shown to do such while some PARP inhibitors were able to kill tumor cells on their own.[14-16] Inactivation of PARP-1 leads to accumulation of SSB that are converted DSB that are mostly repaired by homologous recombination (HR). In breast cancer tumors that do not have functional BRCA1 or BRCA2, DNA repair via HR is not functional and DSBs accumulate. Accumulation of DSBs lead to irreparable DNA damage and cell death.[17] Use of PARP inhibitors in tumors that lacked BRCA1 and BRCA2 was shown to selectively kill tumor cells that led to clinical development of PARP inhibitors in breast cancer.[18,19]

Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) are important markers that are used in prognosis following hormonal and HER2 targeted treatment.[20-22] In close to 70% of breast cancer patients ER and PR are over expressed.[23] HER2 is overexpressed in 20% of breast cancer patients.[24] Therefore, for an effective treatment it is necessary to know the ER, PR and HER2 expression levels in breast cancer patients.

In this study we analyzed the expression of ER, PR, HER2 and PARP1 in a group of breast cancer patients to compare this group to the general population of breast cancer patients.

MATERIAL AND METHODS

Patients

Breast cancer patients were from the southeast region of Turkey with an average age 48 +/-. Patients had grade 1 (4 of them), grade 2 (26 of them), grade 3 (20 of them) or grade 4 (4 of them) cancer (Table 1 and 2).

RNA extraction from breast cancer patients

Normal and breast tumor samples that were obtained from breast cancer patients had been preserved between 2009 and 2011. Fresh-frozen tumor and normal breast samples were cut to 25 gr weight and homogenized by using homogenizer (Kinematica, Gmdh, Switzerland). Total RNA was then extracted using Qiagen miRNeasy kit (Valencia, CA) following manufacturer's protocols. Integrity of total RNA was monitored using NanoDrop (Thermo Scientific, Waltham, MA) and the quality was assessed by Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

Table 1. The demographic information of the patients

Age (median)		48 (24-78)
Premenapozal		44 (24-53)
Postmenapozal		61 (43-78)
Sex	Woman	59 (% 100)
Menopausal Status		
Premenopausal		35 (% 59.3)
Postmenopausal		24 (% 40.7)
Smoker	Yes	8 (% 13.6)
	No	33 (% 55.9)
	Unknown	18 (% 30.5)
Family history of cancer	Yes	7 (%11.9)
	No	29 (% 49.2)
	Unknown	23 (% 39)
History of diabetes mellitus		
Yes		5 (% 8.5)
No		38 (% 64.4)
Unknown		16 (% 27.1)
History of Hypertension	Yes	9 (% 15.3)
	No	34 (% 57.6)
	Unknown	16 (% 27.1)

Table 2. Histopathology of tumors

Histopathology	**Number (percentage)**
Invasive ductal cancer	47 (%79.7)
Invasive papillary	1 (%1.7)
Invasive lobular	4 (% 6.8)
Mixed	3 (% 5.1)
Malignant spindle cell tumor	1 (1.7)
Medullary carcinoma	1 (% 1.7)
High-grade DCIS (Ductal Carcinoma In Sutu)	1 (% 1.7)
Mucinous carcinoma	1 (% 1.7)
Extra-Nodal Invasion in patients With axillary nodal involvement	
Yes	12 (% 32.4)
No	37 (% 67.6)
Lymphatic Invasion Yes	12 (% 20.3)
No	47 (% 79.7)

Table 2. Histopathology of tumors (*continue*)

Extra-Nodal Invasion in Patients With Axillary Nodal Involvement		
Vascular Invasion	Yes	13 (% 22)
	No	46 (% 78)
Perineural Invasion	Yes	3 (% 5.1)
	No	56 (% 94.9)
Estrogen Receptors Status		
Positive		36 (% 61)
Negative		23 (% 39)
Unknown		0
Progesterone Receptor Status		
Positive		32 (% 54.2)
Negative		25 (% 42.4)
Unknown		2 (% 3.4)
Her-2 Status		
Positive		17 (% 28.8)
Negative		41 (% 69.5)
Unknown		1 (% 1.7)
Molecular Classification		
Luminal A		26 (% 44.1)
Luminal B		7 (% 11.9)
Over expression of Her-2		16 (% 27.1)
Basal like		9 (% 15.3)
Unknown		1 (% 1.7)
Distant Metastasis Status		
Yes		4 (% 6.8)
No		51 (% 86.4)
Unknown		4 (% 6.8)
The Number of Metastatic Axillary Lymph Nodes		
N0=No		18 (% 30.5)
N1=1-3		18 (% 28.8)
N2=4-9		13 (% 22)
N3 >10		6 (% 10.2)
Unknown		5 (% 8.5)
Grade	1	4 (% 6.8)
	2	26 (% 44.1)
	3	20 (% 33.9)
	4	4 (% 6.8)
	Unknown	5 (% 8.5)

mRNA reverse transcription

Total RNA (1 μg) was reverse transcribed using Qiagen miScript Reverse Transcription Kit (Valencia, CA). The 20 μl reverse transcription reaction contained 10 μl of total RNA, 0.2 μl of 100 nM dNTP, 0.2 μl of RNase inhibitor 20 U/μl, 1 μl of reverse transcriptase (50 U/μl), 8 μl of 11-or 96-plex reverse primer (mixed to allow a final concentration of 0.05 x of each) and 1.6 μl of H_2O. 2 μl of 5 ng of total RNA and 3 μl of 5X reverse primer were used in the single-plex RT reaction. All reagents were purchased from Applied Biosystems, Inc. (Foster City, CA). The reaction mixture was mixed with RNA and incubated as follows; 16^{0}C for 30 min, 42^{0}C for 30 min and then 85^{0}C for 5 min.

Real-time qPCR

Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR) was carried out using Rotor-Gene 600 (Corbett Research, Australia) Real-time PCR system. PCR reaction of 5 μl contained 2.5 μl of TaqMan PCR Master Mix-UNG (2X), 0.25 μl of each TaqMan assay probe (20X), 1.25 μl of diluted cDNA and 1 μl of H_2O. The PCR was performed at 95^{0}C for 10 min, followed by 50 cycles at 95^{0}C for 15 sec, 50^{0}C for 30 sec and 72^{0}C for 15 sec. Samples were run in duplicate, and mRNA levels were normalized to _-actin. Homo sapiens poly (ADP-ribose) polymerase 1 (PARP-1) (Batch no:11279) primer sequences and corresponding genes were (gene, forward and reverse primer): (5'-CCACACACAATGCGTATGACT-3') and (5'-CCACAGCAATCTTCGGTTATGA-3')

ß-actin (5'-CGTACCACAGGCATTGTGATG-3' and 5'-TTTGATGTCACGCACGATTTC-3')

Statistical Analysis

Statistical Analysis was performed using GraphPad Prism 4 (GraphPad Software, Inc.). The Pearson correlation coefficient(R) was employed to determine the correlation of efficiency of RT reaction and expression of FFPE and FF samples.

RESULTS

The demographic information of the patients is shown in Table 1. Histopathological information about the tumors of the patients is shown in Table 2. In this set of patients, the percentage ER positive and PR positive patients were similar to general population of breast cancer patients (61% and 54.2% respectively). HER2 positive patient ratio was also similar (28.8%) (Table 2).

PARP-1 expression in normal and tumor breast samples from cancer patients was analyzed using real time quantitative PCR. Expression of PARP-1 was determined in 37 samples. The expression of PARP-1 was found to be significantly upregulated in 40.54 % of the patients and significantly downregulated in 51.35 % of the patients. Therefore a change in PARP-1 expression does not correlate with the onset of breast cancer in this set of patients.

DISCUSSION

Use of PARP inhibitors in breast cancer studies have gained attention during the last years and several clinical studies are ongoing that use these inhibitors. PARP inhibitors are predicted to be effective against ER, PR and HER2 negative tumors. In addition, these inhibitors seem to have tolerable side effects. Preliminary studies showed that these inhibitors were highly effective against tumors. PARP inhibitors can be used to sensitize tumor cells by inhibiting repair of DNA damage produced by chemotherapy or radiotherapy.[25] In BRCA deficient tumors, treatment with PARP inhibitors causes synthetic lethality, which is defined by inhibition of functioning of two proteins that leads to cell death while inhibition of only one protein does not. Therefore PARP inhibitors do not have a negative effect on healthy cells with functional BRCA.[17] Clinical studies with tumors that have BRCA mutations have shown PARP inhibitors to be effective against these tumors and it is thought that other tumors with defects in DNA repair pathways might be susceptible to inhibitors of PARP.[26]

Here we have shown that in the group of breast cancer patients studied, the percentages of ER, PR and HER2 positive patients were similar to other patient sets that have been studied. However, PARP1 expression had been shown by others to be upregulated in 58% of breast cancer patients compared to 40.54% found in this study.[27] In addition, PARP1

was significantly downregulated in a high percentage of patients. Therefore PARP1 inhibitors could be used on the breast cancer patients with increased PARP1 expression. Patients with increased PARP1 expression and lack BRCA1 and BRCA2 would be better candidates for treatment as DNA repair will be severely inhibited in these patients. Further study is required to determine the patients who lack BRCA1 and BRCA2 in the PARP1 upregulated group. Effect of PARP inhibitors in PARP1 downregulated breast cancer patients would be hard to predict as the lowest amount of PARP1 required for effective DNA repair is unknown. Studies can be done to determine the effect of PARP inhibitors on cells with downregulated PARP1 in vitro, followed by animal experiments. These experiments would then help determine in which cases of tumor PARP inhibitors could be used. Development of new treatments that include PARP inhibitors have the potential to shorten the regimen and cause less side effects and several clinical trials with hopes of better results are currently being conducted at the moment.

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Correspondence

Beyhan Cengiz

Gaziantep Üniversitesi Tıp Fakültesi

Fizyoloji Anabilim Dalı

GAZİANTEP / TURKEY

Tel: (+90.342) 360 60 60

e-mail: beyhancengiz@hotmail.com