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Original Article

Expression of PGRMC1 in paraffin-embedded tissues of breast cancer

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Abstract: Hormone replacement therapy (HRT) can increase the risk of breast cancer, shown especially in the only double-blind placebo-controlled study, the Women's Health Initiative (WHI). Recent published researches are suggesting that progesterone receptor membrane component 1 (PGRMC1) expression may explain this result. This study aimed at investigating whether paraffin-embedded tissue could be used in PGRMC1-related trials. Samples from 109 breast cancer patients from years 2008 to 2014 were evaluated for the expression of estrogen receptor alpha (ERα), progestrone receptor (PR), Ki67 and PGRMC1 by immunohistochemistry (IHC). Our data indicate that the expression of PGRMC1 is stable in paraffin-embedded tissue stored for different years. The IHC score of Erα (X^2 = 4.40, P = 0.11), PR (X^2 = 2.89, P = 0.24) and Ki67 (X^2 = 0.25, P = 0.88) also had no significant different in the paraffin-embedded tissue from different years. Our data suggest that paraffin embedded tissue can be used in PGRMC1-related trials.

Keywords: Breast cancer, immunohistochemistry, PGRMC1

Introduction

In China breast cancer has become the most frequently diagnosed cancer in females since 2009 [1]. Hormone replacement therapy (HRT) with estrogen alone or in combination with progestogen can alleviate these symptoms in peri- and post-menopause women. However Women's Health Initiative (WHI) and the Million Women Study showed a probable relationship between progestin treatment and an increased risk of breast cancer in postmenopausal women [2]. As can be derived from the most important clinical studies, especially from the results of WHI, as well as from experimental research, progestogens are the main component to be able to increase the risk of breast cancer during hormone therapy. On the other hand after long-term therapy also estrogen-only can increase this risk, as for example has been observed in the Nurses Health Study [3].

PGRMC1 contributes to multiple features of tumor growth. It is known to enhance the progression of breast cancer. It has been shown by our studies that PGRMC1 may be involved in the receptor-mediated carcinogenic effect of oestrogens and progestins. One EDITORIAL on our research clearly stated, that the progestogen action via PGRMC1 may indeed explain the results in the WHI Study, i.e. increased breast cancer risk by combined estrogen/progestogen therapy.

Immunohistochemistry (IHC) is now the globally accepted methodology for detection of hormonal receptors [4], such as estrogen receptor (ER) and progesterone receptor (PR) in breast carcinomas. So here we use this method to test PGRMC1 expression in paraffin-embedded tissue. All of this was to investigate whether we could only use fresh material in PGRMC1 related studies.

Materials and methods

Subjects

109 breast cancer samples from Beijing Obstetrics and Gynecology Hospital, Capital

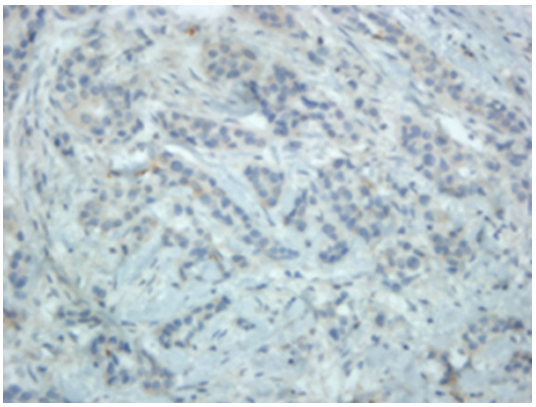

Figure 1. Weak membrane expression of PGRMC1 (Immunohistochemical stain, 200×).

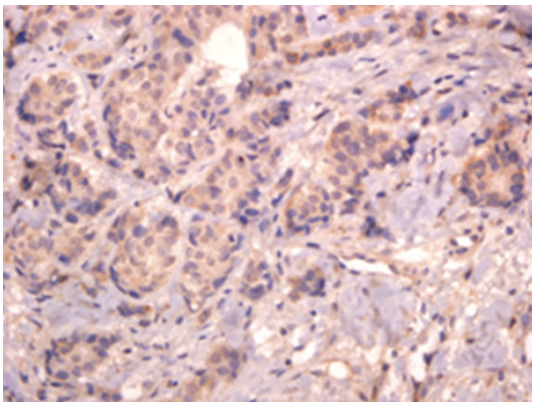

Figure 2. Moderate membrane expression of PGRMC1 (Immunohistochemical stain, 200×).

Medical University from year 2008 to 2014 were obtained. The approval was received from the Human Ethics Committee of Beijing Obstetrics and Gynecology Hospital. Written informed consent was also obtained from the patients.

Breast cancer tissues, which were obtained from pre chemotherapy patients were fixed in 10% neutral buffered formalin and then embedded in paraffin.

Immunohistochemistry

Immunohistochemical staining was carried out manually. Histological assessments were performed on 4-5 μm thick HE-stained sections of formalin-fixed paraffin embedded tumors. Slides were incubated with normal serum (ABC-kit, Vector Labs, Burlingame, CA, USA) for 20 min to block non-specific IgG reactions. And

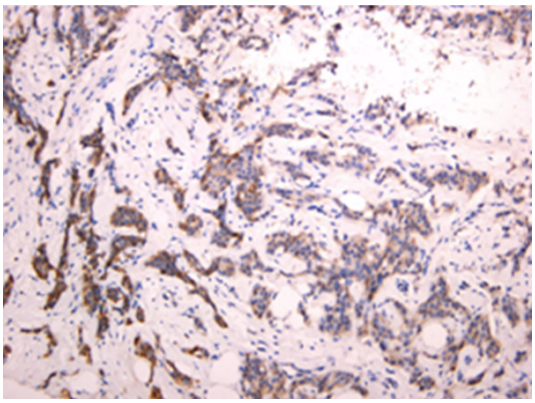

Figure 3. Strong membrane expression of PGRMC1 (Immunohistochemical stain, 200×).

then were incubated at 4°C overnight with a goat polyclonal antibody raised to detect PGRMC1 (8.0 μg/ml; ab48012; Abcam, Cambridge, MA, USA). After overnight incubation with primary antibody, the following day all slides (paraffin-embedded) were rinsed in PBS (containing 0.075% non-ionic detergent BRIJ), incubated with normal serum for 20 min and then incubated with the appropriate biotinylated secondary IgG (donkey anti-goat IgG) for ~30 min. Slides were counter stained with hematoxylin, dehydrated in ascending ethanols, cleared through a series of xylene.

Semi-quantitative pathologic evaluation

Images were captured by an Olympus BX41 light microscope. ERα, PR, Ki67 and PGRMC1 immunolabeling was independently checked by two pathologists. Tumor cells with nuclear and/or membrane immunohistochemical staining were considered to be positive cells. The numbers of positively labeled tumor cells were scored as follows: 0 = 0-4%; 1 = 5%-25%; 2 = 26%-50%; 3 = 51%-75%; and 4 = 75%. The intensity of staining was also evaluated and graded from 1 to 3, where 1 indicates weak staining; 2, moderate staining; and 3, strong staining. The two values obtained were multiplied to calculate a receptor score (maximum value, 12). For statistical analysis, the samples were grouped into negative (score < 2) or positive (score ≥ 2).

Statistical analysis

SPSS 17.0 program for Windows was used for this analysis (SPSS Inc., Chicago, IL). Statistical significance level was set at $P < 0.05$. Cor-

Table 1. Different expression of ERα in breast cancer tissues from different years

Year	Positive		Negative		Media IHC Score	Chi-square	*P* value
	n	%	n	%	$\bar{X}$		
2008-2010	21/36	58.33%	15/36	41.67%	7.00	4.40	0.11
2011-2012	22/39	56.41%	17/39	43.59%	13.00		
2013-2014	17/34	50.00%	17/34	50.00%	5.00		

Table 2. Different expression of PR in breast cancer tissues from different years

Year	Positive		Negative		Media IHC Score	Chi-square	*P* value
	n	%	n	%	$\bar{X}$		
2008-2010	15/36	41.67%	21/36	58.33%	1.00	2.89	0.24
2011-2012	15/39	38.46%	24/39	61.54%	5.00		
2013-2014	13/34	38.24%	21/34	61.76%	3.00		

Table 3. Different expression of Ki67 in breast cancer tissues from different years

Year	Positive		Negative		Media IHC Score	Chi-square	*P* value
	n	%	n	%	$\bar{X}$		
2008-2010	1/36	2.78%	35/36	97.22%	0.00	0.25	0.88
2011-2012	4/39	10.26%	35/39	89.74%	1.00		
2013-2014	1/34	2.94%	33/34	97.06%	0.00		

Table 4. Different expression of PGRMC1 in breast cancer tissues from different years

Year	Positive		Negative		Media IHC Score	Chi-square	*P* value
	n	%	n	%	$\bar{X}$		
2008-2010	28/36	77.78%	35/36	22.22%	11.00	0.33	0.85
2011-2012	28/39	71.79%	11/39	28.21%	11.00		
2013-2014	17/34	50.00%	17/34	50.00%	8.00		

relations were performed by Spearman's rank correlation test. To compare the immunohistochemical profiles of PGRMC1 ERα, PR and Ki67 in paraffin embedded tissue from different years, the Kruskal-Walllis test were utilized.

Results

The median age at the time of primary tumor diagnosis was 54 years of age (range 30-81). Grade 1 was diagnosed in 1 out of 109 cases (0.91%), grade 3 was diagnosed in 4 out of 109 cases (3.67%) and grade 2 in 104 out of 109 cases (95.41%). Invasive lobular carcinoma was diagnosed in 4 out of 109 cases (3.67%), and invasive ductal carcinoma in 104 out of 109 cases (95.41%). One case of invasive ductal carcinoma was mixed with ductal carcinoma in situ. To compare the immunohistochemical profiles of ERα, PR, Ki67 and PGRMC1 among samples from different year, Kruskal-Wallis test were utilized. We did not observe a significant difference in expression of these variables among samples in different years at a *P*-value of 0.05 (**Figures 1-3** and **Tables 1-4**). And our trial showed no correlation between PGRMC1 and age (**Table 5**).

Discussion

PGRMC1 was a new-found receptor homogeneously expressed within the tumors. It is induced in approximately one-half of breast tumors compared to matched nonmalignant tissue [5]. Immunohistochemistry (IHC) is now the globally accepted methodology for detection of hormonal receptors. Our finding did not find any different of PGRMC1 in paraffin embedded tissues from different years use this method. Our present study demonstrates that expression of PGRMC1 is stable in paraffin embedded breast cancer samples which could be used in PGRMC1 related trials.

The current mainstay of hormone receptor assessment (such as ER, PR and Ki67) in breast cancer is immunohistochemistry (IHC) [6]. Calibration of a cut-off that allows 100% sensitivity and specificity is hard to achieve. There is wide variability in how different laboratories perform the tests and interpret the

Table 5. No correlation of PGRMC1 expression with patient's age analyzed by a non-parametric Spearman test

	PGRMC1		
	Spearman r	95% confidence interval	*P* Value (two-tailed)
Age	-0.065	2.73-3.74	0.588

results. Based on current recommendations, the time from tissue acquisition to fixation (ischemic time) should be as short as possible. Samples should be fixed in 10% neutral buffered formalin (NBF) for 6-72 h. The minimum fixation time for reliable IHC ER has been suggested to be 6-8 h, regardless of the type or size of the specimen. PGRMC1 is a new-found receptor and is not routinely evaluated in breast cancer biomarker testing. However researchers have showed that PGRMC1 could regulate gene expression in a way that would increase the cell's susceptibility to undergoing apoptosis [7]. We already have performed an extensive research about the involvement of this receptor in the development of breast cancer. The present trial showed that the PGRMC1 is stable in paraffin embedded tissues made with standardized preparing method mentioned before. So in further study we could use paraffin embedded tissues for PGRMC1 related trial for it is reliable.

The potentially harmful effects of combination hormone therapy (HT) for postmenopausal symptoms have made many women feel fear of HRT since the results of the Women's Health Initiative were published [8]. In the WHI estrogen-only decreased the breast cancer risk. Nurses' Health Study showed after long-term therapy also estrogen-only can increase this risk [1-3]. During these years we found that PGRMC1 possibly plays a significant role in the development of breast cancer. Whereas one EDITORIAL on our research clearly stated, that the progestogen action via PGRMC1 may indeed explain the results in the WHI Study, i.e. increased breast cancer risk by combined estrogen/progestogen therapy [9], the second EDITORIAL in the same journal (Journal of the North American Menopause Society) pointed to the fact, that we still need more research since this receptor might interact with other important mechanisms in the development of breast cancer [10]. In vitro studies showed that certain synthetic progestins will increase the proliferation of PGRMC1 over-expression breast cancer cells and may be involved in tumorigenesis [11-14]. And in almost all experimental models estrogen can increase the proliferation of breast cancer cells, including estriol, estrone and estetrol, as recently we could demonstrate using different cell lines [15].

About PGRMC1 and its relationship with age, in 28 frozen or paraffin-embedded breast cancer samples and ten control benign breast tissue samples by RelqPCR, that PGRMC1 mRNA levels decreased significantly with patient age [16]. Our trial showed no correlation between PGRMC1 and age. The different results may be due to different detection methods, as we know that the expression level of mRNA is not always fully translated into protein levels. Another possible reason may be that different ethnic groups have been investigated, one from China, while the other studies were from the USA [16]. In order to harmonize the data, more studies among different countries using the same methods are necessary.

In conclusion, paraffin embedded tissues could be used for PGRMC1 related trials. Factors like age may have no effect on expression of PGRMC1 in breast cancer tissues.

Limitation

The findings in the present paper did not compare the stable expression of PGRMC1 between paraffin embedded tissues and freshly frozen tissue samples. But the findings in this trial may also contribute to the use of PGRMC1 as marker in breast cancer related trials.

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Disclosure of conflict of interest

None.

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