Increased expression of progesterone receptor membrane component 1 is associated with aggressive phenotype and poor prognosis in ER-positive and negative breast cancer

Xiangyan Ruan, MD, PhD,1,2 Ying Zhang, MD,1 Alfred O. Mueck, MD, PhD, PharmD,1,2 Marina Willibald, MSC,3 Harald Seeger, PhD,2 Tanja Fehm, MD,3 Sara Brucker, MD,4 and Hans Neubauer, PhD3

Abstract

Objective: Expression of progesterone receptor membrane component 1 (PGRMC1) has been shown to be higher in breast cancer than normal tissue. We have previously shown that certain progesterogens strongly stimulate proliferation of breast cancer cells overexpressing PGRMC1, and therefore hypothesize that PGRMC1 may play a critical role in breast cancer progression. Because little information is available if expression of PGRMC1 is also associated with worse prognosis for breast cancer patients, in this study we investigated the clinicopathologic significance of PGRMC1 expression in breast cancer tissue.

Methods: Expression of PGRMC1 was analyzed by immunohistochemical staining of primary tumor tissues obtained from 69 breast cancer patients. A labeling score was developed, and results were correlated with tumor size, lymph node metastasis, and clinical outcome.

Results: Overexpression of PGRMC1 is correlating with larger tumor size and lymph node metastasis. Kaplan-Meier survival curves indicate that patients with PGRMC1high tumors have poorer disease-free and overall survival independent from the estrogen receptor status than breast cancer patients with PGRMC1low tumors.

Conclusions: Our findings suggest that the expression of PGRMC1 might be useful for predicting prognosis in patients with breast cancer.

Key Words: Breast cancer – ER – PGRMC1 – Prognosis.

Breast cancer is the most common tumor type among the female population. It comprises 22.9% invasive cancers in women and 16% of all female cancers, and is the most common cause of cancer death worldwide.1,2 It is known that breast cancer is a heterogeneous disease characterized by diverse clinical behavior and outcomes.3 Although previous studies have provided various prognostic and predictive biomarkers, identification of new biomarkers and therapeutic targets is still important to improve treatment of patients.4-5

Within the last decade, several novel membrane-associated progesterone receptors (PRs) have been identified which can initiate fast-acting membrane-associated, nongenomic signaling.6-8 Among them progesterone receptor membrane component 1 (PGRMC1) has been identified in breast cancer tissue in which its expression seems to be different from that in normal mammary glands. According to the human protein atlas (http://www.proteinatlas.org), PGRMC1 is moderately expressed in normal breast epithelial tissue. In contrast, PGRMC1 is strongly expressed in approximately one-third of the investigated breast cancer tissues. In breast cancer, PGRMC1 is primarily expressed in estrogen receptor (ER)-negative basal epithelial cells of mammary ductules. In ductal in situ breast carcinoma of comedo type, PGRMC1 expression colocalizes with the presence of glucose transporter 1 in poorly oxygenated cells surrounding the necrotic core, whereas more distal ER-positive cells are PGRMC1 negative.9 Furthermore, PGRMC1 also exists in many cancers or cancer cell lines, for example breast cancer,10 and is over-expressed in lung cancer and colon cancer.11
PGRMC1 expression can be found in almost all tissues and is associated with different functions. For example, it is involved in cholesterol synthesis by activating the P450 protein CYP51/lanosterol demethylase, and participates in drug and hormone metabolism by binding directly to P450 proteins.\textsuperscript{12} PGRMC1 is expressed in the nervous system playing a role in neuroendocrine functions which control female reproductive behavior, and is overexpressed after trauma by the neuroprotective progesterone.\textsuperscript{13-15} PGRMC1 has also been implicated in the rapid effects of progesterone in ovarian cells and sperm regulating the effect of corpus luteum.\textsuperscript{16-19}

In our previous research published in \textit{Menopause}, we demonstrated that proliferation of breast cancer cells overexpressing PGRMC1 is strongly increased by certain progestogens.\textsuperscript{20} In an Editorial published in the same journal, the idea was emphasized that our data may explain the increase in breast cancer observed in the Women’s Health Initiative.\textsuperscript{21}

Because little information is available on PGRMC1 and its association with prognosis for breast cancer patients, the present project was planned to investigate the correlation between PGRMC1 expression in breast cancer tissue and clinicopathologic parameters such as tumor size and lymph node metastasis and to determine a correlation of PGRMC1 expression with breast cancer outcome.

\section*{METHODS}

\subsection*{Participants}

Sixty-nine female breast cancer patients who underwent curative surgery from 2008 to 2014 in Beijing Obstetrics and Gynecology Hospital, Capital Medical University, were enrolled after having received their consent (number of ethical approval: BJEC2031115).

Archived slides were stained with hematoxylin & eosin and immunohistochemistry, using monoclonal antibodies against ER, PR, Ki67, and PGRMC1. Pathology reports and other medical records were reviewed to confirm the diagnoses as well as to collect the clinicopathologic characteristics of tumors and participants such as age, histologic grade, tumor size, lymph node metastasis, and participant follow-up information. Participant characteristics are shown in Table 1.

\subsection*{Immunohistochemistry}

Breast cancer tissues were taken from participants before chemotherapy, fixed in 10\% neutral buffered formalin and embedded in paraffin. Immunohistochemical staining was carried out manually by blocking endogenous peroxide with a solution of 6\% hydrogen peroxide for 3 minutes. Antigen retrieval was performed by heating the slides in a pressure cooker for 30 minutes in 0.1\% HCl. Non-specific antigens were blocked with Avidin/Biotin Blocking System (avidin solution for 10 min and biotin solution for another 10 min after rinsing off avidin). Then tissue sections were incubated with primary antibodies specific for ER-\textalpha; (mouse monoclonal, 1:100; Abcam), PR (mouse monoclonal, 1:400; Abcam), Ki67 (rabbit monoclonal, 1:200; Abcam), and PGRMC1 (goat monoclonal, 1:1,000; Abcam) in a humidified chamber. Antibodies were applied at room temperature: ER for 28 minutes, PR for 24 minutes, Ki67 for 30 minutes, and PGRMC1 for 16 minutes. Thereafter, biotinylated anti-mouse (goat anti-mouse; Abcam), anti-rabbit (goat anti-rabbit; Abcam), or anti-goat (rabbit anti-goat) secondary antibodies were applied for 25 minutes in a humidified chamber followed by enzyme labeling with streptavidin-conjugated peroxidase (1:1,000; Pierce) for 25 minutes. Immunohistochemistry was performed twice. Images were captured with an Olympus BX41 light microscope. ER-\textalpha;, PR, Ki67, and PGRMC1 immunolabeling were independently checked by two pathologists in a blinded fashion without any knowledge of the participant data.

\subsection*{Immunohistochemical score}

Tumor cells with complete membranous staining for PGRMC1 and positive nuclear signals (ER-\textalpha;, PR, Ki67) were considered to be “positive” and scored as follows: 0, 0\% of positive cells; 1, 1\% to 10\% of positive cells; 2, 11\% to 33\% of positive cells; 3, 34\% to 66\% of positive cells; and 4, 67\% to 100\% of positive cells. 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 final receptor score was calculated by multiplication of both values (maximum value, 12). For statistical analysis, the scores were grouped into negative (score = 2) or positive (score \geq 2). Slides were evaluated by two blinded observers. The observer variation was less than 5\%.

\subsection*{Statistics}

The software package SPSS 17.0 (SPSS Inc, Chicago, IL) was used for statistical analysis. The comparison of various

\begin{table}[h]
\centering
\caption{Characteristics of the study cohort}
\begin{tabular}{|l|l|l|}
\hline
Variable & Categories & No. & \% \\
\hline
Disease-free survival & Recurrence & 38 & 55.07 \\
& Nonrecurrence & 31 & 44.93 \\
\hline
Survival & Live & 48 & 69.57 \\
& Death & 21 & 30.43 \\
\hline
Age & \leq 45 & 16 & 23.19 \\
& 45 to \leq 60 & 36 & 52.17 \\
& > 60 & 17 & 24.64 \\
\hline
Histological grade & Grade 1 & 1 & 1.45 \\
& Grade 2 & 64 & 92.75 \\
& Grade 3 & 4 & 5.80 \\
\hline
Primary tumor size & \leq 2 cm & 21 & 30.43 \\
& 2 to 5 cm & 40 & 57.97 \\
& > 5 cm & 8 & 11.59 \\
\hline
Lymph node metastasis & Absent & 29 & 42.03 \\
& 1 & 26 & 37.68 \\
& 2 & 14 & 20.29 \\
\hline
ER-\textalpha; expression & Negative & 32 & 46.40 \\
& Positive & 37 & 53.60 \\
\hline
PR expression & Negative & 39 & 56.50 \\
& Positive & 30 & 43.50 \\
\hline
Ki67 expression & Negative & 66 & 95.70 \\
& Positive & 3 & 4.30 \\
\hline
PGRMC1 expression & Negative & 21 & 30.40 \\
& Positive & 48 & 69.60 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a} ER, estrogen receptor; PGRMC1, progesterone receptor membrane component 1; PR, progesterone receptor.
prognostic parameters in the different groups (mortality, recurrence, nonrecurrence) was investigated with independent sample t test. Kruskal-Wallis test was applied to analyze the mean PGRMC1 expression in different groups categorized according to participant characteristics and clinicopathologic factors. Kaplan-Meier method was used to develop disease-free and overall survival curves, and log-ranking test was applied to determine significance levels. The Cox proportional hazard method was used to identify independent predictors of survival. All statistical tests were two-sided. Statistical significance level was set at $P < 0.05$.

**RESULTS**

**Participant and tumor characteristics**

Participant and tumor characteristics are shown in Table 1. There were 21 deaths from breast cancer and 38 recurrent cases within 5 years after diagnosis. ER-$\alpha$ expression (ER-$\alpha$+) was determined in 53.60% of breast cancers, 43.50% were positive for PR expression (PR+), and 4.30% were Ki67 positive (Ki67+). PGRMC1 was expressed in 69.60% of breast cancer cases investigated, whereas PGRMC1 was only expressed in 4.7% of normal breast tissues (Fig. 1).

**TABLE 2. Association between PGRMC1 expression and clinicopathologic parameters in 69 breast cancer patients**

<table>
<thead>
<tr>
<th>Clinicopathologic features</th>
<th>No.</th>
<th>PGRMC1 IHC score $\bar{x}$</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>OR</th>
<th>95% CI</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq$45</td>
<td>16</td>
<td>3.0</td>
<td></td>
<td>1.51</td>
<td>0.47</td>
<td>1.188</td>
<td>0.360-3.919</td>
</tr>
<tr>
<td>45-60</td>
<td>36</td>
<td>3.0</td>
<td></td>
<td>1.12</td>
<td>0.57</td>
<td>5.323</td>
<td>0.722-39.364</td>
</tr>
<tr>
<td>$&gt;60$</td>
<td>17</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>1</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>64</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>4</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq$2 cm</td>
<td>21</td>
<td>1.0</td>
<td></td>
<td>18.52</td>
<td>$&lt;0.001^a$</td>
<td>0.060</td>
<td>0.010-0.374</td>
</tr>
<tr>
<td>2-5 cm</td>
<td>40</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$&gt;5$ cm</td>
<td>8</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>29</td>
<td>1.0</td>
<td></td>
<td>7.23</td>
<td>$0.007^a$</td>
<td>1.01</td>
<td>0.025-0.411</td>
</tr>
<tr>
<td>1-5</td>
<td>26</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\geq$5</td>
<td>14</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease-free survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td>38</td>
<td>6.0</td>
<td></td>
<td>40.32</td>
<td>$&lt;0.001^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonrecurrence</td>
<td>31</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live</td>
<td>48</td>
<td>2.0</td>
<td></td>
<td>13.98</td>
<td>$&lt;0.001^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>21</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PGRMC1, progesterone receptor membrane component 1; OR, odds ratio.

$^a$The correlations were significant at $P < 0.05$. The expression of PGRMC1 was associated with aggressive phenotype of breast cancer, such as larger tumor size and lymph node metastasis.
PGRMC1 expression and clinicopathologic parameters

Correlation of PGRMC1 expression with conventional prognostic factors (Table 2) resulted in direct association with an increased aggressive phenotype of breast cancer, with a larger tumor size ($P = 0.003$) and lymph node metastasis ($P = 0.001$) (Table 2).

PGRMC1 expression and breast cancer prognosis

The Kaplan-Meier survival curves demonstrate a significant association between PGRMC1 expression and clinical outcome. In addition, survival analyses stratified by tumor stage revealed no significant differences. The Kaplan-Meier survival curves stratified by ER status, however, revealed that high PGRMC1 expression is associated with significant poor disease-free survival (log-rank test, $P = 0.017$) and overall survival (log-rank test, $P = 0.025$) in ER-positive and -negative breast cancers (Fig. 2).

Univariate and multivariate Cox regression analyses (stepwise analysis) of the factors influencing prognosis are shown in Table 3. Univariate analyses of conventional clinicopathologic parameters revealed that primary tumor size ($P = 0.017$), lymph node metastasis ($P = 0.002$), PR expression ($P = 0.011$), and PGRMC1 expression ($P = 0.013$) are predictors of poor disease-free survival in breast cancer patients. Predictors of poor overall survival in breast cancer patients were primary tumor size ($P = 0.001$), lymph node metastasis ($P = 0.004$), ER-α expression ($P = 0.018$), PR expression ($P < 0.001$), Ki67 expression ($P = 0.02$), and PGRMC1 expression ($P = 0.021$) as in Cox’s proportional hazard model (Table 3). Cox regression analyses revealed that participants with high PGRMC1 expression had higher recurrence and increased risk of dying than those that had lower or no PGRMC1 expression.

In summary, participants with PGRMC1high breast cancers had a poorer prognosis than those without PGRMC1 expression.

DISCUSSION

There is a longstanding link between PGRMC1 and progesterone signaling. PGRMC1 binds progesterone with a moderately high binding affinity ($K_d \sim 11$ nM), and it can also bind testosterone, glucocorticoids, and molecules such as heme, cholesterol metabolites, and proteins. Because bacterially expressed PGRMC1 does not bind to progesterone, and because the majority of PGRMC1 is not localized to the plasma membrane, it is now, however, tentatively assumed that PGRMC1 does not bind P4 by itself but requires an unknown protein that is associated only in partially purified PGRMC1 preparations.

Increased expression of PGRMC1 has been identified in various human malignancies, including cancers in breast, thyroid, colon, rectum, lung, and cervix. In our study, PGRMC1 was expressed in 69.60% of breast cancer cases which agrees with data from a former study. PGRMC1 overexpression correlated with adverse clinicopathologic features, including large tumor size, lymph node metastasis, and neoplasm recurrence. Univariate analysis revealed PGRMC1 expression as a predictor of poor disease-free survival in breast cancer patients; it also detected PGRMC1 being an independent prognostic marker for poor outcome in breast cancer patients.

PGRMC1 is a cytochrome b5-related protein that is up-regulated in tumors and promotes cancer growth. PGRMC1 was originally identified by its induction by dioxin during liver tumorigenesis, and it is one of six genes comprising a signature predicting nongenotoxic carcinogens. Previous studies have linked PGRMC1 with cancer through its expression and biological activities. It promotes multiple phenotypes in cancer cells, including resistance to apoptosis, anchorage-independent growth, cell invasion, in vivo tumor growth, and metastasis. PGRMC1 is required for key functions in tumor growth, promoting survival of cancer cells particularly after damage from chemotherapeutic drugs. An interesting novel activity of PGRMC1 in cancer is increasing the cell surface stability of epidermal growth factor receptor by interacting with epidermal growth factor receptor to maintain it at the plasma membrane. Furthermore, PGRMC1 expression increases with tumor stage in ovarian cancer.

Our previous studies suggest that PGRMC1 might be involved in the receptor-mediated carcinogenic effects of...
TABLE 3. Association of PGRMC1 expression with prognosis in 69 breast cancer patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease-free survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (&lt;45 vs 45-60 and &gt;60)</td>
<td>0.970</td>
<td>0.936-1.006</td>
<td>0.099</td>
</tr>
<tr>
<td>Histologic grade (G1 vs G2 and G3)</td>
<td>1.354</td>
<td>0.194-9.462</td>
<td>0.760</td>
</tr>
<tr>
<td>Primary tumor size (≤2 cm vs 2-5 cm and &gt;5 cm)</td>
<td>2.832</td>
<td>1.204-6.662</td>
<td>0.017*</td>
</tr>
<tr>
<td>Lymph node metastasis (absent vs 1-5 and &gt;5)</td>
<td>1.118</td>
<td>1.042-1.290</td>
<td>0.062*</td>
</tr>
<tr>
<td>PR expression (negative vs low and high)</td>
<td>1.332</td>
<td>0.986-1.100</td>
<td>0.626</td>
</tr>
<tr>
<td>Ki67 expression (negative vs low and high)</td>
<td>0.580</td>
<td>0.381-0.883</td>
<td>0.011*</td>
</tr>
<tr>
<td>PGRMC1 expression (negative vs low and high)</td>
<td>2.350</td>
<td>0.576-9.598</td>
<td>0.234</td>
</tr>
<tr>
<td>Overall survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (&lt;45 vs 45-60 and &gt;60)</td>
<td>0.982</td>
<td>0.915-1.053</td>
<td>0.605</td>
</tr>
<tr>
<td>Histologic grade (G1 vs G2 and G3)</td>
<td>11.701</td>
<td>0.432-316.800</td>
<td>0.144</td>
</tr>
<tr>
<td>Primary tumor size (≤2 cm vs 2-5 cm and &gt;5 cm)</td>
<td>37.728</td>
<td>7.171-198.513</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Lymph node metastasis (absent vs 1-5 and &gt;5)</td>
<td>1.810</td>
<td>1.051-3.204</td>
<td>0.040*</td>
</tr>
<tr>
<td>ER expression (negative vs low and high)</td>
<td>1.780</td>
<td>1.103-2.873</td>
<td>0.018*</td>
</tr>
<tr>
<td>PR expression (negative vs low and high)</td>
<td>0.144</td>
<td>0.055-0.373</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Ki67 expression (negative vs low and high)</td>
<td>16.184</td>
<td>1.542-169.841</td>
<td>0.020*</td>
</tr>
<tr>
<td>PGRMC1 expression (Negative vs low and high)</td>
<td>2.045</td>
<td>1.116-3.747</td>
<td>0.021*</td>
</tr>
</tbody>
</table>

Cox regression analyses revealed that participants with high PGRMC1 expression had higher recurrence and increased risk to die than those that had lower or no PGRMC1 expression.

ER, estrogen receptor; HR, hazard ratio; PGRMC1, progesterone receptor membrane component 1; PR, progesterone receptor.

*The correlations were significant at P < 0.05.

PGRMC1 was found to be expressed in triple negative breast cancers (TNBCs) at a level consistent with matched nonmalignant breast tissue. Further analysis of PGRMC1 expression using data from The Cancer Genome Atlas database indicated that PGRMC1 mRNA expression was increased by 2- to 3.64-fold in 25% of the available TNBC samples compared with matched normal breast tissue. Overall, PGRMC1 expression is only marginally increased in TNBC compared with normal tissue or is not differentially expressed at all depending upon the sample. Because there is increasing evidence that PGRMC1 and ER-α may cooperate to activate the proliferation of breast cancer cells, histopathological analysis of PGRMC1 in breast cancer is highly intriguing. Our previous studies have suggested that PGRMC1 overexpression might be functionally correlated with the expression pattern of ER-α, and expression of PGRMC1 is positively associated with ER-α expression in breast cancer development. In this study, PGRMC1 expression in both ER-positive and ER-negative breast cancer groups was related to participant poor clinical outcome. Although patients with ER-α-positive breast cancer have a better prognosis than ER-α-negative patients, still some of them develop metastasis and die soon after the operation. In our study, PGRMC1 expression is associated with metastasis and poor prognosis. Maybe PGRMC1 could be a better independent prognosis factor for breast cancer patients in the future.

There are some limitations to our current study. First, this was a retrospective investigation. Despite the strict enrollment criteria applied, we were unable to completely exclude conditions that might influence the expression of PGRMC1 in breast cancer tissue, such as the way to get the biopsy via paracentesis or excision. Second, the participant data were collected from a single institution. Our results need to be validated by prospective research and participant data from multiple medical centers.

CONCLUSIONS

Our data demonstrate for the first time that high PGRMC1 expression in breast cancer is correlated with several adverse prognostic parameters such as tumor size and...
Regional lymph node metastasis. In addition, high PGRMC1 levels point to poor disease-free survival and overall survival for these participants, independent from ER status. These data emphasize our hypothesis that PGRMC1 may increase breast cancer risk as observed in the Women’s Health Initiative trial.

REFERENCES


13. Indelkofer KA, Petersen SI. Distribution of mRNAs encoding classical progestin receptor, progesterone membrane components 1 and 2, serpine mRNA binding protein 1, and progestin and ADIPOQ receptor family members 7 and 8 in rat forebrain. Neuroscience 2011;172:55-63.


