Original Article

Prevalence of Retinoblastoma Protein Loss in Thai Women with Triple-Negative Breast Cancer

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Background: Triple-negative breast cancer (TNBC) has a different epidemiology, histologic features, and clinical behavior, and lacks effective targeted therapies that can improve the benefits gained from chemotherapy compare to other subtypes. Several pathways associated with the retinoblastoma protein (pRB) are perturbed in this aggressive breast cancer.

Objective: To investigate the prevalence of pRB loss among Thai patients with TNBC and to compare clinical outcomes between patients with and without pRB expression.

Materials and Methods: The pRB status was evaluated by immunohistochemistry in 71 patients who were diagnosed with TNBC. The clinicopathologic features and the association between pRB expression and clinical outcomes were analyzed retrospectively.

Results: The prevalence of pRB loss was 63.4% (45 out of 71). No differences were observed in the patients and/or tumors characteristics in terms of pRB expression, but there was a trend toward a high frequency of high Ki-67 expression in pRB-negative tumors (88.9% versus 73.3%, 2P=0.052). However, the differences between two groups were not statistically significant. After a median follow-up of 67.7 months, 5-year disease free survival estimates were 73% and 65% (2P=0.610) and 5-year overall survival estimates were 76% and 65%, (2P=0.500) in patients with pRB loss and with pRB-positive tumors, respectively. According to multivariable Cox proportional hazards models, pRB expression did not independently correlated with DFS or OS in cases of TNBC (HR 1.32, 95% CI 0.56 to 3.13, 2P=0.530; for DFS and HR 1.19, 95% CI 0.49 to 2.9, 2P=0.710; for OS).

Conclusion: The prevalence of RB loss in Thai women with TNBC was significantly higher than in previous studies. RB loss was associated with more aggressive behavior; however, it did not translate into inferior survival outcomes.

Keywords: Retinoblastoma protein, Triple-negative breast cancer

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The retinoblastoma tumor suppressor gene (RB gene) is primarily known for its regulation of cell cycle progression in various cancers. The phosphorylation of RB, which is initiated by cyclin-dependent kinase (CDK)-cyclin complexes, is a significant driver of cancer cell proliferation. Aberrant expression of RB in breast cancer has been identified in approximately 20% to 35% of cases[1,2]. For instance, several alterations in the RB pathway (e.g., RB mutations and deletions), INK4a (e.g., mutations, deletions, or methylation) and in cyclins or CDKs (e.g., over-expression) are observed in most cancers, including breast cancer[3-9]. The most recent genomic study demonstrated that the RB pathway plays distinct roles that have a significant impact on the progression of cancer, which has implications across multiple breast cancer subtypes[10].

Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype that is defined by the lack of expression of estrogen receptor (ER),

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progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). This breast cancer subtype not only has a different epidemiology, histologic features, and clinical behavior, but it also lacks effective targeted therapies that can improve the benefits gained from chemotherapy. Although patients with TNBC exhibit a high response rate to chemotherapy, early recurrence occurs frequently within the first three years, which leads to poorer survival outcomes[11-13]. Therefore, a better understanding of the molecular biology of this challenging breast cancer subgroup may help physicians improve and customize drug development to improve patient outcomes.

Unlike the ER-positive subtype, in which RB aberrations such as the amplification or overexpression of cyclin D1 are dominant and contribute to a more proliferative subset of this subgroup, TNBCs exhibit frequent dysregulation of RB due to RB loss[10]. Previously, Treré et al demonstrated that RB loss occurred more frequently in the TNBC subtype than in other subtypes ($p<0.001$)[7]. Similarly, in another study, retinoblastoma protein (pRB) loss was observed in 42% of TNBC cases compared with 3% to 5% in the other subtypes, ($p<0.001$)[8]. Moreover, recent genomic, transcriptome, epigenetic, and proteomic results from The Cancer Genome Atlas Network (TCGA) demonstrated RB1 mutations or loss in 20% of TNBCs[14]. Furthermore, patients with TNBC whose tumors were negative for pRB had a very favorable clinical outcome if they were treated with conventional adjuvant chemotherapy, which further improved overall outcomes[7,15-17]. Therefore, these findings have prompted most scientists to extensively explore and validate the implications of RB analysis with the intention of defining patients with TNBC who might receive a greater benefit from chemotherapy. In addition, little is known about the epidemiology of RB pathway aberrations in Thai patients with TNBC. Therefore, the objectives of the present study were to characterize the RB alterations via the identification of the frequency of pRB protein loss in Thai patients with TNBC and the association between pRB, clinical characteristics, and outcomes. Moreover, clinical outcomes following neoadjuvant treatment were compared between patients whose tumors were either negative or positive for pRB.

Materials and Methods

Patients

One hundred sixty women with TNBC who were diagnosed and received systemic chemotherapy between August 2006 and August 2010 were reviewed. Patients with a lack of tumor tissues for further immunohistochemical (IHC) staining were excluded. Tumors were considered triple receptor-negative if nuclear staining was 1% or less for ER and PR, and HER2 receptor expression according to IHC (membranous staining in less than 0% of cells or IHC 0 or 1+), and/or if no gene amplification was found on fluorescence in situ hybridization. Adjuvant and neoadjuvant chemotherapies with anthracycline-based, taxane-based, anthracycline and taxane-based, and non-anthracycline and taxane-based regimens were administered depending on the staging and preferences of the patients and/or the physicians. Patients received adjuvant radiation therapy if indicated. The Institutional Review Board of King Chulalongkorn Memorial Hospital and Chulalongkorn University approved the present retrospective study.

RB immunohistochemistry and scoring

Seventy-one formalin-fixed, paraffin-embedded tissues were processed for microarray testing. To detect pRB, immunohistochemistry was performed on the tissue microarray with the following monoclonal antibody (mAb): clone 1F8/Rb1 (Neomarkers, Thermo Scientific Lab Vision, Waltham, MA, USA), which identifies all forms of RB (phosphorylated as well as unphosphorylated and hypophosphorylated). Immunostained tissue sections were evaluated independently by a single pathologist. The results were scored on a scale of 0 to 4+. Additionally, the numerical values corresponded to the following: 0 indicated undetectable expression, 1 indicated less than 25% expression, 2 indicated 25% to 50%, 3 indicated 50% to 75% and 4 indicated more than 75% expression in the nuclei of malignant cells examined. A grade of 0 was reported as negative, and grades 1+ to 4+ were considered positive for pRB expression.

Statistical analysis

Patients with TNBC were categorized into one of two groups according to their pRB expression and pRB loss. Patient characteristics, including age, tumor size, lymph node involvement, pathologic stage (early stage: stage I-II, or locally advanced stage: stage III, or advanced stage: stage IV), histology, grade, lymphovascular invasion, Ki-67 (low was less than 30% or high was 30% or more) and adjuvant chemotherapy, were tabulated and compared between the groups using the Fisher exact test. Overall survival (OS) was measured from the date of surgery to the date of death or when the patients were lost to follow-

up. Disease-free survival (DFS) was measured from the date of surgery to the date of the first reported local or distant recurrence or when the patients were lost to follow-up or when death occurred from any cause. Patients who died before they experienced the relevant events were considered censored on the dates of their last follow-up. The Kaplan-Meier product limit method was used to estimate the 5-year OS and DFS with 95% confidence intervals (CIs) of all patients according to pRB expression and other clinical characteristics; the groups were compared using the log-rank statistic. All statistical analyses were performed with the SPSS statistical software package (Statistical Package for Social Science, SPSS, Chicago, IL) version 16.0. Values for which p-value was smaller than 0.05 were regarded as statistically significant.

Results

Between August 2006 and August 2010, 160 patients were diagnosed with TNBC. Only 71 cases had adequate tumor tissues for the pRB immunohistochemical study. In the overall TNBC cohort (n = 160), the median age was 48.6 years (range 26 to 79). In all, 10.8% had a known family history of breast cancer. In terms of histological data, 63.1% had grade III, 80% had T1 to T2 tumors, 61.7% were node-negative, 83.6% had high Ki-67 expression, and 67.8% had high TP53 expression. In the 48 cases who received neoadjuvant chemotherapy with anthracycline and/or taxane or other chemotherapy treatments, 12 (25%) and 23 cases (47.9%) did show a pathologic complete response and a partial response, respectively. The overall response rate was 72.9% in this cohort, (data not shown). In the subset of 71 patients, the median age was 48.6 years (range 30 to 79). Similarly, 14.3% had a known family history of breast cancer. Approximately 77.3% had grade III, 82.8% had T1 to T2 tumors, 62.9% were node-negative and 76.1% were stage I or II breast cancer. Likewise, 84.3% had high Ki-67 expression and 65.3% had high TP53 expression. Additional baseline characteristics of all patients are shown in Table 1.

The pRB loss by immunohistochemistry was reported in 45 out of 71 (63.4%) patients with TNBC. Grade 0 was reported as negative and grades 1+ to 4+ were considered positive for pRB expression (Figure 1).

The baseline characteristics of patients in both the pRB loss and pRB-positive groups were shown in Table 1. No differences were observed in the characteristics of the patients and/or the tumors in terms of pRB expression, but a trend toward high frequency of high Ki-67 expression in pRB-negative tumors (88.9% versus 73.3%, 2P=0.052) was observed. Additionally, the authors found some aggressive features such as younger age, histological grade 3, and positive TP53 expression, were more frequent in the pRB loss group compared with the pRB-positive

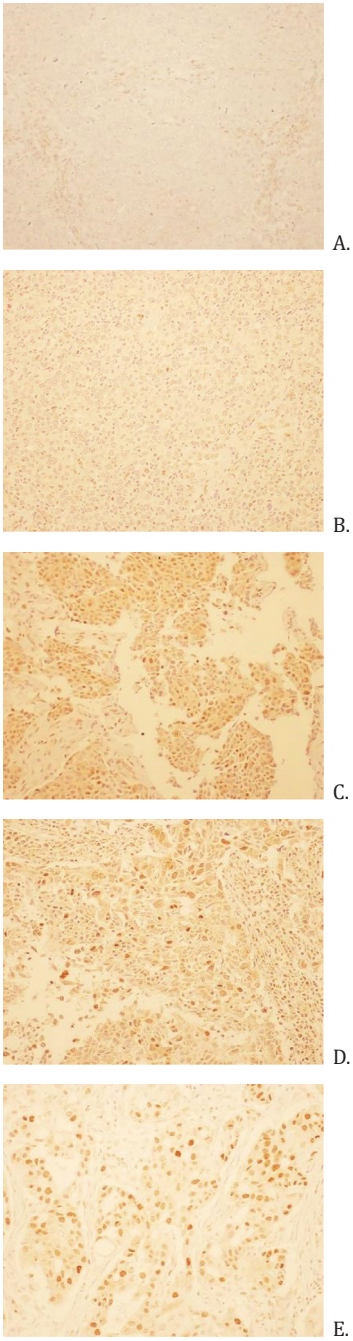

Figure 1. pRB immunohistochemistry staining (magnification ×100).

Table 1. Baseline patient characteristics

Characteristics	Total n (%)	pRB– n (%)	pRB+ n (%)	p-value
Number of patients	71 (100)	45 (63.4)	26 (36.6)	
Age (years)				0.804
Mean±SD	48.64±11.62	47.58±11.40	50.65±12.09	
Range	30 to 79	30 to 79	31 to 72	
ECOG				0.223
0	42 (59.2)	28 (62.2)	14 (53.8)	
1	29 (40.8)	17 (37.8)	12 (46.2)	
Reproductive status				0.084
Pre-menopause	36 (50.7)	25 (55.6)	11 (42.3)	
Post-menopause	35 (49.3)	20 (44.4)	15 (57.7)	
Family history				0.736
Yes	10 (14.3)	7 (15.9)	3 (11.5)	
No	60 (85.7)	37 (84.1)	23 (88.5)	
Histological grade				0.308
1	1 (1.5)	0 (0.0)	1 (4.6)	
2	14 (21.2)	9 (20.5)	5 (22.7)	
3	51 (77.3)	35 (79.5)	16 (72.7)	
Tumor size				0.261
T1 (≤2 cm)	18 (25.7)	13 (29.5)	5 (19.2)	
T2 (>2 to 5 cm)	40 (57.1)	26 (59.1)	14 (53.9)	
T3 (>5 cm)	12 (17.2)	5 (11.4)	7 (26.9)	
Nodal involvement				0.844
N0 (no axillary LN+)	44 (62.9)	29 (65.9)	15 (57.7)	
N1 (1 to 3)	18 (25.7)	10 (22.7)	8 (30.8)	
N2 (4 to 9)	6 (8.6)	3 (6.8)	3 (11.5)	
N3 (≥10)	2 (2.8)	2 (4.6)	0 (0.0)	
Lymphovascular invasion				0.063
Yes	15 (22.7)	6 (14.3)	9 (37.5)	
No	24 (36.4)	17 (40.5)	7 (29.2)	
Unknown	27 (40.9)	19 (45.2)	8 (33.3)	
P53 expression (n = 49)				0.281
Positive	32 (65.3)	25 (71.4)	7 (50.0)	
Negative	17 (34.7)	10 (28.6)	7 (50.0)	
Ki-67 (n = 51)				0.052
<30%	8 (15.7)	4 (11.1)	4 (26.7)	
≥30%	43 (84.3)	32 (88.9)	11 (73.3)	
Type of surgery				0.251
Wide local excision	19 (29.7)	15 (34.9)	4 (19.0)	
Simple mastectomy+MRM	45 (70.3)	28 (65.1)	17 (81.0)	
Chemotherapy				1.00
Yes	69 (98.6)	43 (97.7)	26 (100)	
No	1 (1.4)	1 (2.3)	0 (0.0)	
Anthracycline-based CMT				0.675
Yes	59 (85.5)	38 (88.4)	21 (80.8)	
No	10 (14.5)	5 (11.6)	5 (19.2)	
Taxane-based CMT				0.489
Yes	25 (37.9)	13 (31.7)	12 (48.0)	
No	41 (62.1)	28 (68.3)	13 (52.0)	
Radiotherapy				0.780
Yes	44 (67.7)	30 (69.8)	14 (63.6)	
No	21 (32.3)	13 (30.2)	8 (36.4)	
First recurrent site (n = 70)		14/44 (31.8)	9/26 (34.6)	0.302
Loco-regional	10 (33.3)	6 (31.6)	4 (36.4)	
Lung	10 (33.3)	6 (31.6)	4 (36.4)	
Brain	5 (16.7)	4 (21.0)	1 (9.0)	
Bone	5 (16.7)	3 (15.8)	2 (18.2)	
Dead				0.511
Yes	22 (31.0)	13(28.9)	9 (34.6)	
No	49 (69.0)	32 (71.1)	17 (65.4)	

ECOG=Eastern Cooperative Oncology Group Score; pRB–=pRB loss; pRB+ =pRB expression; LN=lymph node; MRM=modified radical mastectomy; CMT=chemotherapy

group. However, the differences between the two groups were not statistically significant.

Considering the 48 patients who received neo-adjuvant treatment, a retinoblastoma analysis was performed in only 16 patient samples. Moreover, the authors found a higher pathologic complete response (pCR) in the RB loss group (two of six or 33.3%) compared with the RB expression group (2% of 10% or 20%) (p=0.604), but this difference was not statistically significant.

At the end of the study, as of December 31, 2014, 23 patients (32.9%) had experienced relapse. The most common was multiple organ relapse (11/23, 47.8%), but other sites of relapse were the chest wall, axillary lymph nodes, bone, lung, brain, and liver. In all, 22 patients (31%) died. The mean follow-up time of the present study was 67.7 months. After a comparison between each pRB group, the authors found that the pRB loss group had a 9.2-month longer follow-up time than the pRB-positive group (71.1 versus 61.9 months). The authors also found that 32 out of 45 (71.1%) patients in the pRB loss group compared with 17 out of 26 (65.4%) patients in the pRB-positive group were still disease-free at the end of the study. The 5-year DFS estimates were 73% and 65% in patients whose tumors were pRB-negative and in those whose tumors were pRB-positive tumors, respectively (2P=0.610). The 5-year OS estimates were 76% and 65% in patients with pRB loss and in those with pRB-positive tumors (2P=0.500), respectively. The pRB expression was not an independent predictor of survival outcomes. The 5-year DFS and 5-year OS estimates according to the patient and tumor characteristics are summarized in Table 2, and the Kaplan-Meier curves for the DFS and the OS according to pRB expression are shown in Figure 2A and 2B.

According to multivariable Cox proportional hazards models, pRB expression was not independently correlated with DFS or OS in TNBCs (HR 1.32, 95% CI 0.56 to 3.13, 2P=0.530; for DFS and HR 1.19, 95% CI 0.49 to 2.9, 2P=0.710; for OS) after adjustment for various important risk factors such as tumor grade, pathologic stage, lymphovascular invasion, and treatment with adjuvant chemotherapy. The multivariable Cox proportional hazards models of DFS and OS that were adjusted for pRB group, grade, stage, lymphovascular invasion, and adjuvant chemotherapy are summarized in Table 3.

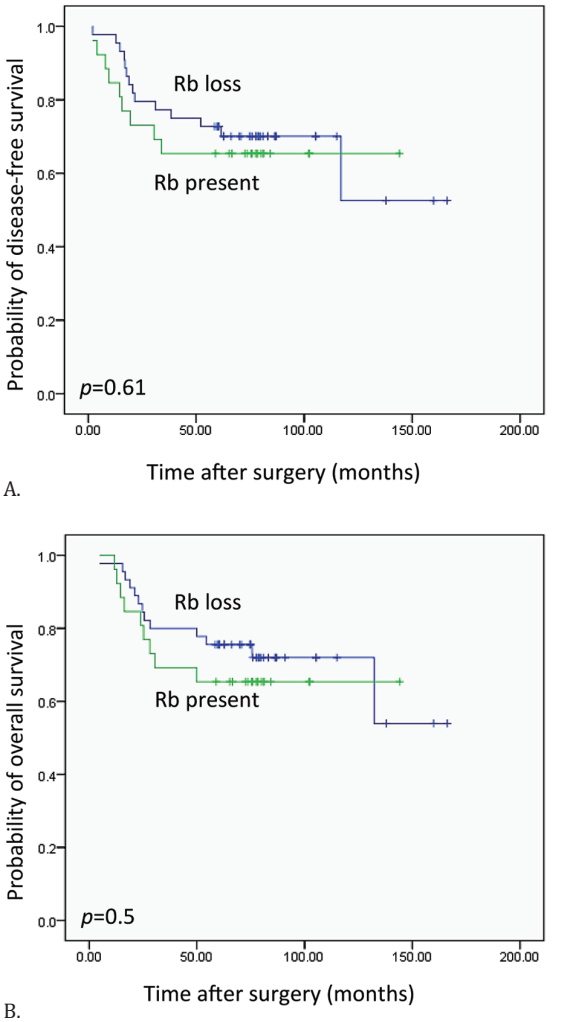

Figure 2. Disease free survival (A) and overall survival (B) by retinoblastoma protein expression in triple negative breast cancer patients. The 5-year disease-free survival estimates were 73% and 65% in patients whose tumors were pRB-negative and in those whose tumors were pRB-positive tumors, respectively (2P=0.61). The 5-year overall survival estimates were 76% and 65% in patients with pRB loss and in those with pRB-positive tumors (2P=0.5), respectively.

Discussion

The aims of the present study were to evaluate the frequency of pRB loss in Thai TNBC patients as well as the association between pRB expression, clinical characteristics, and outcomes. In the 71 patients with TNBC in our series, the prevalence of retinoblastoma protein loss was 63.4%. RB loss was associated with more aggressive baseline characteristics such as younger age at onset, higher grade tumors, higher TP53 positivity, and higher rate of high Ki-67 positivity; however, this did not translate to better

Table 2. Survival estimates based on patient characteristics

	No. patients	No. events	5-year overall survival estimate (95% CI)	p-value	No. events	5-year disease-free survival estimate (95% CI)	p-value
All patients	71	20	0.72 (0.67 to 0.77)		21	0.70 (0.64 to 0.76)	
Retinoblastoma				0.500			0.610
Loss	45	11	0.76 (0.70 to 0.82)		12	0.73 (0.66 to 0.80)	
Presence	26	9	0.65 (0.57 to 0.73)		9	0.65 (0.56 to 0.74)	
Age (years)				0.100			0.090
≤50	40	14	0.65 (0.56 to 0.74)		15	0.63 (0.55 to 0.71)	
>50	29	6	0.79 (0.71 to 0.87)		6	0.79 (0.71 to 0.87)	
Pathologic stage				<0.001			<0.001
Stage I/II	54	10	0.82 (0.77 to 0.87)		12	0.78 (0.72 to 0.84)	
Stage III	15	8	0.47 (0.34 to 0.60)		8	0.47 (0.34 to 0.60)	
Stage IV	2	2	0.00		1	0.00	
Nuclear grade				0.900			0.700
Grade II	14	4	0.71 (0.59 to 0.83)		4	0.71 (0.69 to 0.83)	
Grade III	51	12	0.77 (0.71 to 0.83)		13	0.74 (0.68 to 0.80)	
Tumor size				0.100			0.400
T1 to T2	59	15	0.75 (0.69 to 0.81)		17	0.71 (0.65 to 0.77)	
T3 to T4	11	5	0.55 (0.40 to 0.70)		4	0.60 (0.44 to 0.76)	
Lymph nodes							
N0	41	5	0.88 (0.93 to 0.83)	<0.001	6	0.85 (0.79 to 0.91)	<0.001
N1	23	10	0.57 (0.47 to 0.67)		10	0.57 (0.47 to 0.67)	
N2 to N3	6	4	0.33 (0.14 to 0.52)		4	0.20 (0.02 to 0.38)	
Lymphovascular invasion				<0.001			<0.001
Negative	20	1	0.95 (0.90 to 1.00)		1	0.95 (0.90 to 1.00)	
Positive	13	9	0.31 (0.18 to 0.44)		9	0.31 (0.18 to 0.44)	
Adjuvant chemotherapy				<0.001			<0.001
Anthracycline	35	5	0.86 (0.80 to 0.92)		6	0.83 (0.77 to 0.89)	
Anthracycline/taxane	22	12	0.46 (0.35 to 0.57)		12	0.46 (0.35 to 0.57)	

CI=confidence interval

Table 3. Multivariable cox proportional hazards model

Factor	Overall survival			Disease-free survival		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Retinoblastoma protein: presence vs. loss	1.19	0.49 to 2.9	0.710	1.32	0.56 to 3.13	0.530
Age: >50 years vs. ≤50 years	0.79	0.15 to 4.01	0.770	1.18	0.24 to 5.94	0.840
Grade: 2 vs. 3	0.48	0.07 to 3.30	0.460	0.47	0.07 to 3.30	0.450
Tumor: T3 to T4 vs. T1 to T2	4.71	0.60 to 37.13	0.140	2.76	0.38 to 20.26	0.320
Node: N2 to N3 vs. N1 vs. N0	1.12	0.32 to 3.95	0.860	0.86	0.26 to 2.85	0.800
Lymphovascular invasion: yes vs. no	28.69	1.08 to 764.48	0.050	40.06	1.99 to 808.61	0.020
Adjuvant chemotherapy: anthracyclines/taxanes vs. anthracyclines	1.34	0.21 to 8.42	0.750	1.27	0.26 to 6.32	0.770

CI=confidence interval

survival outcomes after treatment with chemotherapy. At a median of 67.7 months of follow-up, the 5-year OS and DFS estimated for those whose tumors were RB-negative versus RB-positive were 76% and 73% versus 65% and 65% (OS; p=0.5, DFS; p=0.61), respectively.

The retinoblastoma gene is one tumor suppressor gene that plays various crucial roles in the development

of breast cancers. In TNBC, pRB loss is defined by lack of protein expression, but genomic, transcriptomic and epigenetic analyses have been recognized in several recent studies. Treré et al[7] demonstrated that RB loss occurred more frequently in the TNBC subtype than in other subtypes (64.5% versus HER-2 positive; 22.6% versus luminal A and B; 6.5% and 6.5%, respectively, p<0.001), and 37.7% of 53 triple-negative tumors exhibited pRB loss compared with 2.3% observed in other subtypes (p<0.001). The authors also found that patients with TNBC whose tumors lacked pRB had very good clinical outcomes[7]. Similarly, Stefansson et al reported that the frequency of pRB loss was approximately 42% in TNBC compared with 3% to 5% in other subtypes[8]. Here, the authors found a higher frequency of pRB loss among Thai women with TNBC (63.4%) compared with two previous studies. Approximately 64.2% of the patients in Treré et al's study[7] were at least 50 years of age or older at diagnosis whereas approximately 58% of the patients in our study were younger than 50 years at diagnosis. The difference in the patient population between each study might be one of the reasons that diverse RB rates were observed. Likewise, different ethnicities, different patient characteristics, and the use of different pRB antibodies and scoring systems might support why the frequencies of RB in various studies were not identical.

Earlier studies have suggested that RB pathway aberrations are the significant marker for the identification of patients who might show improvement in response to neoadjuvant chemotherapy[15,18-20]. Derenzini et al[18] investigated the RB activity in terms of therapeutic responses and clinical outcomes of breast cancer patients. They found that the absence of pRB expression was a predictive factor for good clinical outcomes in patients who were treated with standard systemic chemotherapy (CMF regimen i.e., cyclophosphamide, methotrexate, and 5-FU) but not in patients who were treated with endocrine therapy alone according to a multivariate analysis[18]. Herschkowitz et al[15] also examined functional loss of RB based on loss of heterozygosity of RB1, low expression of the RB1 transcript, and pRB loss or p16INK4a loss in breast cancer samples and reported that the frequency of pRB protein loss in 31 TNBCs was 42.9%. Additionally, high expression of each of the RB pathway signatures was correlated with pCR in primary breast and lymph nodes[15]. Recently, Witkiewicz et al[17] explored dysregulation of the RB pathway using a RB loss gene signature in correlation with an improved response to neoadjuvant chemotherapy in patients with breast cancer. They demonstrated that a RB loss gene signature was associated with increased pCR in breast cancer patients who were treated with 5 fluorouracil/Adriamycin/Cytoxan (FAC, T/FAC), and taxane/Adriamycin neoadjuvant therapy independently of ER status[17]. Similar to our 16 patients who were treated with neoadjuvant chemotherapy, a higher pCR rate in the RB loss group (33.3%) compared with the RB-positive group (20%) (p=0.604) was observed, although this difference was not statistically significant. The results from the present study replicated previous findings and supported the hypothesis that patients with TNBC whose tumors lack pRB expression exhibit chemotherapeutic sensitivity. Due to the small subset of patients in our study, subsequent studies that involve a large neoadjuvant subgroup would be helpful to validate role of RB for tailored chemotherapy treatment in Thai women with TNBC.

Several studies showed an inconsistent relationship between RB expression and clinical outcomes[7,8,21]. Treré et al reported that patients whose tumors expressed pRB had a significantly poorer DFS than patients with loss of pRB (p=0.008)[7]. Conversely, the study by Bogina et al[21], which aimed to evaluate the association between RB and p16 protein expression and clinical outcomes in 117 unselected triple-negative breast carcinomas, found that pRB was not associated with DFS and OS (p=0.66 and 0.89, respectively). Conversely, p16 expression was associated with a good response to therapy with a significantly increased DFS (p=0.001) and a trend toward an increased OS (p=0.056)[21]. Here, the authors showed that pRB loss was not associated with better 5-year OS and better 5-year DFS estimates compared with pRB expression (5-year OS; p=0.5 and 5-year DFS, p=0.61, respectively). Until now, RB expression has not been demonstrated to be strongly predictive of survival outcomes.

To our knowledge, this is the first study on molecular aberrations and RB pathway disruption in Thai cases of TNBC. This work presented a great opportunity for further extensive exploration of the biology of TNBCs, which may provide both an understanding of tumor cell heterogeneity and possible therapeutic implications in this difficult-to-treat subgroup. Finally, a better understanding of the molecular characterization of TNBCs in the Thai population may lead to personalized drug development, to better meet our patients' needs.

Our retrospective single-center study had several limitations. First, although the baseline characteristics

of the subgroup of the RB test population showed similar results to the entire population, the small sample size might affect the reported frequency of pRB expression. Second, with regards to pRB immunohistochemical scoring, no definite standard of pRB measurement has been recommended; therefore, the authors elected to use a different pRB scoring system (0, 1+ to 4+) compared with that used in other studies; this difference might be responsible for the diverse frequencies of RB loss observed in previous studies. Additionally, our scoring needs to be validated in a large independent cohort population. Lastly, only a single RB aberration, pRB expression, may not be sufficient to entirely understand the variations in the RB-CDK-cyclin complex in TNBCs. Hence, the use of a comprehensive analysis such as a RB loss signature along with other molecules such as p16ink4a and/or cyclin E1 may efficiently identify additional information and distinguish a new chemotherapy-sensitive subgroup of patients with TNBC.

Conclusion

The present study showed the prevalence of RB loss in Thai patients with TNBC was significantly higher than in previous studies. RB loss was associated with more aggressive behaviors and superior response to neoadjuvant chemotherapy. However, pRB expression was not an independent predictor of either OS or DFS outcomes.

What is already known on this topic?

The retinoblastoma tumor suppressor gene (RB gene) is recognized for promoting cancer proliferation by regulation of cell cycle progression in various cancers. Similarly, several alterations in the RB pathway have been identified in breast cancer and have a significant impact on the breast cancer progression. In the aggressive breast cancer, triple negative subtype, dysregulation of RB due to RB loss are frequently reported in previous studies. Additionally, pRB loss TNBC patients had favorable clinical outcomes after receiving adjuvant chemotherapy. Hence, understanding RB analysis might help defining subgroup of patients who will have more advantage from chemotherapy.

However, little is known about the epidemiology of RB pathway aberrations in Thai patients with TNBC. Therefore, the information in molecular biology of this challenging TNBC may support and customize drug development to improve Thai patient outcomes.

What this study adds?

The prevalence of RB loss in Thai patients with TNBC was significantly higher than in previous studies. Different ethnicities and different pRB analysis might influence the different outcomes. RB loss was associated with more aggressive behaviors and greater response to neoadjuvant chemotherapy. Conversely, pRB expression was not an independent predictor of either OS or DFS outcomes.

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Conflicts of interest

The authors declare no conflict of interest.

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