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Article

ARTICLE

PTHrP, A Biomarker for CNS Metastasis in Triple-Negative Breast Cancer and Selection for Adjuvant Chemotherapy in Node-Negative Disease

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Abstract

Background: Triple-negative breast cancer (TNBC) is characterized by poor prognosis and lack of targeted therapies and biomarkers to guide decisions on adjuvant chemotherapy. Parathyroid hormone-related protein (PTHrP) is frequently overexpressed in breast cancer and involved in proliferation and metastasis, two hallmarks of poor prognosis for node-negative breast cancer. We investigated the prognostic value of PTHrP with respect to organ-specific metastasis and nodal status in TNBC.
Methods: We assessed PTHrP expression using immunohistochemistry in a clinically annotated tissue microarray for a population-based study of 314 patients newly diagnosed with TNBC, then analyzed its correlation to progression and survival using Kaplan-Meier and Cox regression analyses. The Cancer Genome Atlas (TCGA) validation analysis was performed through Bioconductor. All statistical tests were two-sided.
Results: PTHrP overexpression (160 of 290 scorable cases, 55.2%) was statistically significantly associated in univariate analysis with decreased overall survival (OS) in our cohort ($P = .0055$) and The Cancer Genome Atlas ($P = .0018$) and decreased central nervous system (CNS)-progression-free survival ($P = .0029$). In multivariate analysis, PTHrP was a statistically significant independent prognostic factor for CNS-progression-free survival in TNBC (hazard ratio [HR] = 5.014, 95% confidence interval [CI] = 1.421 to 17.692, $P = .0122$) and for OS selectively in node-negative TNBC (HR = 2.423, 95% CI = 1.129 to 5.197, $P = .0231$). Strikingly, PTHrP emerged as the only statistically significant prognostic factor (HR = 2.576, 95% CI = 1.019 to 6.513, $P = .0456$) for OS of low-clinical risk node-negative patients who did not receive adjuvant chemotherapy.
Conclusions: PTHrP is a novel independent prognostic factor for CNS metastasis and adjuvant chemotherapy selection of low-clinical risk node-negative TNBC. Its predictive value needs to be prospectively assessed in clinical trials.

Triple-negative breast cancers (TNBCs), defined by absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), represent 15–20% of all breast cancer (BC) cases and are characterized by aggressive clinical course, increased rate of metastases, and lack of targeted therapy (1–3). Several clinico-pathologic variables have been shown to affect patient outcome in TNBC, including tumor size, lymph node (LN) status, tumor grade, age at diagnosis, and type of surgery and chemotherapy (4,5). Nodal status represents an important prognostic factor for adjuvant chemotherapy decision-making for women with node-positive BC and high-risk women with node-negative BC (6). Therapeutic decisions

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for node-negative BC are mostly guided using clinico-pathologic risk classifications such as age, tumor grade, lympho-vascular invasion (LVI), and tumor size (7,8). Novel prognostic biomarkers are required for the identification of node-negative TNBC patients at high risk of distant metastasis and death (9).

Parathyroid hormone-related protein (PTHrP), ubiquitously expressed in normal adult and fetal tissues, is involved in a wide range of developmental processes, including the development of mammary glands (10–12). PTHrP also displays growth-promoting and antiapoptotic properties (13) and is frequently overexpressed in BC and other solid tumors (14–22). Using the Mouse Mammary Tumor Virus-Polyoma Middle T antigen mouse model of mammary cancer, we showed that PTHrP is implicated in all stages of BC initiation, progression, and metastasis, where it regulates the expression of key signaling molecules involved in proliferation and metastasis (23). Although increased cell proliferation and propensity for metastasis are key determinants for prognosis of node-negative patients (24,25), the prognostic value of PTHrP in TNBC and node-negative disease in particular remains unknown.

The purpose of this study was to investigate the clinical significance of PTHrP specifically in TNBC. We identified a subset of 314 patients with available tumor tissue and clinical data in a population-based cohort of treatment-naive patients newly diagnosed with TNBC, previously characterized by our group (26). We investigated the correlation between immunohistochemical expression of PTHrP and clinical outcomes, including progression, organ-specific metastasis, and survival based on LN status and adjuvant chemotherapy treatment. Survival prognostic value of PTHrP was validated using a publicly available dataset of The Cancer Genome Atlas (TCGA).

Methods

Study Population

Patients with newly diagnosed TNBC between January 1998 and December 2008 in a single cancer center were included. Immunohistochemical staining for ER, PR, and HER2 was performed centrally and prospectively using standard methods (27,28). Patients with neoadjuvant chemotherapy, metastatic BC at presentation, or multiple primary malignancies were excluded. Patients were offered guideline-based staging, surgery, adjuvant chemotherapy, and radiotherapy (Supplementary Methods, available online) as per published recommendations (6,29,30).

Ethics Statement

The Alberta Cancer Research Ethics Committee has waived patient consent (impractical, unreasonable, or not feasible to obtain). The Health Research Ethics Board of Alberta-Cancer Committee, McGill University Health Centre and Human Research Protection Office of the US Army-Department of Defense approved this study.

Tissue Microarray and Immunohistochemistry

We constructed a tissue microarray (TMA) containing triplicate cores for 523 formalin-fixed paraffin-embedded pretreatment TNBC tissue specimens on seven paraffin blocks. Cores from normal tissues were incorporated as internal controls. Immunohistochemistry (IHC) staining of PTHrP was optimized for automated IHC then performed on TMA sections using anti-PTHrP antibody (Santa Cruz, sc20728, 1:10 dilution; Supplementary Methods, available online). A practicing breast pathologist (A.O.) blinded to clinical outcomes performed PTHrP scoring. PTHrP expression in tumor cores was evaluated relative to its expression in normal breast tissue. PTHrP-high refers to tumor tissues with stronger staining than normal breast tissue and PTHrP-low to tumor tissues with staining equal to or lower than normal breast tissue. The strongest PTHrP staining intensity obtained among tumor cores for the same patient was used for scoring analysis.

End Points and Statistical Analysis

The variables analyzed included age at diagnosis, tumor size, grade, nodal status, LVI, adjuvant chemotherapy, surgery type, and adjuvant radiotherapy. Patients with missing information for tumor grade (two cases), adjuvant chemotherapy (two cases), and PTHrP expression (24 cases) were excluded from statistical analysis of these variables. The end points of this study were progression-free survival (PFS), central nervous system (CNS)-PFS, brain metastasis (BM)-free survival (BMFS), and overall survival (OS). OS was measured from the date of surgery to the date of death from any cause. Patients last known to be alive were censored at the date of last follow-up. PFS, CNS-PFS, and BMFS were measured from the date of surgery to the date of progression, CNS-progression, or BM, respectively. Patients who did not progress (for PFS) or did not experience CNS metastasis (for CNS-PFS), or BM (for BMFS) were censored at either the date of death or date of last follow-up if they were still alive. We used the χ^2 test and Fisher exact test, when appropriate, to evaluate the association of PTHrP expression with clinicopathologic characteristics, progression, organ-specific metastasis, and survival. PFS, CNS-PFS, BMFS, and OS curves were constructed according to the Kaplan-Meier method. The log-rank test was used to compare patient survival probability between different groups. The median follow-up time for OS was calculated based on the "reverse Kaplan–Meier" method (31). Cox proportional hazards regression models were used for univariate and multivariable survival analyses. We assessed the assumption of proportional hazards by examining graphs of scaled Schoenfeld residuals. Only the variables with a statistically significant P value ($P \leq .05$) in univariate analysis were included in the multivariable model. All analyses were two-sided with $P \leq .05$ being considered statistically significant. Statistical analysis was conducted using SAS software version 9.4 (SAS Institute Inc., Cary, NC).

TCGA Data Analysis

The breast invasive carcinoma associated datasets containing clinical information and mRNA expression were collected from the Genomic Data Commons data portal of TCGA using the UCSC Cancer Genome Browser (https://genome-cancer.ucsc.edu/proj/site/hgHeatmap/). The level 3 TCGA breast-based gene expression profile was measured using the Agilent G4502A_07_3 platform. Of 565 BC patients, we identified 55 TNBC patients (negative IHC staining for ER, PR, and HER2) with available gene expression datasets. The mRNA expression profile of *PTHLH*, which encodes PTHrP, was filtered from 17 815 expressed genes, and the data were validated across all platforms using ±1.5-fold change as a cutoff to determine high and low *PTHLH* levels. Survival data were retrieved from TCGA clinical data and

analyzed through Bioconductor (package TCGAbiolinks) using R statistical software.

Results

Patients and Treatment Characteristics

We analyzed a subset of patients with available tissue specimens from a large cohort of treatment-naive women newly diagnosed with TNBC (n = 768) characterized by our group (26). This subset (n = 314) displayed similar proportions of all clinico-pathologic characteristics analyzed compared with the entire cohort. In agreement with previous reports (32,33), this population presented a high percentage of young women with a median age of 52 years (range 27–90 years) and 151 patients (48.1%) aged 50 years or less at diagnosis. The median follow-up time for OS was 3.6 years (range 0.1–9.8 years). Sixty-six patients (21%) developed disease progression, similar to the recurrence rate observed in other TNBC cohorts (34). Analysis of the patterns of recurrence showed that 21 patients (6.7%) experienced locoregional recurrence, 34 (10.8%) developed distant metastasis, and 11 (3.5%) experienced both. Eighteen patients developed CNS metastasis (5.7% of the entire cohort), with 12 patients developing BM (3.8%), five patients developing leptomeningeal metastasis (1.6%), and three patients developing spinal cord metastasis (0.9%) associated with BM or leptomeningeal metastasis for two of them. Thirty-five patients (11.1%) developed visceral organ metastasis (lung or liver). Nineteen patients (6.1%) developed bone metastasis. Thirteen patients (4.1%) presented distant LN metastasis (Table 1). Consistent with reports of increased likelihood of death in TNBC patients within 5 years of diagnosis (35,36), the 5-year OS for this population was 70%. Median OS following CNS progression was only 3.2 months (range 0–14.3 months).

Table 1. Distribution of clinical and treatment characteristics among TNBC patients (n = 314)

Clinico-pathologic characteristic	Patients, No. (%)
Age at diagnosis, y	314 (100.0)
≤50	151 (48.1)
>50	163 (51.9)
Tumor size, cm	314 (100.0)
T1 (<2)	174 (55.4)
T2 (2–5)	126 (40.1)
T3 (>5)	14 (4.5)
Grade*	312 (100.0)
1	6 (1.9)
2	41 (13.1)
3	265 (85.0)
LN status	314 (100.0)
N0	216 (68.8)
N1	39 (12.4)
N2	59 (18.8)
LVI	314 (100.0)
Negative	206 (65.6)
Positive	108 (34.4)
Type of surgery	314 (100.0)
MRM	178 (56.7)
Breast conserving (lumpectomy)	136 (43.3)
Adjuvant chemotherapy*	312 (100.0)
No	92 (29.5)
Yes	220 (70.5)
Adjuvant RT	314 (100.0)
No	122 (38.9)
Yes	192 (61.1)
RT area	314 (100.0)
Breast/chest wall alone	121 (38.5)
Locoregional	71 (22.6)
None	122 (38.9)
Progression	314 (100.0)
No	248 (79.0)
Yes	66 (21.0)
Type of progression	314 (100.0)
None	248 (79.0)
Locoregional	21 (6.7)
Distant	34 (10.8)
Both	11 (3.5)
Distant metastasis†	314 (100)
CNS	18 (5.7)
Brain	12 (3.8)
Leptomeninges	5 (1.6)
Spinal cord	3 (0.9)
Visceral organ (liver, lung)	35 (11.1)
Bone	19 (6.1)
Distant LN	13 (4.1)

*Cases with missing information: grade (two cases), adjuvant chemotherapy (two cases). CNS = central nervous system; LN = lymph node; LVI = lympho-vascular invasion; MRM = modified radical mastectomy; N0 = LN-negative; N1 = 1 to 3 positive LN; N2 ≥3 positive LN; RT = radiotherapy; TNBC = triple-negative breast cancer.

†Patients with multiple sites of distant progression are included in more than one category.

PTHrP Expression in a TMA From a Population-Based Study of Patients With TNBC

We assessed for the first time, to our knowledge, immunohistochemical expression of PTHrP in TNBC. We scored PTHrP expression in 430 of the 523 TMA cases; 233 of the 430 scorable cases (54.2%) displayed high PTHrP expression levels compared with normal breast. Accordingly, among 314 patients with available clinical information, 160 of 290 TNBC patients with scorable tumor cores (55.2%) displayed high PTHrP expression. Consistent with previous data regarding PTHrP expression in BC patients (37,38), PTHrP was mainly localized to the cytoplasm of TNBC tumor cells, whereas stromal regions showed negative PTHrP staining (Figure 1). Staining of TMA internal control cores from various normal tissues (Supplementary Figure 1, available online) showed PTHrP expression levels similar to the "Human Protein Atlas" PTHrP scoring analysis (39,40).

Expression of PTHrP and Rate of Metastasis in TNBC

Next, we examined the relationship between immunohistochemical PTHrP expression levels and clinico-pathologic characteristics of TNBC patients. PTHrP expression was not statistically significantly correlated with the rate and type of progression of TNBC patients (Supplementary Table 2, available online). However, analysis of distant progression sites revealed a statistically significant association between high PTHrP expression and increased rate of CNS metastasis in all patients (P = .0232) and in patients with distant metastasis (P = .0238) (Figure 2, A and B). Incidence rates of CNS metastasis were 2.3% (3 of 130 patients) in the PTHrP-low compared with 8.7% (14 of 160 patients) in the PTHrP-high subgroup. Incidence rates of BM were 0.8% (1 of 130 patients) in the PTHrP-low compared with 6.2% (10 of 160 patients) in the PTHrP-high subgroup

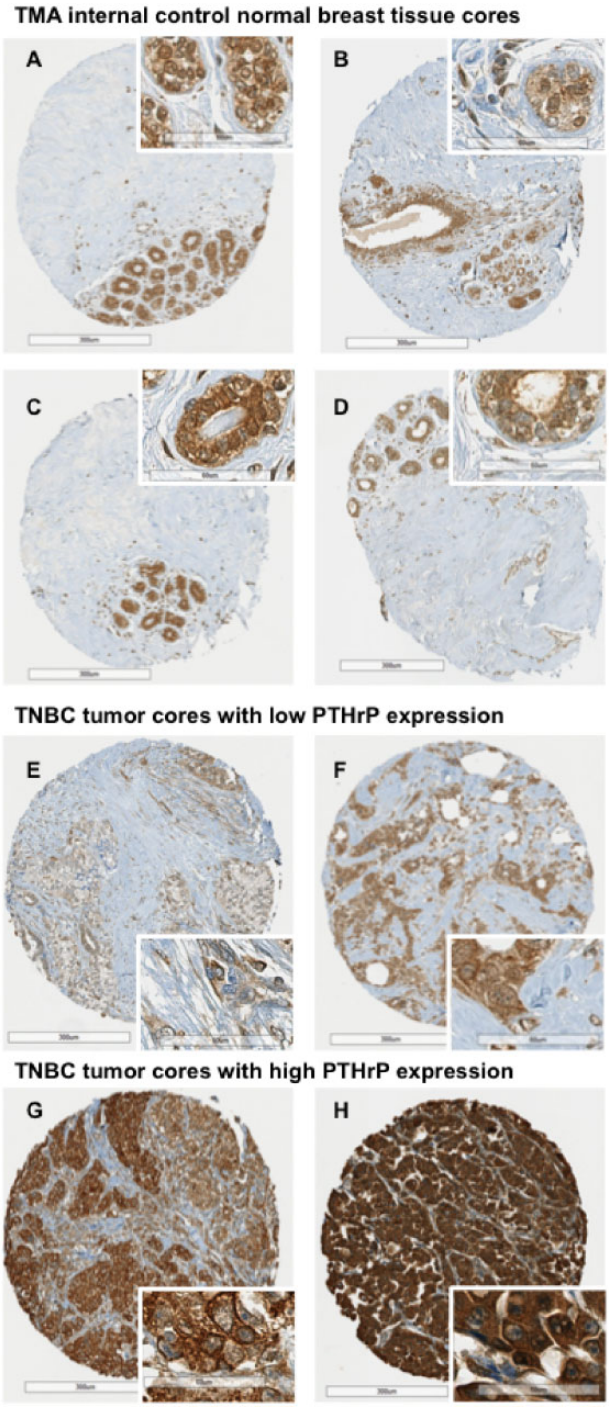

Figure 1. Representative immunohistochemistry staining results for parathyroid hormone-related protein (PTHrP) in the tissue microarray. (**A–D**) Representative control normal breast cores; (**E,F**) representative triple-negative breast cancer (TNBC) tumor cores with low PTHrP staining (lower or equal to normal breast); (**G,H**) representative TNBC tumor cores with high PTHrP staining (higher than normal breast). Stromal cells are used as negative internal controls as opposed to normal glandular and myoepithelial cells (**A–D**) or positively stained tumor cells (E–H) (**insets**). Original magnification ×400. TMA = tissue microarray.

(Supplementary Table 1, available online). To gain further insight into the role of PTHrP in BM, we examined gene signatures previously known to be involved in BC metastatic progression to the brain (41). Using the "Breast Cancer Gene-Expression Miner" microarray-based tool (42), we conducted in silico analyses of publicly available BC datasets to evaluate the correlation between the expression of the *PTHLH* gene, which encodes PTHrP, and these gene signatures across several BC subtypes. Interestingly, *PTHLH* correlates with the BM genes *HBEGF* (heparin-binding EGF-like growth factor) and *ANGPTL4* (angiopoietin-like 4) selectively in TNBC and basal-like subtypes (Supplementary Table 2, available online) known for their common morphological and genetic features and their increased rate of BM (43). These data suggest for the first time, to our knowledge, a correlation between PTHrP expression and CNS metastasis specifically in TNBC.

Prognostic Value of PTHrP Expression in TNBC

Next, we evaluated the prognostic significance of PTHrP expression in TNBC in terms of PFS, CNS-PFS, and OS (n = 290). In univariate analysis, PTHrP had statistically significant prognostic value in CNS-PFS (hazard ratio [HR] = 5.519, 95% confidence interval [CI] = 1.570 to 19.398, $P = .0077$) and OS (HR = 2.033, 95% CI = 1.221 to 3.386, $P = .0064$) but not in PFS (HR = 1.487, 95% CI = 0.890 to 2.485, $P = .1295$). Kaplan-Meier analysis also showed statistically significant prognostic value for PTHrP in CNS-PFS ($P = .0029$) and OS ($P = .0055$). Five-year CNS-PFS was 97% and 81% for the low and high PTHrP groups, respectively, and 5-year OS was 76% and 61% for the low and high PTHrP groups, respectively (Figure 2, D and E). PTHrP had statistically significant prognostic value for BMFS as well ($P = .0019$). Five-year BMFS was 100% and 84% for the low and high PTHrP groups, respectively (Supplementary Figure 2, available online). Consistent with this study population, TCGA analysis revealed statistically significant prognostic value for PTHrP in OS ($P = .0018$) in TNBC (Figure 2F). Analysis of TNBC patients' outcome at different timepoints revealed that high PTHrP expression is statistically significantly associated with shorter PFS, CNS-PFS, and OS up to 7 years follow-up period starting from the early 2-year timepoint for PFS and CNS-PFS (Supplementary Table 3, available online). In multivariable analysis, we analyzed the prognostic association of PTHrP with CNS-PFS and OS when adjusted to the covariates found statistically significant in univariate analysis (Supplementary Table 4, available online). Both high PTHrP expression (HR = 5.014, 95% CI = 1.421 to 17.692, $P = .0122$) and LN positivity (N2 > 3 positive LN) (HR = 3.262, 95% CI = 1.218 to 8.733, $P = .0186$) emerged as independent prognostic factors for short CNS-PFS. As expected, LN positivity (N2) independently predicted poor OS (HR = 2.970, 95% CI = 1.672 to 5.276, $P = .0002$), and treatment with adjuvant chemotherapy was an independent prognostic factor for improved OS (HR = 0.362, 95% CI = 0.208 to 0.631, $P = .0003$) in TNBC patients. High PTHrP expression was not an independent prognostic factor for poor OS (HR = 1.590, 95% CI = 0.925 to 2.736, $P = .0936$) (Table 2). These results indicate that PTHrP has statistically significant independent prognostic value for CNS-PFS but not OS.

Prognostic Significance of PTHrP Expression Based on Lymph Node Status in TNBC

To minimize the considerable influence of LN positivity on patients' outcome, we analyzed the prognostic value of PTHrP in our cohort based on LN stratification. Indeed, in accordance with other TNBC cohorts (5), LN status showed a strong statistically significant prognostic value in our

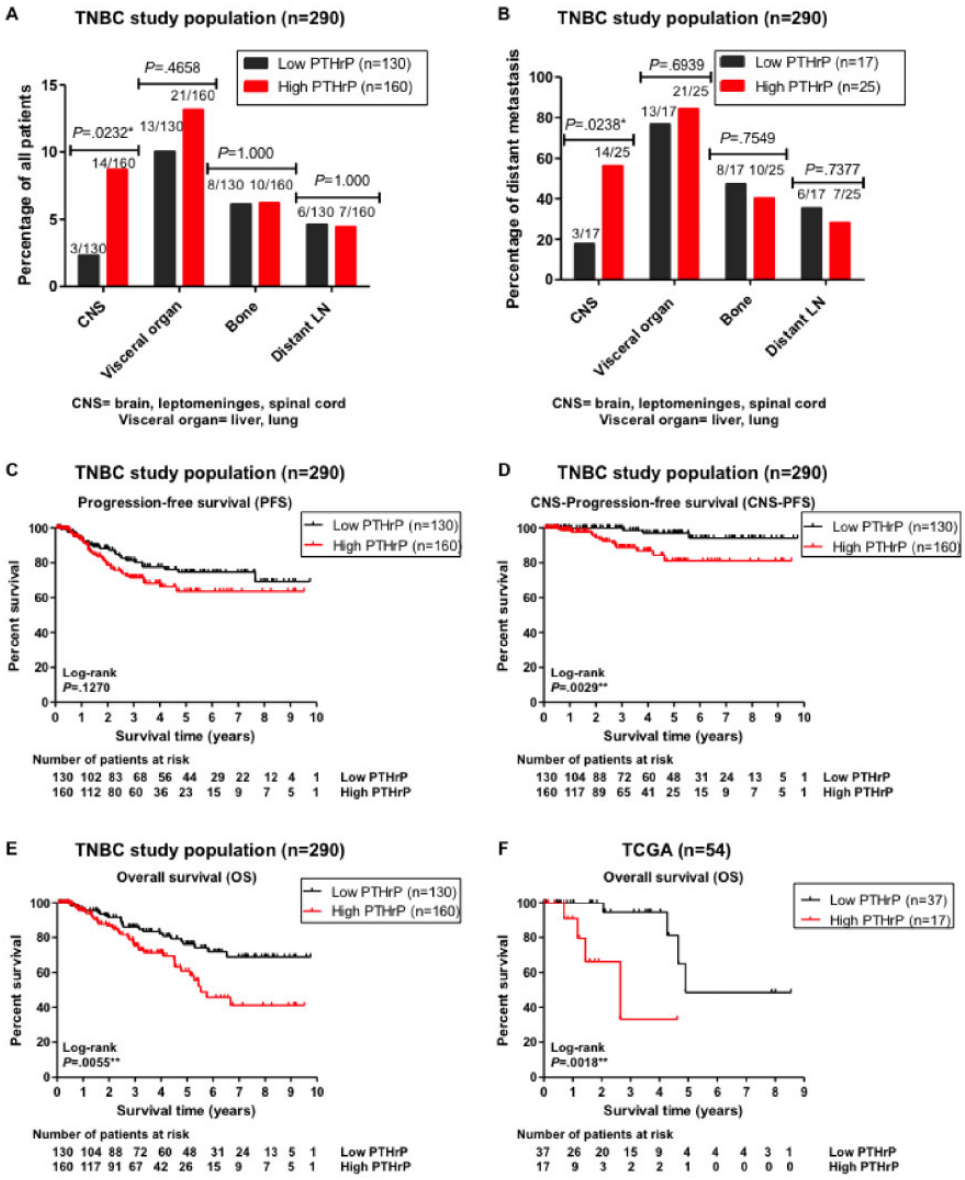

Figure 2. High parathyroid hormone-related protein (PTHrP) expression is statistically significantly associated with increased rate of central nervous system (CNS) metastasis and with short CNS-progression-free survival (PFS) and overall survival (OS) in triple-negative breast cancer (TNBC) in univariate analysis. (**A,B**) Association using Fisher exact test between PTHrP expression and organ-specific metastasis in all TNBC patients (n = 290) (A) and in TNBC patients with distant metastasis (n = 42) (B). Values above the histobars indicate the number of patients in each category. (**C–E**) Kaplan-Meier survival curves of PFS (C), CNS-PFS (D), and OS (E) according to PTHrP expression in our TNBC study population (n = 290). (**F**) Kaplan-Meier survival curve of OS according to PTHrP expression in TNBC using The Cancer Genome Atlas data (n = 54 of 55 patients). One patient was excluded due to missing survival time information. *P* values ≤ .05 were considered statistically significant. *P ≤ .05; **P ≤ .01. LN = lymph node.

population ($P < .0001$) (Supplementary Figure 3, available online). We found that PTHrP had statistically significant prognostic value for OS only in the LN-negative subgroup using Kaplan-Meier analysis ($P = .0272$) (Figure 3A; Supplementary Figure 4, available online). Strikingly, high PTHrP expression emerged as an independent negative prognostic factor for OS (HR = 2.423, 95% CI = 1.129 to 5.197, $P = .0231$) in multivariable analysis including co-variates statistically significant in univariate analysis of the LN-negative subpopulation (Table 3), with a HR comparable with that of LN-positivity (N2) in the whole population (Table 2). Treatment with adjuvant chemotherapy was an independent prognostic factor for improved OS (HR = 0.285, 95% CI = 0.111 to 0.733, $P = .0091$) in node-negative disease (Table 3). These results indicate that PTHrP independently predicts OS in LN-negative TNBC.

Prognostic Significance of PTHrP Expression in Lymph Node-Negative TNBC Based on Adjuvant Chemotherapy

Clinico-pathologic risk factors including age, tumor grade, LVI, and tumor size are mostly used to inform therapeutic decisions in node-negative BC (7). Accordingly, analysis of the association between adjuvant chemotherapy and clinico-pathologic parameters of LN-negative patients in our cohort recapitulates these standard clinical risk stratification criteria (Supplementary

Table 2. Multivariable proportional hazards regression analyses of predictors for CNS-PFS and OS of all TNBC patients (288 ≤ n ≤ 290)

TNBC patients	Multivariable analysis CNS-PFS (n = 290)		Multivariable analysis‡ OS (n = 288)	
Parameter†	HR (95% CI)	P§	HR (95% CI)	P§
PTHrP*				
High	5.014 (1.421 to 17.692)	.0122	1.590 (0.925 to 2.736)	.0936
Age at diagnosis				
≤50 y	–	–	0.777 (0.443 to 1.363)	.3790
Tumor size, cm				
T2 (2–5)	–	–	1.305 (0.744 to 2.290)	.3525
T3 (>5)	–	–	2.246 (0.897 to 5.627)	.0841
LN status				
N1	0.769 (0.096 to 6.163)	.8045	2.191 (0.958 to 5.012)	.0633
N2	3.262 (1.218 to 8.733)	.0186	2.970 (1.672 to 5.276)	.0002
Type of surgery				
MRM	–	–	1.685 (0.955 to 2.972)	.0715
Adjuvant chemotherapy*				
Yes	–	–	0.362 (0.208 to 0.631)	.0003

*Cases with missing information: PTHrP expression (24 cases), adjuvant chemotherapy (two cases). CI = confidence interval; CNS-PFS = central nervous system-progression-free survival; HR = hazard ratio; LN = lymph node; MRM = modified radical mastectomy; N1 = 1 to 3 positive LN; N2 ≥3 positive LN; OS = overall survival; PTHrP = parathyroid hormone-related protein; TNBC = triple-negative breast cancer.

†The multivariable model included only statistically significant variables that converged from the univariate analysis.

‡Multivariable analysis was performed on n = 290 (for CNS-PFS) or n = 288 (for OS) of 314 TNBC patients from whom matching data were available for PTHrP expression and for all the included clinico-pathologic factors.

§Likelihood-ratio test analyses were two-sided. P values ≤ .05 were considered statistically significant.

Table 5, available online). Patients who did not receive adjuvant chemotherapy are referred to as low-clinical risk and those who did as high-clinical risk patients.

Because PTHrP overexpression is an independent predictor of OS in the LN-negative subgroup, we sought to investigate its prognostic value based on adjuvant chemotherapy treatment. We found that PTHrP was a statistically significant prognostic factor selectively in the low-clinical risk subgroup ($P = .0387$) (Figure 3, B and C), wherein it is the only statistically significant factor for OS (HR = 2.576, 95% CI = 1.019 to 6.513, $P = .0456$) (Supplementary Table 6, available online). Collectively, these data suggest that LN-negative patients with low clinical risk who have high PTHrP expression and poor OS should have been eligible for adjuvant chemotherapy. Hence, PTHrP IHC scoring analysis could be potentially added to standard clinico-pathologic criteria to select newly diagnosed LN-negative TNBC patients for adjuvant chemotherapy.

Discussion

We investigated the clinical significance of PTHrP in TNBC using immunohistochemical analysis of PTHrP expression in a TMA constructed from archived primary tumors of patients newly diagnosed with TNBC. In this population-based study, although PTHrP was not statistically significantly correlated to PFS, we uncovered for the first time, to the best of our knowledge, a statistically significant association between high PTHrP expression and increased rate of CNS metastasis in TNBC patients. Remarkably, PTHrP overexpression emerged as a statistically significant independent negative prognostic factor for CNS-PFS.

Interestingly, our analysis of transcriptomic data from bc-GenExMiner correlating gene expression with BC molecular subtypes showed a statistically significant positive correlation between *PTHLH* and *TGF-β1* (specifically in TNBC) and between *PTHLH* and *ANGPTL4* (specifically in TNBC and basal-like subtypes) (Supplementary Table 2, available online). A previous study showed that the brain metastatic TNBC cell line MDA-231BR increased PTHrP production in response to TGF-β1 compared with the parental MDA-MB-231 cell line (44). *ANGPTL4* is known to promote BC cells extravasation through the nonfenestrated capillaries of the brain and lungs (45–47). Furthermore, TGF-β1 has been shown to induce ANGPTL4 in breast cancer cells, which disrupts vascular endothelial junctions and promotes metastasis (46). The relationship identified in our in silico analysis between *PTHLH* and *TGF-β1* and between *PTHLH* and *ANGPTL4* could be related to the tumor-suppressive function of the microRNA-520/373 family in ER-negative BC. Expression of miR-520c was inversely correlated to LN metastasis in ER-negative but not ER-positive tumors, and miR-520/373 mediated direct suppression of *TGFBR2* and decreased expression of Smad-dependent tumor-promoting genes, including *PTHLH* and *ANGPTL4* (48). Additional studies are warranted to provide mechanistic insights into the potential context-specific TGF-β-mediated regulation of PTHrP and ANGPTL4 to promote BM in TNBC.

The association between BM and decreased survival in TNBC (49) highlights the need to elucidate the mechanisms underlying BM in TNBC (43,47,50) and identify new targets to hinder tumor progression to the brain. Our findings, if validated in other cohorts of early-stage, newly diagnosed TNBC patients, raise the hypothesis that monitoring TNBC patients with high PTHrP expression using brain imaging screening to detect early stages of BM might identify patients with first recurrent BM. This might ultimately increase their benefit from whole-brain radiotherapy, stereotactic radiosurgery, surgical resection, or chemotherapy in combination with novel targeted therapies or immunotherapy (51,52).

Intriguingly, although PTHrP has been previously linked to bone metastasis in BC (14,38,53,54), we did not identify a

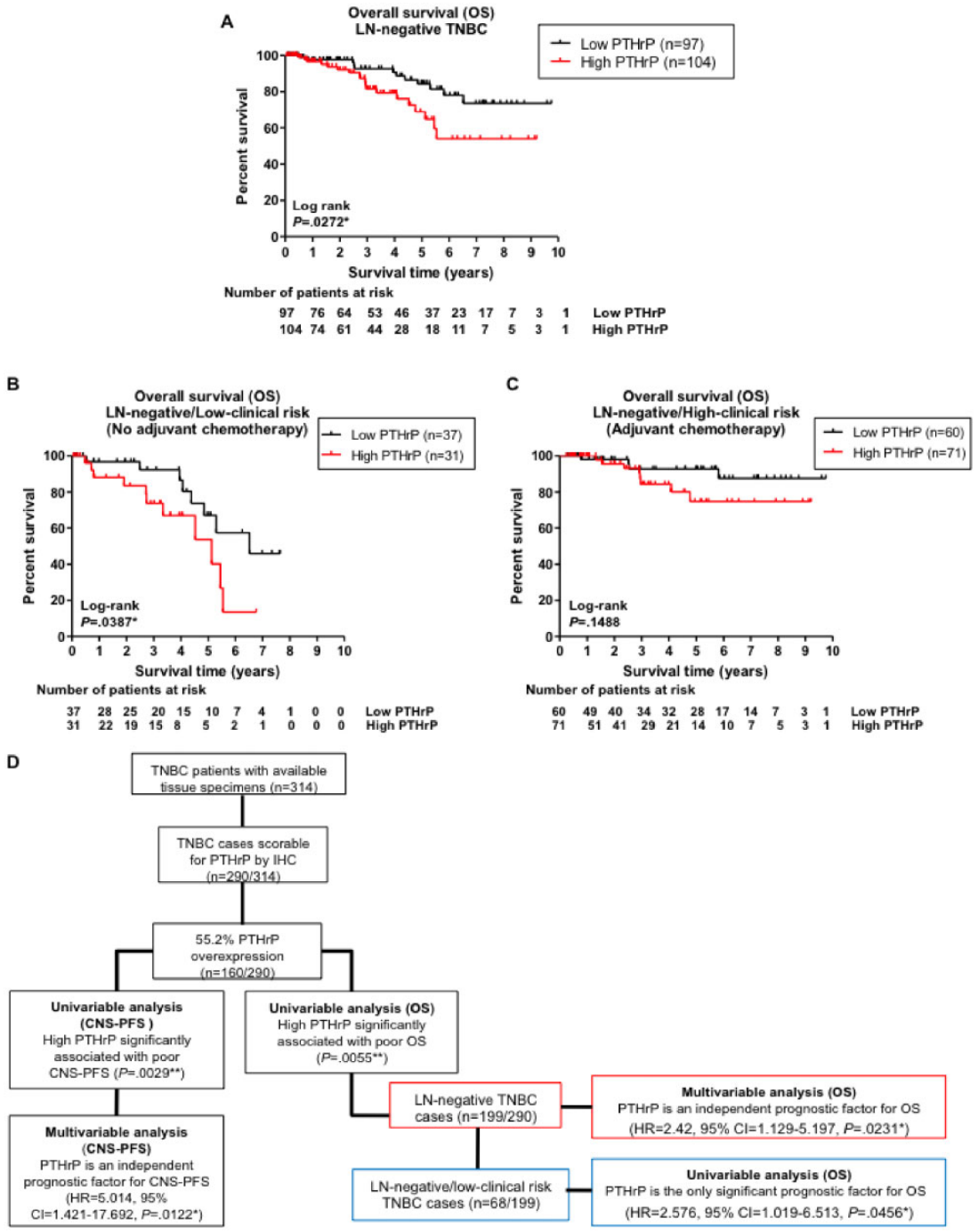

Figure 3. High parathyroid hormone-related protein (PTHrP) expression is statistically significantly associated with short overall survival (OS) in lymph-node (LN)-negative and LN-negative/low-clinical risk triple-negative breast cancer (TNBC) in univariate analysis. (**A**) Kaplan-Meier curves of overall survival based on PTHrP status in LN-negative TNBC (n = 201). (**B**,**C**) OS based on PTHrP status in LN-negative TNBC patients with low clinical risk (n = 68) (B) or high clinical risk (n = 131) (C). Low-clinical risk patients refer to patients who did not receive adjuvant chemotherapy, whereas high-clinical risk patients are the ones who received adjuvant chemotherapy. (**D**) Diagram representing the analysis workflow and summarizing the main findings of this study. CI = confidence interval; CNS-PFS = central nervous system progression-free survival; HR = hazard ratio; IHC = immunohistochemistry. *P* values ≤ .05 were considered statistically significant. *$P \leq .05$; **$P \leq .01$.

statistically significant relationship between PTHrP overexpression and increased incidence of bone metastasis in the TNBC study population. This could be attributed to a potential difference between PTHrP expression in the primary tumors of early-stage TNBC patients and bone metastatic sites. Of note, TNBC is known to have the lowest incidence of bone metastasis compared with other BC subtypes (55).

Our results demonstrated that PTHrP expression had a statistically significant prognostic value in TNBC, where it was associated with worse OS of patients in univariate analysis. These data are in line with several studies showing that PTHrP expression in BC is correlated with poor patient survival (14,38,53,54,56). PTHrP overexpression was an independent negative prognostic factor for OS specifically in the LN-negative

Table 3. Univariate and multivariable proportional hazards regression analyses of predictors for overall survival of TNBC patients with lymph node-negative status ($197 \leq n \leq 216$)

OS LN-negative TNBC patients	Univariate analysis ($201 \leq n \leq 216$)		Multivariable analysis‡ ($n = 197$)	
Parameter†	HR (95% CI)	P§	HR (95% CI)	P§
PTHrP*				
High	2.238 (1.076 to 4.656)	.0312	2.423 (1.129 to 5.197)	.0231
Age at diagnosis				
≤50 y	0.350 (0.165 to 0.741)	.0061	0.919 (0.359 to 2.348)	.8591
Grade*				
2	0.405 (0.101 to 1.621)	.2016	0.562 (0.139 to 2.273)	.4189
3	0.246 (0.073 to 0.823)	.0228	0.587 (0.158 to 2.172)	.4245
Adjuvant chemotherapy*				
Yes	0.314 (0.155 to 0.638)	.0014	0.285 (0.111 to 0.733)	.0091
Tumor size, cm				
T2 (2–5)	0.853 (0.405 to 1.797)	.6758		
T3 (>5)	1.879 (0.433 to 8.150)	.3992		
LVI				
Positive	0.690 (0.284 to 1.682)	.4160		
Type of surgery				
MRM	1.007 (0.502 to 2.021)	.9837		
Adjuvant RT				
Yes	1.062 (0.527 to 2.142)	.8658		

*Cases with missing information: PTHrP expression (15 cases), grade (two cases), adjuvant chemotherapy (two cases). CI = confidence interval; HR = hazard ratio; LN = lymph node; LVI = lympho-vascular invasion; MRM = modified radical mastectomy; OS = overall survival; PTHrP = parathyroid hormone-related protein; RT = radiotherapy; TNBC = triple-negative breast cancer.

†The multivariable model included only statistically significant variables from the univariate analysis.

‡Multivariable analysis was performed on 197 of 216 LN-negative TNBC patients from whom matching data were available for PTHrP expression and for all the included clinico-pathologic factors.

§Likelihood-ratio test analyses were two-sided. *P* values ≤ .05 were considered statistically significant.

subgroup in multivariable analysis, wherein it increased the risk of death (HR = 2.423) to an extent similar to LN-positivity N2 in the whole population (HR = 2.970), suggesting potential implications in clinical management of TNBC. Indeed, current prognostic factors fail to accurately identify high-risk node-negative BC patients, and microarray-based biomarkers for adjuvant chemotherapy decisions, including those from the MammaPrint assay, are not useful for patients with TNBC (9). Although previous IHC studies have successfully identified biomarkers with statistically significant prognostic value for predicting recurrence and survival in node-negative BC and TNBC (24,57), their findings failed to translate into the clinical setting to tailor patients' treatment and surveillance strategies. Our discovery that a PTHrP biomarker-based test might independently stratify survival of LN-negative patients with low clinical risk in our cohort suggests its potential clinical utility to inform decisions on adjuvant systemic chemotherapy in node-negative TNBC, if confirmed in other independent datasets.

Limitations to our study include its retrospective design, which underscores the need to investigate the predictive value of PTHrP for adjuvant chemotherapy selection of node-negative patients in a future prospective randomized clinical trial. Our single-cancer institution analysis offers the advantage of standardized diagnostic, treatment, and follow-up procedures in a large sample size cohort including only triple-negative subtype. However, we cannot extend our findings to other TNBC cohorts with predominant representation of a specific ethnic/race group (58). Noteworthy, we validated the prognostic value of PTHrP and its association with CNS metastasis using TCGA and bcGenExMiner, respectively.

In conclusion, we identified PTHrP as a novel independent prognostic biomarker for CNS metastasis with potential clinical value for adjuvant chemotherapy selection of node-negative patients in TNBC. PTHrP, a secreted factor detectable in the serum (15), could be used to noninvasively investigate therapeutic monitoring of patients diagnosed with TNBC. Future PTHrP-targeted strategies might provide an alternative treatment option for this molecularly and clinically distinct group of TNBC.

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