Parathyroid hormone-related protein expression, in combination with nodal status, predicts bone metastasis and prognosis of breast cancer patients

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Abstract. Parathyroid hormone-related protein (PTHrP) has been known to play an important role in the formation of metastatic lesions in the bone. However, there remains controversy over its practical role in predicting the occurrence of bone metastasis and the prognosis of breast cancer patients. In this study, we attempted to investigate the clinical value of PTHrP expression status in the primary lesions of breast cancer patients. We immunohistochemically investigated PTHrP expression in surgically resected specimens from 125 primary breast cancer patients whose clinicopathological background and long-term prognosis were available. Positive PTHrP staining was demonstrated in 79 (63.2%) tumors. PTHrP was expressed significantly more frequently in the tumors of premenopausal patients. Bone metastases were significantly more common in patients with T4 tumors, with a positive node, with distant metastasis and with PTHrP-positive tumors. Multivariate logistic analysis revealed positive PTHrP expression as an independent risk factor for predicting bone metastasis. PTHrP expression was significantly related to a shorter overall survival. Bone metastasis was found significantly more frequently (28.3%) in PTHrP- and node-positive cases than in double-negative cases, and the rate was more pronounced in postmenopausal cases (32.1%). Expression of PTHrP in primary lesions, in combination with positive nodal status, is indicative of an increased risk of bone metastasis in breast cancer patients.

Introduction

Bone is one of the most preferential metastatic locations in patients with breast cancer. Although bone metastasis itself rarely becomes a life-threatening lesion, it often impairs the quality of a patient's life due to pathological fracture and/or severe pain (1). To develop bone metastasis, cancer cells have to invade the surrounding tissues, escape from the primary site, survive within the blood stream, adhere to the target, and migrate out of the vessel into the bone marrow. Subsequent to going through these common stages of metastasis, certain special features of the cancer cells in cooperation with the microenvironment of the bone marrow are considered to be prerequisite in establishing a metastatic bone lesion. One of the most important capacities required is the potential to produce parathyroid hormone-related protein (PTHrP) (2,3).

PTHrP is a cytokine, which was originally identified as a causative factor of malignant humoral hypercalcemia (4). The bone matrix contains a variety of growth factors, such as insulin-like growth factor (IGF), transforming growth factor β (TGF-β), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and Ca^{++}. These factors are released by bone resorption. Among these, TGF-β and Ca^{++} bind to the TGF-β receptor and calcium-sensing receptor of the cancer cells, respectively, to stimulate PTHrP production (5,6). PTHrP then stimulates the osteoblasts to upregulate the ligand of receptor activator of nuclear factor κ β (RANKL) expression. RANKL bound with RANK is expressed in premature osteoclasts, and promotes differentiation into mature osteoclasts (7). Finally, bone resorption is activated by mature osteoclasts, completing a 'vicious cycle' that accelerates bone resorption, coinciding with the growth of a metastatic bone lesion stimulated by other growth factors.

PTHrP is reported to be produced in various malignant tumors such as breast, prostate and lung cancer. These are the cancers that frequently result in bone metastasis (2,8). More than 60% of cancerous lesions of the breast were reported to secrete PTHrP (9-16). In several studies, the patients who subsequently develop bone metastasis showed higher or more frequent PTHrP expression than those without bone metastasis (9,11,14,17-19). Several previous studies have, thus, suggested that PTHrP expression contributes to the formation and development of bone metastasis in breast cancer patients (2,5,18,20,21). However, controversy still exists over the clinical or prognostic significance of PTHrP expression in cancer cells in the primary site. Several studies have reported that PTHrP expression in the primary lesion correlates with

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the decreased risk of developing bone metastasis (13) or the favorable prognosis in breast cancer patients (9,15,16).

Herein, we investigated the expression of PTHrP in primary lesions of breast cancer, and attempted to reveal the role of its expression by analyzing its relationship to clinicopathological characteristics by enrolling patients at various stages of primary breast cancer, treated in a university hospital, whose long-term prognosis was available. We found a potential role of PTHrP expression in the formation of bone metastasis according to the status of local disease.

Materials and methods

Clinical materials. One hundred and twenty-five surgical specimens from patients with primary breast cancer who underwent surgery at the Department of Surgical Oncology, Osaka City University Hospital, between 1996 and 1999 were investigated. All the patients were women. Their ages ranged from 31 to 88 years (mean 57.4) at the time of surgery. Cases who received either preoperative chemotherapy or endocrine therapy were excluded from this study. The patients were followed up for 5 to 243 months. The median follow-up period was 97 months. The patients were followed up with ultrasonography for determining local recurrence, computed tomography for evaluating metastatic lesions in the lung and the liver, and bone scintigraphy to detect bone metastasis annually, as well as physical examination every six months. The patient clinicopathological features are presented in Table I. Postoperative adjuvant therapies, including chemotherapy, hormonal therapy, and radiation therapy were conducted according to the status of the disease and the condition of the patient. No patient had received elective administration of bisphosphonates. Thirty-five patients were premenopausal and 90 patients were postmenopausal. Thirty-four patients had a tumor without skin or chest wall invasion equal to or less than 2 cm in diameter (T1), 63 patients had a tumor of 2.1 to 5 cm (T2) and 14 patients had a tumor of more than 5 cm (T3). Fourteen patients had a tumor with skin or chest wall invasion of any size (T4). Seventy patients had lymph node metastasis and 9 had distant metastasis at the time of initial surgery. Estrogen receptor (ER) was expressed in 61 patients. Twenty cases (16.0%) developed bone metastasis, 13 developed visceral metastasis, 9 developed local recurrence and 25 succumbed to breast cancer during follow-up.

Immunohistochemical techniques. To study PTHrP expression in the tissues, immunohistochemical staining was used (21). Formalin-fixed, paraffin-embedded tissue blocks were obtained from each patient including normal mammary glands and cancerous lesions. Sections were dewaxed, and antigen retrieval was performed by autoclaving in Target Retrieval Solution (Dako Cytomation, Carpinteria, CA, USA) at 105˚C for 15 min, followed by incubation with 0.3% hydrogen peroxide in methanol for 30 min. Following blocking with 10% normal rabbit serum to reduce nonspecific antibody binding, mouse monoclonal antibody against PTHrP (100 μg/ml) (Oncogene Science Inc., Uniondale, NY, USA) was reacted with tissue sections overnight at 4˚C followed by three washes with PBS. The sections were incubated with biotinylated rabbit anti-mouse immunoglobulin G (IgG), then reacted with streptoavidin-biotin peroxidase reagent (Histofine Kit; Nichirei Co. Tokyo, Japan). Finally, diaminobenzidine and 1% hydrogen peroxide were applied as a chromogen, and counterstaining was carried out with hematoxylin. Normal mouse IgG was substituted for the primary antibody as the negative control. The sections were then assessed independently by two investigators without knowledge of the clinical outcome of the patient. Tumors were classified as being PTHrP-positive when more than 10% of the cancer cell was positively stained.

Statistics. Mann-Whitney's U test was used to define statistical difference. Survival curves for patients were calculated using the Kaplan-Meier method and analyzed using the log-rank test. The multivariate analysis concerning bone metastasis was evaluated by logistic regression analysis, and multiple factors concerning overall survival were calculated using the Cox regression test. Statistical significance was defined as $P<0.05$. All analyses were carried out using the analytical software SPSS (SPSS Inc., Chicago, IL, USA).

Results

Expression of PTHrP. PTHrP expression was observed in 79 out of 125 cases (63.2%) and 46 (36.8%) were negative for PTHrP expression in tumor cells. The relationship between PTHrP expression and clinicopathological findings is presented in Table I. No relationship was demonstrated between PTHrP expression and tumor size, lymph node metastasis, estrogen receptor status, lymphatic infiltration, or vascular invasion. PTHrP was statistically more frequently expressed in the tumors of premenopausal women (80%) compared with those of postmenopausal women (57%). There were three cases that had bone metastasis at the initial diagnosis. PTHrP expression in the primary lesion was observed in each of the three cases.

Factors related to bone metastasis. The relationship between initial (at the time of surgery) and late (during the postoperative follow-up period) bone metastasis and clinicopathological findings is presented in Table II. There was no factor that significantly related to the occurrence of initial bone metastasis. However, patients with a T4 tumor, or with positive nodal status significantly more commonly developed late bone metastasis. Patients with a PTHrP-expressing tumor tended to develop late bone metastasis more often than those with a PTHrP-negative tumor, and positive PTHrP expression in the tumor was significantly related to the development of bone metastasis at any time.

Multivariate logistic regression analysis. Multivariate logistic regression analysis between bone metastasis and clinicopathological findings is presented in Table III. PTHrP status was an independent risk factor for bone metastasis (HR, 7.104; 95% CI, 1.782-48.110; P=0.0037). Having a T4-tumor was also an independent risk factor for bone metastasis (HR, 5.124; 95% CI, 1.169-24.243; P=0.0303). Menopausal status, lymph node metastasis, estrogen receptor status, lymphatic involvement and venous involvement did not affect bone metastasis.

Nodal involvement status and bone metastasis. The relationship between bone metastasis and PTHrP expression in

Table I. Relationship between PTHrP expression and clinicopathological findings.

Factor	No. of patients	PTHrP expression		P-value
		Negative, n (%)	Positive, n (%)	
Menopausal status				
Premenopausal	35	7 (20.0)	28 (80.0)	0.015
Postmenopausal	90	39 (53.3)	51 (56.7)	
T category				
T1	34	17 (50.0)	17 (50.0)	n.s.
T2	63	20 (31.7)	43 (68.3)	
T3	14	5 (35.7)	9 (64.3)	
T4	14	4 (18.6)	10 (71.4)	
Nodal status				
(-)	55	22 (40.0)	33 (60.0)	n.s.
(+)	70	24 (34.3)	46 (65.7)	
Distant metastasis				
M0	116	46 (39.7)	70 (60.3)	0.044
M1	9	0 (0)	9 (100)	
Estrogen receptor				
(-)	64	22 (34.4)	42 (65.6)	n.s.
(+)	61	24 (39.3)	37 (60.7)	
Lymphatic infiltration[a]				
(-)	59	24 (41.7)	35 (59.3)	n.s.
(+)	61	19 (31.1)	42 (68.9)	
Vascular invasion[a]				
(-)	116	39 (42.7)	67 (57.3)	n.s.
(+)	14	4 (28.6)	10 (71.4)	
Total	125	46 (36.8)	79 (63.2)	

[a]Lymphatic infiltration and vascular invasion were not evaluated in 5 cases. n.s., not significant.

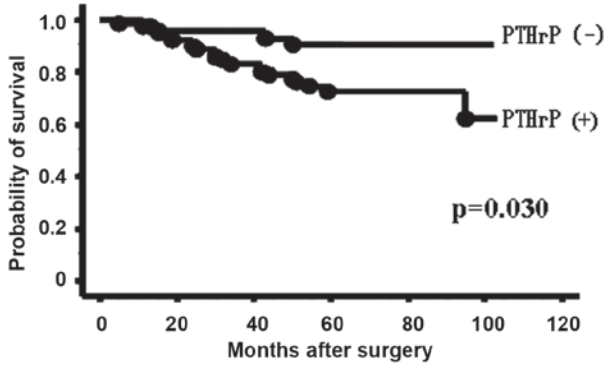

Figure. 1. Overall survival of 116 patients without initial metastatic disease according to PTHrP status.

combination with nodal involvement status is presented in Table IV. Patients were divided into four groups according to the status of PTHrP expression and lymph node metastasis. Initial bone metastasis was not observed in patients of the double-negative group, on the other hand the highest rate (4.3%) of bone metastasis was shown in those of the double-positive group. This tendency was pronounced when the relationship between late bone metastasis was examined. In the double-positive group, the risk of late bone metastasis (28.3%) was significantly higher than that in the double-negative group (0%). The occurrence of bone metastasis was increased by adding nodal and menopausal status to the PTHrP expression status, and as high as 32% of the cases with PTHrP-positive tumor, node-positive, and postmenopausal status developed late bone metastasis.

Overall survival. Fig. 1 presents the overall survival of the 116 patients without initial metastatic disease according to PTHrP status. The patients with PTHrP expression had significantly poorer outcomes than those without PTHrP expression (P=0.030). Five-year survival rate was 94.0% in the patients without PTHrP expression, falling to 77.3% in those with PTHrP expression. Multivariate analysis for overall survival (Table V) showed that PTHrP expression (RR, 3.644; 95% CI, 1.182-15.880; P=0.022) and tumor size (RR, 5.028; 95% CI, 1.665-15.589; P=0.005) were independently correlated with shorter overall survival.

Table II. Relationship between bone metastasis and clinicopathological findings.

Factors at the time of surgery	No. of patients	Bone metastasis (BM)			P-value	
		Initial [n (%)]	Late [n (%)]	Total [n (%)]	Late vs. no BM	Total vs. no BM
Menopausal status						
Premenopausal	35	1 (2.9)	6 (17.1)	7 (20.0)	n.s.	n.s.
Postmenopausal	90	2 (2.2)	14 (15.6)	16 (17.8)		
T category						
T1	34	0 (-)	3 (8.8)	3 (8.8)	0.014[b]	0.004[b]
T2	63	2 (3.2)	11 (17.5)	13 (20.6)		
T3	14	0 (-)	1 (7.1)	1 (7.1)		
T4	14	1 (7.1)	5 (35.6)	6 (42.9)		
Nodal status						
(-)	55	1 (1.8)	4 (7.3)	5 (9.1)	0.018	0.017
(+)	70	2 (2.9)	16 (22.9)	18 (25.7)		
Distant metastasis						
M0	116	0 (-)	15 (12.9)	15 (12.9)	0.004	0.000
M1	9	3 (33.3)	5 (55.6)	8 (88.9)		
Estrogen receptor						
(-)	64	3 (4.7)	7 (10.9)	10 (15.6)	n.s.	n.s.
(+)	61	0 (-)	13 (21.3)	13 (21.3)		
Lymphatic infiltration[a]						
(-)	59	1 (1.7)	10 (17.0)	11 (18.6)	n.s.	n.s.
(+)	61	2 (3.3)	8 (13.1)	10 (16.4)		
Vascular invasion[a]						
(-)	116	2 (1.7)	16 (13.8)	18 (15.5)	n.s.	n.s.
(+)	14	1 (7.1)	2 (14.3)	3 (21.4)		
PTHrP expression						
(-)	46	0 (-)	3 (6.5)	3 (6.5)	0.051	0.018
(+)	79	3 (3.8)	17 (21.5)	20 (25.3)		
Total	125	3 (2.4)	20 (16.0)	23 (18.4)		

[a]Lymphatic infiltration and vascular invasion were not evaluated in 5 case. [b]P-value of T1-3 vs 4, otherwise not significant.

Discussion

Previous studies have demonstrated that the expression of PTHrP in a primary breast tumor had a negative impact on the prognosis of the patient (14), as demonstrated by the positive correlation with advanced clinical stages (12,17) and with developing bone metastasis (11,12). These observations were in line with our results. PTHrP expression in the primary breast lesion in cases who had already developed bone metastasis has also been frequently demonstrated (12,23), as in the present series. Moreover, it has been clearly described that PTHrP was almost always expressed in the metastatic lesions in the bone (9,11,14,18,23). These investigations clearly demonstrate the essential role of PTHrP expression in the formation of bone metastasis. However, several studies have suggested PTHrP expression in the tumor might act against its progression, and be associated with better prognosis during early stage (15,23), and non-invasive breast cancer (16). Experimental analyses have also suggested that PTHrP has little involvement in the

Table III. Multivariate logistic regression analysis between bone metastasis and clinicopathological findings.

Factor	Odds ratio	95% CI	P-value
PTHrP	7.104	1.7815-48.1101	0.0037
Menopausal status	1.244	0.4059-4.1746	0.7083
T stage	5.123	1.1690-24.2426	0.0303[a]
Nodal status	3.229	0.9912-12.1161	0.0518
Estrogen receptor	1.577	0.5323-4.8187	0.4097
Lymphatic infiltration	0.331	0.0870-1.1042	0.0728
Vascular invasion	0.557	0.0704-2.8288	0.5024

[a]P-value of T1-3 vs 4, otherwise not significant.

Table IV. Relationship between bone metastasis and PTHrP expression in combination with nodal involvement status.

PTHrP	Nodal status	No. of patients	Initial bone metastasis (%)	Late bone metastasis (%)	P-value
(-)	(-)	22	0 (0.0)	0 (0.0)	
	(+)	24	0 (0.0)	3 (12.5)	
(+)	(-)	33	1 (3.0)	4 (12.1)	
	(+)	46	2 (4.3)	13 (28.3)	
Total		125	3 (2.4)	20 (16.0)	<0.01[a]

[a]P-value of PTHrP(-)/N(-) vs PTHrP(+)/N(+), otherwise not significant.

Table V. Multivariate analysis for overall survival was performed using Cox regression analysis.

Factor	Relative Risk	95% CI	P-value
PTHrP	3.644	1.1821-15.8803	0.0226
Menopausal status	0.753	0.3200-1.8149	0.5188
T stage	5.028	1.6654-15.5891	0.0046[a]
Nodal status	1.506	0.5759-4.1956	0.4058
Estrogen receptor	0.807	0.3134-2.0563	0.6509
Lymphatic infiltration	0.828	0.2969-2.2623	0.7108
Vascular invasion	0.683	0.1039-2.5606	0.6083

[a]P-value of T1-3 vs 4, otherwise not significant. CI, confidence interval.

initial stages of metastasis (24,25). These observations suggest that PTHrP expression plays a role in preventing breast cancer progression while the disease remains *in situ*. Taken together, these findings suggest that PTHrP expression in breast cancer has dual roles according to the disease stage; inhibiting progression in the primary site and promoting it in the bone marrow.

Similarly to previous studies (11,12,14,17), we were able to demonstrate that PTHrP expression in the primary lesion was indicative of poorer outcome. A significantly worse survival was also demonstrated when patients with stage IV disease were excluded. However, one previous study involving 526 patients suggested the opposite result (23). There were certain unique points to their study, compared with the others. Tumors were judged positive for PTHrP when staining was observed in any cell. This criterion led to a positive rate of as high as 80% in their study. As a consequence the representative figures might be altered significantly by a small number of highly malignant patients in the PTHrP-negative group. The occurrence of bone metastasis was more than 30% and the survival rate was 51% at 10 years in the PTHrP-negative cases, which differed from our observation. We also assumed there were differences in the nature of the tumor according to the race or the volume of therapeutic intervention following surgery.

In the present study, we found that a combination analysis of PTHrP expression with nodal status clearly indicated the risk of bone metastasis during the post-operative follow-up period. In breast cancer, lymph node involvement is known as the most reliable clinical indicator for prognosis. Nodal status may also be practically used as a decision maker in performing adjuvant chemotherapy. Cancer cells found in the regional lymph node may be interpreted as the disease having already spread to the whole body. Braun *et al* reported that patients with lymph node involvement significantly more often had bone marrow micrometastasis than node-negative patients (26). Furthermore, Wulf *et al* demonstrated the evidence of disseminated PTHrP-expressing breast cancer cells to the peripheral blood and also bone marrow (27). It is easy to imagine that, when disseminated cancer cells have the ability to express PTHrP in response to the microenvironment of the bone marrow, forming metastatic lesions in the bone might confer an advantage to these cases. Therefore, by combining positive nodal status as an indicator of cancer cell dissemination to the circulation, the potential value of PTHrP expression in the primary lesion is pronounced in predicting bone metastasis, as shown in this study.

Aromatase inhibitors and LH-RH agonists are the key drugs for patients with hormone receptor-positive breast cancer and they reduce the recurrence rate significantly by regulating estrogen production. At the same time, they have the adverse effects on the bone of decreasing bone mineral density. Early menopause induced by adjuvant chemotherapy may also have a similar effect on bone. Bone resorption caused by these anti-cancer therapies results in the release of bone growth factors, which may unintentionally activate the formation of bone metastases by beginning the 'vicious cycle' (28). In the present study, we demonstrated that menopausal status might have a role in increasing the risk of late bone metastasis, especially in node-positive patients with a PTHrP-positive tumor. Thus, not only avoiding osteoporosis or fracture, but also to protect against bone metastasis, careful surveillance of bone resorption might be advisable.

Several strategies have been devised to prevent bone metastasis during breast cancer treatments. Bisphosphonate is able to protect against resorption of the bone by downregulating the function of osteoclasts (29), preventing cancer cell activation by reducing cytokine release from the bone marrow and resulting in protection against skeletal bone events (30). Recent studies have also demonstrated the possible impact of bisphosphonates in protecting against bone metastasis in breast cancer patients (31). Experimental evidence shows that humanized monoclonal antibody against PTHrP suppresses osteolytic bone metastasis of human breast cancer cells (32). Anti-RANKL antibody is also a promising drug being investigated for protection against the progression of bone metastasis (33). These novel agents could be useful in preventing development or progression of bone metastasis in high risk cases.

In conclusion, our results indicate that patients with PTHrP-producing tumors potentially risk development of bone metastasis. The risk increased predominantly in patients with lymph node metastasis who may already have systemic micrometastasis, especially when undergoing hormonal changes associated with postmenopausal status. Investigation of PTHrP expression of the primary lesion, in combination with lymph node metastasis is, thus, suggested to be a novel useful marker for predicting the risk of bone metastasis in breast cancer patients.

References

1. Coleman RE: Skeletal complications of malignancy. Cancer 80: 1588-1594, 1997.
2. Mundy GR: Metastasis to bone: causes, consequences and therapeutic opportunities. Nat Rev Cancer 2: 584-593, 2002.
3. Yoneda T and Hiraga T: Crosstalk between cancer cells and bone microenvironment in bone metastasis. Biochem Biophys Res Commun 328: 679-687, 2005.
4. Suva LJ, Winslow GA, Wettenhall RE, *et al*: A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. Science 237: 893-896, 1987.
5. Yin JJ, Selander K, Chirgwin JM, *et al*: TGF-β signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. J Clin Invest 103: 197-206, 1999.
6. Sanders JL, Chattopadhyay N, Kifor O, Yamaguchi T, Butters RR and Brown EM: Extracellular calcium-sensing receptor expression and its potential role in regulating parathyroid hormone-related peptide secretion in human breast cancer cell lines. Endocrinology 141: 4357-4364, 2000
7. Suda T, Takahashi N, Udagawa N Jimi E, Gillespie MT and Martin TJ: Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 20: 345-357, 1999.
8. Rubens RD: Clinical aspects of bone metastases. In: Tumor Bone Diseases and Osteoporosis in Cancer Patients. Body JJ (ed). Marcel Dekker, Inc., Brussels, p85, 2000.
9. Southby J, Kissin MW, Danks JA, *et al*: Immunohistochemical localization of parathyroid hormone-related protein in human breast cancer. Cancer Res 50: 7710-7716, 1990.
10. Liapis H, Crouch EC, Grosso LE, Kitazawa S and Wick MR: Expression of parathyroidlike protein in normal, proliferative, and neoplastic human breast tissues. Am J Pathol 143: 1169-1178, 1993.
11. Kohno N, Kitazawa S, Fukase M, *et al*: The expression of parathyroid hormone-related protein in human breast cancer with skeletal metastases. Surg Today 24: 215-220, 1994.
12. Downey SE, Hoyland J, Freemont AJ, Knox F, Walls J and Bundred NJ: Expression of the receptor for parathyroid hormone-related protein in normal and malignant breast tissue. J Pathol 183: 212-217, 1997.
13. Henderson M, Danks J, Moseley J, *et al*: Parathyroid hormone-related protein production by breast cancers, improved survival, and reduced bone metastases. J Natl Cancer Inst 93: 234-237, 2001.
14. Linforth R, Anderson N, Hoey R, *et al*: Coexpression of parathyroid hormone related protein and its receptor in early breast cancer predicts poor patient survival. Clin Cancer Res 8: 3172-3177, 2002.
15. Surowiak P, Dziegiel P, Matkowski R, Sopel M, Wojnar A, Kornafel J and Zabel M: Prognostic value of immunocytochemical determination of parathyroid hormone-related peptide expression in cells of mammary ductal carcinoma. Analysis of 7 years of the disease course. Virchows Arch 442: 245-251, 2003.
16. Fleming NI, Trivett MK, George J, *et al*: Parathyroid hormone-related protein protects against mammary tumor emergence and is associated with monocyte infiltration in ductal carcinoma in situ. Cancer Res 69: 7473-7479, 2009.
17. Bouizar Z, Spyratos F, Deytieux S, de Vernejoul MC and Jullienne A: Polymerase chain reaction analysis of parathyroid hormone-related protein gene expression in breast cancer patients and occurrence of bone metastasis. Cancer Res 53: 5076-5078, 1993.
18. Powell GJ, Southby J, Danks JA, *et al*: Localization of parathyroid hormone-related protein in breast cancer metastases: increased incidence in bone compared with other sites. Cancer Res 51: 3059-3061, 1991.
19. Kohno N, Kitazawa S, Sakoda Y, Kanbara Y, Furuya Y, Ohashi O and Kitazawa R: Parathyroid hormone-related protein in breast cancer tissues: relationship between primary and metastatic sites. Breast Cancer 1: 43-49, 1994.
20. Guise TA, Yin JJ, Taylor SD, *et al*: Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. J Clin Invest 98: 1544-1549, 1996.
21. Thomas RJ, Guise TA, Yin JJ, Elliot J, Horwood NJ, Martin TJ and Gillespie MT: Breast cancer cells interact with osteoblasts to support osteoclast formation. Endocrinology 140: 4451-4458, 1999.
22. Tezuka K, Onoda N, Takashima T, Ishikawa T, Wakasa T, Wakasa K and Hirakawa K: Clinical significance of intra-tumoral sinusoidal structures showing lympho-endothelial immunoreactivity in breast cancer. Oncol Rep 20: 25-32, 2008.
23. Henderson MA, Danks JA, Slavin JL, *et al*: Parathyroid hormone-related protein localization in breast cancers predict improved prognosis. Cancer Res 66: 2250-2256, 2006.
24. Torricelli C, Fortino V, Capurro E, *et al*: Role of PTHrp and PTHrp-engaged pathways in MCF-7 cells migration/invasion. Matrix Biol 25: 104-111, 2006.
25. Dittmer A, Vetter M, Schunke D, Span PN, Sweep F, Thomssen C and Dittmer J: Parathyroid hormone-related protein regulates tumor-relevant genes in breast cancer cells. J Biol Chem 281: 14563-14572, 2006.
26. Braun S, Vogl FD, Naume B, *et al*: A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med 353: 793-802, 2005.
27. Wulf GG, Jürgens B, Liersch T, *et al*: Reverse transcriptase/polymerase chain reaction analysis of parathyroid hormone-related protein for the detection of tumor cell dissemination in the peripheral blood and bone marrow of patients with breast cancer. J Cancer Res Clin Oncol 123: 514-521, 1997.
28. Guise TA, Kozlow WM, Heras-Herzig A, Padalecki SS, Yin JJ and Cirgwin JM: Molecular mechanisms of breast cancer metastases to bone. Clin Breast Cancer 5 (Suppl 2): 46-53, 2005.
29. Russell RG, Rogers MJ, Frith JC, *et al*: The pharmacology of bisphosphonates and new insights into their mechanisms of action. J Bone Miner Res 14 (Suppl 2): 53-65, 1999.
30. Gnant M: Bisphosphonates in the prevention of disease recurrence: current results and ongoing trials. Curr Cancer Drug Targets 9: 824-833, 2009.
31. Aft R, Naughton M, Trinkaus K, *et al*: Effect of zoledronic acid on disseminated tumour cells in women with locally advanced breast cancer: an open label, randomised, phase 2 trial. Lancet Oncol 11: 421-428, 2010.
32. Saito H, Tsunenari T, Onuma E, Sato K, Ogata E and Yamada-Okabe H: Humanized monoclonal antibody against parathyroid hormone-related protein suppresses osteolytic bone metastasis of human breast cancer cells derived from MDA-MB-231. Anticancer Res 25: 3817-3824, 2005.
33. Body JJ, Facon T, Coleman RE, *et al*: A study of the biological receptor activator of nuclear factor-kappaB ligand inhibitor, denosumab, in patients with multiple myeloma or bone metastases from breast cancer. Clin Cancer Res 12: 1221-1228, 2006.