Expression of Bone Morphogenetic Protein-7 Significantly Correlates With Non-small Cell Lung Cancer Progression and Prognosis: A Retrospective Cohort Study

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ABSTRACT

BACKGROUND: Bone morphogenetic protein-7 (BMP-7) is a signaling molecule belonging to the transforming growth factor-β superfamily. Recent studies have demonstrated that BMP-7 is expressed in various human cancers and plays an important role in the progression of their cancers. The purpose of this study was to investigate the clinicopathologic and prognostic impact of BMP-7 expression in clinical samples of non-small cell lung cancer.

METHODS: This study enrolled 160 patients with non-small cell lung cancer who underwent complete resection. Expression of BMP-7 in cancer tissue was evaluated by immunohistochemistry. Correlations between expression of BMP-7 and clinicopathologic factors and prognosis were analyzed.

RESULTS: In non-small cell lung cancer, BMP-7 expression was identified not only in cell membranes but also in the cytoplasm of cancer cells. Expression of BMP-7 correlated with p-T ($P = .047$), N factor ($P = .013$), and p-stage ($P = .046$). Overall survival rate was significantly lower in the BMP-7-positive group than in the BMP-7-negative group ($P = .004$). Multivariate analysis indicated that BMP-7 expression was one of the independent prognosis factors of overall survival ($P = .021$). Furthermore, among patients with postoperative recurrence (n = 58), the BMP-7-positive group (n = 29) had a significantly poorer prognosis than the BMP-7-negative group (n = 29) ($P = .012$).

CONCLUSIONS: Expression of BMP-7 in non-small cell lung cancer was correlated with clinicopathologic factors and poorer prognosis. BMP-7 expression may be a useful predictor of aggressive activity of tumor behavior and postoperative outcome of patients with non-small cell lung cancer.

KEYWORDS: BMP-7, non-small cell lung cancer, complete resection, lymph node metastasis, prognosis

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Background

Lung cancer has a high incidence and mortality for all cancers worldwide.[1] The reasons for the high mortality of non-small cell lung cancer (NSCLC) are that patients are diagnosed in advanced stages and recurrence is common, even among patients who have received curative surgery. Selecting appropriate treatment for patients with NSCLC requires that novel molecular markers for diagnosis and prediction of prognosis be found.

BMPs (bone morphogenetic proteins) are signaling molecules belonging to the transforming growth factor-β superfamily, with more than 30 subtypes in mammals, *Drosophila*, *Xenopus*, and sea urchins. The function of BMPs is linked to bone tissue morphogenesis as well as cellular homeostasis and embryonic development.[2-5]

Expression of bone morphogenetic protein-7 (BMP-7) is highest in the kidney, and it is thought that BMP-7 itself is related to kidney and eye development as well as skeletal patterning.[3,6] Recent studies have demonstrated that BMP-7 is also expressed in various human solid cancers and regulates cell proliferation, migration, invasion, and apoptosis.[7-9] However, to our knowledge, there have been few reports investigating an association between BMP-7 expression and clinicopathologic factors, including prognosis, in NSCLC. Thus, the purpose of this study was to investigate BMP-7 expression by immunohistochemistry in NSCLC and evaluate the clinical impact of BMP-7-positivity in NSCLC.

Methods

Patients and specimens

This retrospective cohort study enrolled 160 patients with NSCLC. All patients had undergone complete resection at Kagoshima University Hospital from January 2001 to December

Table 1. Patient baseline characteristics.

CLINICOPATHOLIGICAL FACTORS		
Sex	Men	102
	Women	58
Age (years)	Mean (range)	69 ± 9 (26-84)
Operation	Pneumonectomy	2
	Bilobectomy	4
	Lobectomy	154
Stage	IA	50
	IB	52
	IIA	16
	IIB	15
	IIIA	27
Histology	Adenocarcinoma	112
	Squamous cell carcinoma	40
	Others	8

2007 (Table 1). Patients had undergone lobectomy (n = 154), bilobectomy (n = 4), or pneumonectomy (n = 2) with mediastinal lymphadenectomy. In this study, cases receiving neoadjuvant therapy were not included. Of the 160 patients with NSCLC, 102 were men and 58 were women, and the mean age was 69 years (range = 26-84 years). The final pathologic examination disclosed NSCLC stages IA (n = 50), IB (n = 52), IIA (n = 16), IIB (n = 15), and IIIA (n = 27). The patients were histopathologically classified as adenocarcinomas (n = 112), squamous cell carcinomas (n = 40), or "others" (adenosquamous carcinoma, large cell carcinoma, mucoepidermoid carcinoma, pleomorphic carcinoma; n = 8) according to the Association for the Study of Lung Cancer TNM (tumor-node-metastasis) classification, 7th edition.[10] Written informed consent was obtained from the patients and the study was approved by the Ethical Committee of Kagoshima University Hospital (registration number 351). This investigation conformed to the principles outlined in the Declaration of Helsinki.

Immunohistochemistry of BMP-7 in NSCLC

NSCLC specimens were fixed in formalin, embedded in paraffin, and then cut into 3-μm-thick sections and mounted on glass slides for immunohistochemistry. Specimens were then deparaffinized in xylene and dehydrated with a series of graded ethanol. The endogenous peroxidase activity of specimens was blocked by immersing the slides in a 0.3% hydrogen peroxide solution in methanol for 30 minutes at room temperature. After the sections were washed 3 times with phosphate-buffered saline (PBS) for 5 minutes each, they were treated with 1% bovine serum albumin for 30 minutes to block nonspecific reactions at room temperature. The blocked sections were incubated with the mouse monoclonal antibody against human BMP-7 (1:500; R&D Systems, Inc., Minneapolis, MN, USA) and left overnight at 4°C, followed by staining with a streptavidin-biotin peroxidase kit (Vector Laboratories, Inc., Burlingame, CA, USA). The sections were washed in PBS for 5 minutes 3 times and the immune complex was visualized by incubating the sections with diaminobenzidine tetrahydrochloride. The sections were rinsed briefly in water, counterstained with hematoxylin, and mounted. Noncancerous kidney samples were used as positive controls for BMP-7. BMP-7 expression was determined by counting the number of cancer cells in which the cytoplasm was stained with the anti-BMP-7 antibody. Two investigators (MA and MS) independently evaluated BMP-7 expression via immunohistochemistry within each tumor by assessing a total of 1000 cancer cells in 10 selected fields (100 cells/field) using high-power (×200) microscopy. The average labeling index of BMP-7 was assessed according to the proportion of positive cells in each field. The average expression rate of BMP-7 in all cases was 25.3%. BMP-7 expression was graded as the BMP-7-positive group if more than 30% of cancer cells were stained or as the BMP-7-negative group if less than 30% of cancer cells were stained.

Statistical analysis

A statistical analysis of group differences was performed using the chi-square test. Survival was analyzed using the Kaplan-Meier method and evaluated by the log-rank test. The Cox proportional hazard model was used in the multivariate analysis. A P value of $<.05$ was considered statistically significant.

Results

Bone morphogenetic protein-7 expression in NSCLC and its association with clinicopathologic factors

In NSCLC, BMP-7 expression was identified not only in cell membranes but also in the cytoplasm of cancer cells (Figure 1). According to immunohistochemical evaluation of the samples, the patients were classified into a BMP-7-positive group (n = 68, 43%) and a BMP-7-negative group (n = 92). Table 2 shows the expression of BMP-7 and correlation with clinicopathologic factors. Comparing the BMP-7-positive group with the BMP-7-negative group, BMP-7 expression correlated significantly with several clinicopathologic variables, namely, p-T factor ($P = .047$), p-N factor ($P = .013$), and p-stage ($P = .046$). The BMP-7-positive group tend to be more in men than women ($P = .069$). The other clinicopathologic factors such as age, tumor size, pleural invasion, pulmonary metastasis, and histology were not correlated with the expression of BMP-7 ($P > .05$ for all).

Univariate and multivariate analyses of survival

In term of prognosis of patients, although recurrence-free survival was not correlated with the expression of BMP-7 ($P = .273$; Figure 2A), the overall survival rate was significantly lower in

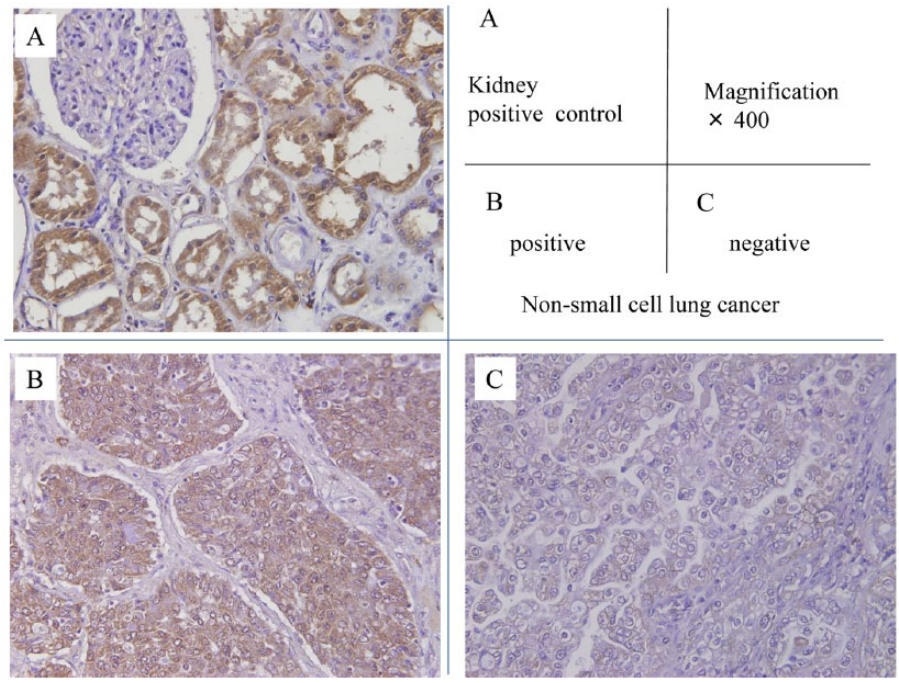

Figure 1. Expression of BMP-7 in clinical samples. Immunohistochemical staining of BMP-7 (original magnification, ×400): examples of (A) noncancerous kidney tissue, (B) BMP-7-positive NSCLC, and (C) BMP-7-negative NSCLC. Staining is detected in both cell membranes and the cytoplasm. BMP-7 indicates bone morphogenetic protein-7; NSCLC, non-small cell lung cancer.

Table 2. Expression of BMP-7 and correlation with clinicopathologic factors.

CLINICOPATHOLOGIC FACTORS		EXPRESSION OF BMP-7		
		POSITIVE N = 68 (43%)	NEGATIVE N = 92	*P* VALUE
Age	<70 years	27 (36%)	45	.265
	⩾70 years	41 (47%)	47	
Sex	Men	49 (48%)	53	.069
	Women	19 (33%)	39	
Tumor size	<30 mm	33 (42%)	46	.874
	⩾30 mm	35 (43%)	46	
Pleural invasion (pl)	Yes	28 (48%)	30	.319
	No	40 (39%)	62	
Pulmonary metastasis (pm)	Yes	7 (39%)	11	.805
	No	61 (43%)	81	
T factor	T1	17 (31%)	38	.047
	⩾T2	51 (49%)	54	
N factor	Yes	22 (61%)	14	.013
	No	46 (34%)	78	
Stage	I	37 (36%)	65	.046
	⩾II	31 (53%)	27	
Histology	Adenocarcinoma	50 (45%)	62	.610
	Others	18 (38%)	30	

Abbreviation: BPM-7, bone morphogenetic protein-7.

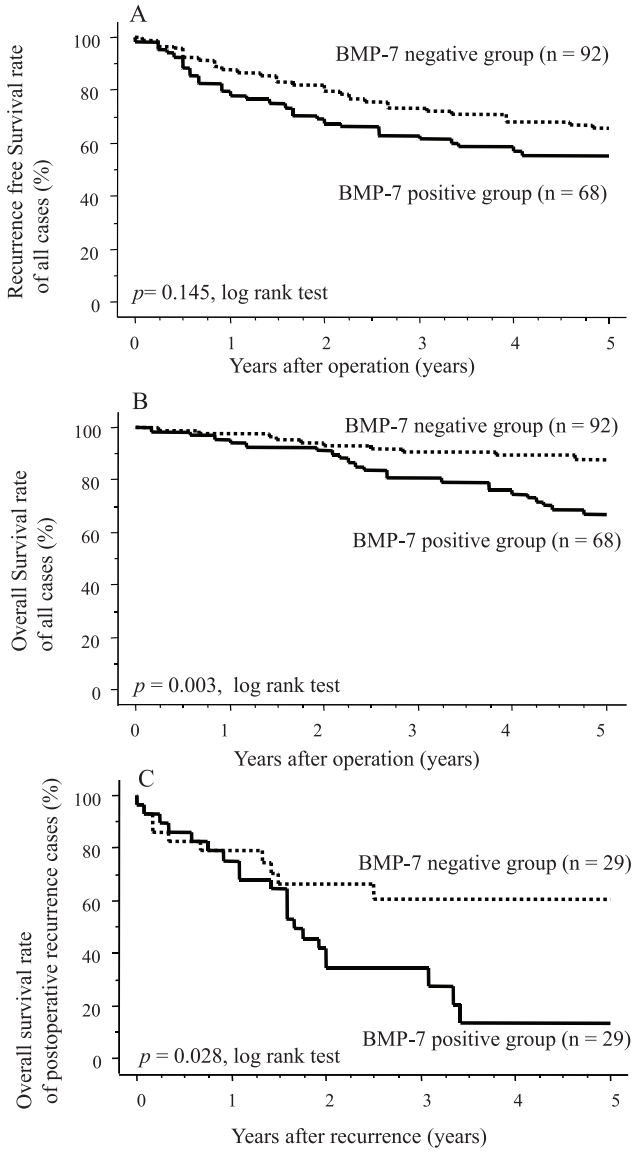

Figure 2. Postoperative survival curves of patients according to their expression of BMP-7 in NSCLC. Although recurrence-free survival was not correlated with BMP-7 expression ($P = .273$), (A) overall survival rate was significantly lower in the BMP-7-positive group than in the (B) BMP-7-negative group ($P = .004$). (C) In the postoperative recurrence cases, BMP-7-positive group was a significantly poorer prognosis than BMP-7-negative group ($P = .012$). BMP-7 indicates bone morphogenetic protein-7; NSCLC, non-small cell lung cancer.

the BMP-7-positive group than in the BMP-7-negative group ($P = .004$; Figure 2B). Furthermore, among patients with postoperative recurrence (n = 58), patients with BMP-7-positive (n = 29) had a significantly poorer prognosis than patients with BMP-7-negative (n = 29) ($P = .012$; Figure 2C).

Table 3 shows the results of univariate and multivariate analyses of factors related to the overall survival. Univariate analysis showed that age ($P = .035$), tumor size ($P < .001$), p-T factor ($P = .057$), p-N factor ($P = .003$), and BMP-7 expression ($P = .004$) were significantly related to postoperative survival. Multivariate analysis indicated that BMP-7 expression was one of the independent prognostic factors of overall survival for the patients with NSCLC ($P = .021$) next to the tumor size ($P = .002$) and p-N factor ($P = .003$).

Discussion

Recently, BMP-7 expression has been identified, and clinical features of its expression in several human solid cancers such as osteosarcoma, prostate cancer, colorectal cancer, malignant melanoma, breast cancer, esophageal cancer, gastric cancer, and renal cell cancer have been discussed.[11-18] In this study, we showed that BMP-7 expression was significantly associated with several clinicopathologic factors such as p-T, N factor, and p-stage. Liu et al[19] demonstrated that downregulation of BMP-7 expression significantly inhibited the invasiveness of SPC-A1 cells whereas forced expression of BMP-7 dramatically increased the motility of A549 cells. Furthermore, previous studies have demonstrated that BMP-7 promoted breast cancer cell migration and invasion, prostate cancer cell mobility, and related metastasis in colorectal cancer.[7-9] In NSCLC, Yang et al[20] reported that restoration of BMP-7 remarkably reversed the tumor suppressive effects of miR-137 on NSCLC tissues. On the contrary, X Ying et al[21] reported that BMP-7 suppresses epithelial mesenchymal transition (EMT) of breast cancer cells, which is opposite to this study. It is difficult to prove the contradiction of this result in this study. However, there is no doubt that BMP-7 controls the tumor progression by various ways beyond the specific organ of cancer origin.

In prognosis, Motoyama et al[16] reported that overexpression of BMP-7 messenger RNA was significantly associated with poorer overall survival in colorectal cancer. Moreover, BMP-7 expression demonstrated by immunohistochemistry was also significantly associated with poorer survival in malignant melanoma, breast cancer, esophageal cancer, and gastric cancer.[11,12,15,17] We showed that BMP-7 expression in NSCLC was an independent prognostic marker in accordance with results for other cancers. Only in renal cell cancer, BMP-7 expression has been significantly associated with better surgical outcome.[13] Unlike normal lung tissue, normal kidney cells usually express BMP-7 at high levels, and this difference might result in the variation in prognostic implication for this marker.

An interesting finding of this study was that although recurrence-free survival was not correlated with BMP-7 expression, the overall survival rate was significantly lower in the BMP-7-positive group than in the BMP-7-negative group. According to the subset analysis of patients with postoperative recurrence, the BMP-7-positive group had a significantly poorer prognosis than the BMP-7-negative group. Moreover, as mentioned above, BMP-7 expression was significantly associated with clinical factors such as p-T, N factor, and p-stage. These facts suggest that BMP-7 has a role in the NSCLC progression.

BMPs mediate their effects through activation of type I/II serine/threonine kinase receptors on cell membranes. After

Table 3. Univariate and multivariate analysis of prognostic factors in NSCLC.

CLINICOPATHOLOGIC FACTORS	UNIVARIATE *P*	MULTIVARIATE *P*	HAZARD RATIO	95% CONFIDENCE INTERVAL
Age	.035	.077	1.75	0.94-3.25
Sex	.159	—	—	—
Tumor size	<.001	.002	2.75	1.44-5.25
Pleural invasion (pl)	.794	—	—	—
Pulmonary metastasis (pm)	.225	—	—	—
T factor	.057	—	—	—
N factor	.003	.003	2.48	1.35-4.57
Histology	.077	—	—	—
BMP-7	.004	.021	2.07	1.12-3.85

Abbreviations: BMP-7, bone morphogenetic protein-7; NSCLC, non-small cell lung cancer.

BMPs bind these receptors via an autocrine or paracrine route, Smad1/5/8 is induced by phosphorylation and assembled into heteromeric complexes with Smad4 in the cytoplasm. Then, these complexes translocate to the nucleus, and mediate the transcription of downstream target genes. The BMP/Smad signaling pathway is intricately regulated by extracellular or intracellular factors. Extracellular factors are BMP antagonists such as Noggin and sclerostin, and intracellular factors are Smads and their related molecules such as Smad6, Smad7, Tob, Ski, Smurf1, and Smurf2. Moreover, BMPs mediate the activation and crosstalk with other signaling pathways such as transforming growth factor-β and mitogen-activated protein kinase.[22-24] Recently, Bieniasz et al demonstrated that there was a positive correlation between gene expression of vascular endothelial growth factor and BMP-2 in patients with lung cancer.[25] In this study, we did not investigate how BMP-7 affects NSCLC progression at a molecular biological level. Therefore, in NSCLC progression, we still need to clarify the role of BMP-7 and factors related to the BMP signaling pathway.

Conclusions

In conclusion, the expression of BMP-7 in NSCLC was significantly associated with tumor progression and poorer prognosis. BMP-7 expression may be used as a predictor of postoperative outcome in NSCLC. Because BMP-7 activates many signaling molecules intricately involved in intracellular and extracellular factors and crosstalk with other signaling pathways, further analysis is needed to determine the exact mechanisms by which BMP-7 affects NSCLC progression.

Author Contributions

MA, KK, MY and MS contributed to the design of the study. MA, GK, NI, SM, YN, TT, AHT and KM contributed to data collection and data analysis. MA, TU, YW, TN, TO and NY contributed to statistical analysis. MA and TU contributed to writing of the study. All authors gave final approval for the publication of the study.

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REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61:69–90.
2. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature*. 1997;387:83–90.
3. Solloway MJ, Robertson EJ. Early embryonic lethality in Bmp5: Bmp7 double mutant mice suggests functional redundancy within the 60A subgroup. *Development*. 1999;126:1753–1768.
4. Urist MR. Bone: formation by autoinduction. *Science*. 1965;150:893–899.
5. Wozney JM, Rosen V, Celeste AJ, et al. Novel regulators of bone formation: molecular clones and activities. *Science*. 1988;242:1528–1534.
6. Luo G, Hofmann C, Bronckers AL, Sohocki M, Bradley A, Karsenty G. BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes Dev*. 1995;9:2808–2820.
7. Alarmo EL, Parssinen J, Ketolainen JM, Savinainen K, Karhu R, Kallioniemi A. BMP7 influences proliferation, migration, and invasion of breast cancer cells. *Cancer Lett*. 2009;275:35–43.
8. Grijelmo C, Rodrigue C, Svrcek M, et al. Proinvasive activity of BMP-7 through SMAD4/src-independent and ERK/Rac/JNK-dependent signaling pathways in colon cancer cells. *Cell Signal*. 2007;19:1722–1732.
9. Yang S, Zhong C, Frenkel B, Reddi AH, Roy-Burman P. Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells. *Cancer Res*. 2005;65:5769–5777.
10. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol*. 2007;2:706–714.
11. Alarmo EL, Korhonen T, Kuukasjarvi T, Huhtala H, Holli K, Kallioniemi A. Bone morphogenetic protein 7 expression associates with bone metastasis in breast carcinomas. *Ann Oncol*. 2008;19:308–314.
12. Aoki M, Ishigami S, Uenosono Y, et al. Expression of BMP-7 in human gastric cancer and its clinical significance. *Br J Cancer*. 2011;104:714–718.
13. Kwak C, Park YH, Kim IY, Moon KC, Ku JH. Expression of bone morphogenetic proteins, the subfamily of the transforming growth factor-beta superfamily, in renal cell carcinoma. *J Urol*. 2007;178(3, Pt. 1):1062–1067.
14. Masuda H, Fukabori Y, Nakano K, Shimizu N, Yamanaka H. Expression of bone morphogenetic protein-7 (BMP-7) in human prostate. *Prostate*. 2004;59:101–106.
15. Megumi K, Ishigami S, Uchikado Y, et al. Clinicopathological significance of BMP7 expression in esophageal squamous cell carcinoma. *Ann Surg Oncol*. 2012;19:2066–2071.

16. Motoyama K, Tanaka F, Kosaka Y, et al. Clinical significance of BMP7 in human colorectal cancer. *Ann Surg Oncol*. 2008;15:1530–1537.
17. Rothhammer T, Wild PJ, Meyer S, et al. Bone morphogenetic protein 7 (BMP7) expression is a potential novel prognostic marker for recurrence in patients with primary melanoma. *Cancer Biomark*. 2007;3:111–117.
18. Sulzbacher I, Birner P, Trieb K, Pichlbauer E, Lang S. The expression of bone morphogenetic proteins in osteosarcoma and its relevance as a prognostic parameter. *J Clin Pathol*. 2002;55:381–385.
19. Liu Y, Chen J, Yang Y, Zhang L, Jiang WG. Molecular impact of bone morphogenetic protein 7, on lung cancer cells and its clinical significance. *Int J Mol Med*. 2012;29:1016–1024.
20. Yang YR, Li YX, Gao XY, Zhao SS, Zang SZ, Zhang ZQ. MicroRNA-137 inhibits cell migration and invasion by targeting bone morphogenetic protein-7 (BMP7) in non-small cell lung cancer cells. *Int J Clin Exp Pathol*. 2015;8:10847–10853.
21. Ying X, Sun Y, He P. MicroRNA-137 inhibits BMP7 to enhance the epithelial-mesenchymal transition of breast cancer cells. *Oncotarget*. 2017;8:18348–18358.
22. Cao X, Chen D. The BMP signaling in vivo bone formation. *Gene*. 2005;357:1–8.
23. Gazzerro E, Canalis E. Bone morphogenetic proteins and their antagonists. *Rev Endocr Metab Disord*. 2006;7:51–65.
24. Zwijsen A, Verschueren K, Huylebroeck D. New intracellular components of bone morphogenetic protein/Smad signaling cascades. *FEBS Lett*. 2003;546:133–139.
25. Bieniasz M, Oszajca K, Eusebio M, Kordiak J, Bartkowiak J, Szemraj J. The positive correlation between gene expression of the two angiogenic factors: VEGF and BMP-2 in lung cancer patients. *Lung Cancer*. 2009;66:319–326.