Expression of Bone Morphogenetic Protein 7 in Lung Cancer and its Biological Impact on Lung Cancer Cells

JINFENG CHEN[1,2], LIN YE[2], FEI XIE[1], YUE YANG[1], LIJIAN ZHANG[1] and WEN G. JIANG[2]

[1]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education),
Department of Thoracic Surgery, Peking University School of Oncology,
Beijing Cancer Hospital and Institute, Beijing 100142, P.R. China;
[2]Metastasis and Angiogenesis Research Group, Department of Surgery,
Cardiff University School of Medicine, Heath Park, Cardiff, CF14 4XN, U.K.

Abstract. *Background: Lung cancer is the leading cause of cancer-related death in the world, with metastasis being the main reason for the mortality. As a member of the bone morphogenetic protein (BMP) family, BMP7 has important biological functions in malignant tumours. As yet, however, there is little knowledge of the role of BMP7 and its cellular function in lung cancer. This study aimed to investigate the biological impact of BMP7 on lung cancer cells and the expression pattern of the molecule in clinical lung carcinomas. Materials and Methods: The expression pattern of BMP7 and its receptors was examined in different lung cancer cell lines and normal cells. The biological function of BMP7 in lung cancer cells was further evaluated. The impact of BMP7 on the downstream signalling, namely the activation of SMAD1 status, was assessed. BMP7 expression level was assessed in a cohort of human lung cancer samples (tumour, n=70; matched normal tissues, n=70), in association with patient clinical variables, using quantitative analysis of BMP7. Results: In vitro, it was found that recombinant human (rh)BMP7 significantly reduced cellular motility of lung cancer cell line, SK-MES1, and its adhesion to Matrigel (p<0.05). rhBMP7 was also able to significantly reduce invasion of lung cancer cells (p<0.05). We found that rhBMP7 had little effect on actual growth of the lung cells.*

In addition, BMP7 was able to regulate the phosphorylation of SMAD1, a downstream signalling intermediate of the BMP7 signalling pathway. The study further revealed that reduced levels of BMP7 in lung cancer tissues significantly correlated with lymph node metastasis for lung cancer patients (p<0.05). Conclusion: BMP7 protein is a pivotal regulator of cell motility in lung cancer with little impact on the growth of tumour cells. BMP7 expression is linked to lymph node involvement in patients with lung cancer. These results indicate that BMP7 plays a key role in regulating the progression of lung cancer.

Lung cancer is the most prevalent type of cancer and is also one of the principal causes of death in the world (1). There has been little improvement in 5-year survival over the last 30 years (2). Distant metastasis, which is regulated by a complex network of molecules and cellular behaviours, is an important cause for the poor prognosis of lung cancer patients. Of the factors related to metastasis, bone morphogenetic proteins (BMPs), crucial in embryonic development including lung development and airway branching (3, 4), have been recently shown to regulate the aggressiveness of cancer cells.

BMPs are essential in a variety of processes of vertebrate embryonic development and the epithelial–mesenchymal interactions. They are multifunctional signal molecules and belong to the transforming growth factor-β (TGF-β) superfamily. BMPs were first identified as being regulators of bone forming in extraskeletal sites (5, 6). BMPs exert biological functions by interaction with a heteromeric complex of membrane receptors, type I and type II BMP receptors. BMP proteins bind the type-I serine kinase receptors (BMPIA/BMPIB), leading to the recruitment of the BMP II receptors, which in turn activates the transcription factors, SMAD (homolog of mothers against decapentaplegic, Drosophila) 1, 5 or 8 (7), known as the SMAD-dependent pathway. BMPs can stimulate epithelial branching in

Correspondence to: Professor Lijian Zhang, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Thoracic Surgery, Peking University School of Oncology, Beijing Cancer Hospital and Institute, Beijing 100142, P.R. China. Tel: +86 1088196209, Fax: +86 1088141036, e-mail: lijzhang@yeah.net / Professor Wen G. Jiang, Metastasis and Angiogenesis Research Group, Cardiff University School of Medicine, Heath Park, Cardiff, CF14 4XN, U.K. Tel: +44 292074 2895, Fax: +44 292074 2896, e-mail: jiangw@cf.ac.uk

Key Words: Bone morphogenetic protein 7, Smad1, lung cancer, motility.

embryonic lung (8-10), and their abnormal expression can lead to abnormal lung development (11). The role of BMPs in cancer has also been reported in recent years. Overexpression of a specific BMP inhibitor, noggin, inhibits the formation of the osteoblastic aspect of the bone lesions and the tumour growth (12). BMPs are also regulators during angiogenesis, by such mechanism as activating vascular endothelial growth factor (VEGF) promoter which can result in the formation of skeletal metastases (13).

In lung cancer, expression of the BMP family members has also been reported. For example, BMP2 is overexpressed in virtually all types of lung cancer, including non-small cell lung cancer (NSCLC) and small cell lung cancer (14, 15). BMP2 protein can stimulate growth, migration and invasion of lung cancer cells (14). This is in contrast to its effect on neuroblastoma-derived cell lines, in which $p27^{kip1}$ expression is up-regulated and growth arrest and differentiation are induced by BMP2 (16). Thus, there may be a contrasting response to any given BMP, depending on the tumour and cell type. Interestingly, the status of *K-Ras* mutation is a factor determining the response of the cells to BMP3b and BMP6 (17). In NSCLC, BMP3b promoter is frequently methylated, resulting in silencing of BMP3b expression during tumour development (18, 19). BMP4, on the other hand, is able to induce senescence of A549 lung adenocarcinoma cell line (20). This leads to less telomerase and invasive activity, low degree of extracellular signal-regulated kinase (ERK) activation, reduced VEGF and BCL2 expression. Finally, BMP4 pathway signalling can negatively regulate the growth of lung cancer cells (20).

Studies on BMP7 in lung cancer have not been reported. However, BMP7 has been shown to play a role in other tumour types. BMP7 is strongly expressed in breast cancer and malignant melanoma (21-23). BMP7 regulates the proliferation and apoptosis of mammary epithelial cells, by acting as a LIM domaonim-only 4 (LMO4) responsive gene (24). In gastric cancer, however, BMP7 promoter has been shown to be methylated (25), suggesting a role in the carcinogenic process. In prostate cancer, BMP7 exposure can modulate the biological behaviour in a cell type-specific manner (26). It can up-regulate survivin activity, restore the starvation-induced suppression of c-jun NH-2 terminal kinase (JNK) activity, and affect prostate cancer cell apoptosis (27).

In the present study, we investigated the expression characteristics of BMP7 in lung cancer cells and tissues. Furthermore, we also investigate the biological impact of BMP7 protein on lung cancer cells.

Materials and Methods

Cell lines, materials and human lung specimens. Human lung cancer A549, SK-MES1, CorL77, CorL88, CorL25, and CorL47 cell lines (European Collection of Animal Cell Culture, Salisbury, UK) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS) and antibiotics (100 U penicillin and 50 µg streptomycin per ml). Recombinant human BMP7 (rhBMP7) protein was purchased from Sigma-Aldrich (Poole, Dorset, UK). Goat anti-human phospho-SMAD1 antibody (P-SMAD1), anti-SMAD1, and mouse anti-human GAPDH antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Matrigel (reconstituted basement membrane) was purchased from Collaborative Research Product (Bedford, MA, USA). Other kits and reagents were obtained from Sigma-Aldrich, Poole, UK, unless otherwise stated.

Fresh frozen lung tissues, matched normal and tumour pairs from the same patients (n=70 pairs) were obtained from patients who attended Beijing Cancer Hospital from January 2004 to August 2007, under Ethical Committee approval. None of the patients received any neoadjuvant therapy prior to surgery. These tissues were collected immediately after surgical resection at the Beijing Cancer Hospital and were stored at the Tissue Bank of Peking University Oncology School. Clinico-pathological characteristics of the tumours were defined according to the TNM criteria of UICC (28). Clinico-pathologic factors, including age, sex, histological type of tumours, TNM stage, and lymph node metastasis were recorded and stored in the patients' database and are shown in Table I.

RNA isolation and RT-PCR. RNA was obtained using Total RNA Isolation Reagent (ABgene™) according to manufacturer's instructions (Abgene, Surrey, UK). Total RNA was converted to cDNA using DuraScript™, a commercial RT kit from Sigma-Aldrich. PCR was carried out using a REDTaq™ ReadyMix PCR reaction mix (primers listed in Table II). Cycling conditions were 94°C for 5 minutes, followed by 36 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 40 seconds. This was followed by final extension for 10 minutes at 72°C. Products were visualized on 2% agarose gel stained with ethidium bromide.

In vitro cell growth assay. This was based on a previously reported method (29). SK-MES1 cells (3,000 cell per well) were seeded in a 96-well plate with the 10% FCS and cells were incubated with or without rhBMP7 protein at a concentration from 2.5 ng/ml to 100 ng/ml. The relative cell number was determined at 48, 72 and 96 hours. Crystal violet was used to stain cells and absorbance was determined at a wavelength of 540 nm using an ELx800 spectrophotometer (Bio-tek, Wolf Laboratories, York, UK). The absorbance measured represents the cell number.

Cell matrix adhesion assay. This was based on a method we previously reported (30, 31). A total of 40,000 cells were added to each well of a 96-well plate pre-coated with Matrigel at 5 µg per well. After 40 minutes of incubation, non-adherent cells were washed off using balanced salt solution (BSS) buffer. The remaining cells were fixed with 4% formalin and stained with 0.5% crystal violet. The number of adherent cells was then counted.

In vitro motility assay using cytodex-2 beads. The protocol described by Rosen *et al*. (32) was followed. Cells (1×10^6) were incubated with 100 µl Cytodex-2 beads (Pharmacia, Piscataway, New Jersey) in 10 ml DMEM overnight. After washing, 100 µl beads per cell were transferred into each well of a 24-well plate, in the presence or absence of BMP7 (2.5-100 ng/ml). After 24 hours incubation, floating cells and beads were washed off with BSS. The adherent cells were fixed in 4% formalin for 5 minutes and then stained with crystal violet for counting. Three independent experiments were performed.

Table I. *The clinicopathological details of the patients.*

Clinical information	No.
Gender	
Male	49
Female	21
Histological type	
Squamous	27
Adenocarcinoma	34
Small cell lung cancer	2
Others	7
Tumour staus	
T1	7
T2	37
T3	14
T4	12
Lymph node status	
N0	29
N1	16
N2	25

Table II. *Primer sequences used in PCR in this study.*

BMP7	Forward	AGAGCATCAACCCCAAGT
	Reverse	CTACTCAGGCCCCAGCTT
BMPR1B	Forward	ATGGAACTTGCTGTATTGCT
	Reverse	CAACTCGAGTGTTAGGTGGT
BMPR2	Forward	TTTGGGAAAGAAACAAATCT
	Reverse	TGGATAAGGACCAATTTTTG
β-Actin	Forward	ATGATATCGCCGCGCTCG
	Reverse	CGCTCGGTGAGGATCTTCA
GAPDH	Forward	AGGTCGGAGTCAACGGATTTG
	Reverse	GTGATGGCATGGACTGTGGT
BMP7	Forward	AGAACCACGAGCTCTATGTC
qPCR	Reverse	*ACTGAACCTGACCGTACA*CTTC
		ACAGTAATACGCAGCA

Figure 1. *The expression of BMP7 and BMP receptors in lung cancer cell lines and lung cancer tissue using RT-PCR. Lung cancer cell lines and HECV cell line had almost no BMP7 expression while lung tumour and paired normal tissues had different levels of BMP7 expression. BMPR1B was detected in SkMes1 cell; it was practically not detected in other cell lines. BMPR2 was detected in A549, CorL47, CorL25 and HECV cell lines, while it was not detected in SkMes1 and CorL88 cell lines. Lung cancer tissues have different levels of BMP7 expression compared with the corresponding normal tissue.*

In vitro invasion assay. Transwell inserts with an 8 μm pore size were coated with 50 μg Matrigel™ and air dired. Matrigel was rehydrated before use. A total of 20,000 cells were added to each well with or without rhBMP7. After 96 hour's incubation, matrix proteins and non-invading cells were removed by cotton swabs. Cells that had migrated through the matrix and pores were fixed, stained in crystal violet and counted.

Immunocytochemical staining for P-SMAD1 protein. To determine the phosphorylation status of SMAD1, cells were first serum starved for 4 hours, followed by one hour's exposure to rhBMP7 protein (5 ng/ml, 10 ng/ml, 50 ng/ml) in serum-free medium. Cells were fixed with 4% formaldehyde and then permeabilized with 0.1% Triton-X100 for 5 minutes. After blocking with horse serum for 60 min, the cells were probed with anti-P-SMAD1 antibody for 1 hour, followed by extensive washing. Horseradish peroxidase-conjugated anti-mouse antibody was then added for 1 hour and visualized using the Vectastain™ ABC system (Nottingham, UK). Slides were mounted with Sterilyte mounting media.
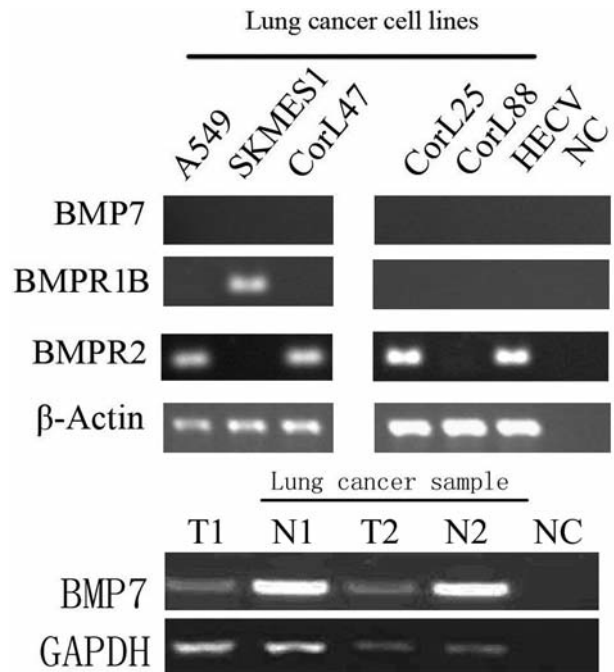
Western blotting and immunoprecipitation in detecting SMAD-1 and p-SMAD1. SK-MES1 cells were treated with different concentrations of rhBMP7 protein (5-100 ng/ml) then pelleted and extracted in HCMF buffer (160 mM NaCl, 0.6 mM Na_2HPO_4, 0.1% w/v glucose and 0.01 M HEPES, pH 7.4) containing 1.5% Triton X-100, 2 mM $CaCl_2$, 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml aprotinin for 30 min. Protein concentration in cell lysates was quantified using as DC Protein Assay kit (Bio-Rad™, Hemmal Hamstead, England, UK) and an ELx800 spectrophotometer. Equal amount of proteins prepared in a loading buffer were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto nitrocellulose sheets. Proteins were probed with the respective primary and peroxidase-conjugated secondary antibodies. For immunoprecipitation experiments, cell lysate was collected. Equal amounts of cell lysate were incubated with anti-SMAD1 IgG (4 μg/ml) to precipitate SMAD1. The immune complexes subsequently formed were then precipitated by incubating with protein A/G-agarose conjugate for 1 hour and subsequent centrifugation at 13,000g for 15 minutes. After washing, samples were boiled in SDS-PAGE sample buffer for 5 minutes prior to separation using SDS-PAGE. The membrane was probed with an anti-phosphoserine/threonine antibody (Sigma-Aldrich) and subsequently with a peroxidase-conjugated secondary antibody (1:1000). Protein
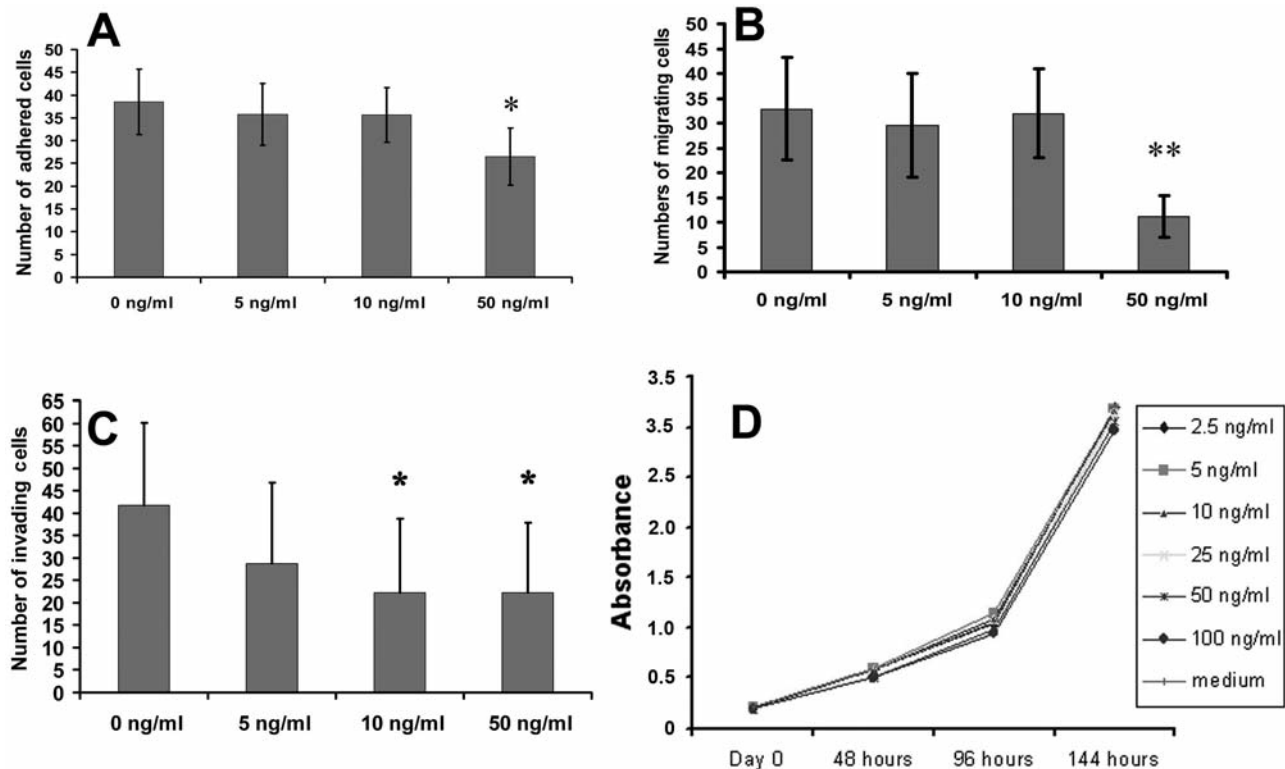
Figure 2. *Biological function of ectogenous BMP7 protein in lung cancer cells. A and B: High concentration of BMP7 (50 ng/ml) significantly reduced lung cancer adhesion (A) and motility (B). C: Only BMP7 ≥10 ng/ml significantly reduced lung cancer invasion. D: Exogenous BMP7 protein did not affect the growth of lung cancer cells.*

bands were visualized by a Supersignal™ Western Dura system and documented using a gel documentation system (UVITech, Cambridge, United Kingdom). The band densities on the photographic film were analysed with a densitometer and are shown as relative values.

BMP7 expression level in lung cancer and normal lung tissue assessed by real time RT-PCR. Levels of BMP7 transcript in cDNA samples prepared from the fresh frozen lung tissues were determined using real-time quantitative PCR based on the Amplifluor™ technology, modified from a method reported previously (33). PCR primers were designed using Beacon Design software but to the reverse primer an additional sequence, known as the Z sequence (5'-actgaacctgaccgtaca-3'), which is complementary to the universal Z probe (Intergen Inc., Oxford, UK) was added (Table II). The reaction was carried out using the following conditions: Hot-start Q-master mix (Abgene, Surrey, England, UK), 10 pmol of specific forward primer, 1 pmol reverse primer which has the Z sequence, 10 pmol of FAM tagged probe (Intergen Inc.) and cDNA. The reaction was carried out using an IcyclerIQ (BioRad) which is equipped with an optic unit that allows real time detection of 96 reactions under the following conditions: 94°C for 12 minutes and 60 cycles of 94°C for 15 seconds, 55°C for 60 seconds and 72°C for 20 seconds. The level of *BMP7* transcript was generated from a standard that was simultaneously amplified with the samples. The expression level of BMP7 gene was then normalized against cytokeratin 19 (CK19) expression already

measured in these specimens, to correct for varying amounts of epithelial tissue between samples (34).

Statistical analysis. One-way ANVOA was used to determine the difference in BMP7 mRNA expression level observed between the group of cancer tissues and the normal tissues. The samples were grouped according to the tissue stage, lymph node stage and bone metastasis. Differences were considered statistically significant at $p<0.05$. Statistical tests were performed using SPSS software version 12.0.0 (SPSS Inc., Chicago, IL, USA).

Results

Expression characteristic of BMP7 gene in different cell lines. Figure 1 shows the expression status of *BMP7* and the receptor transcripts in the cell lines tested. None of the lung cell lines showed a signal for *BMP7*. SK-MES1 was positive for *BMPR1B*, but negative for *BMPR2*. Normal lung tissues displayed a stronger signal for BMP7 than lung tumour tissues (Figure 1 bottom).

Effect of exogenous BMP7 on adhesion, motility and invasion ability of lung cancer cells in vitro. SK-MES1 cells showed significant change in cell motility and adhesion, depending on BMP7 concentration using the *in vitro* cell matrix adhesion technique. The number of adherent cells for the
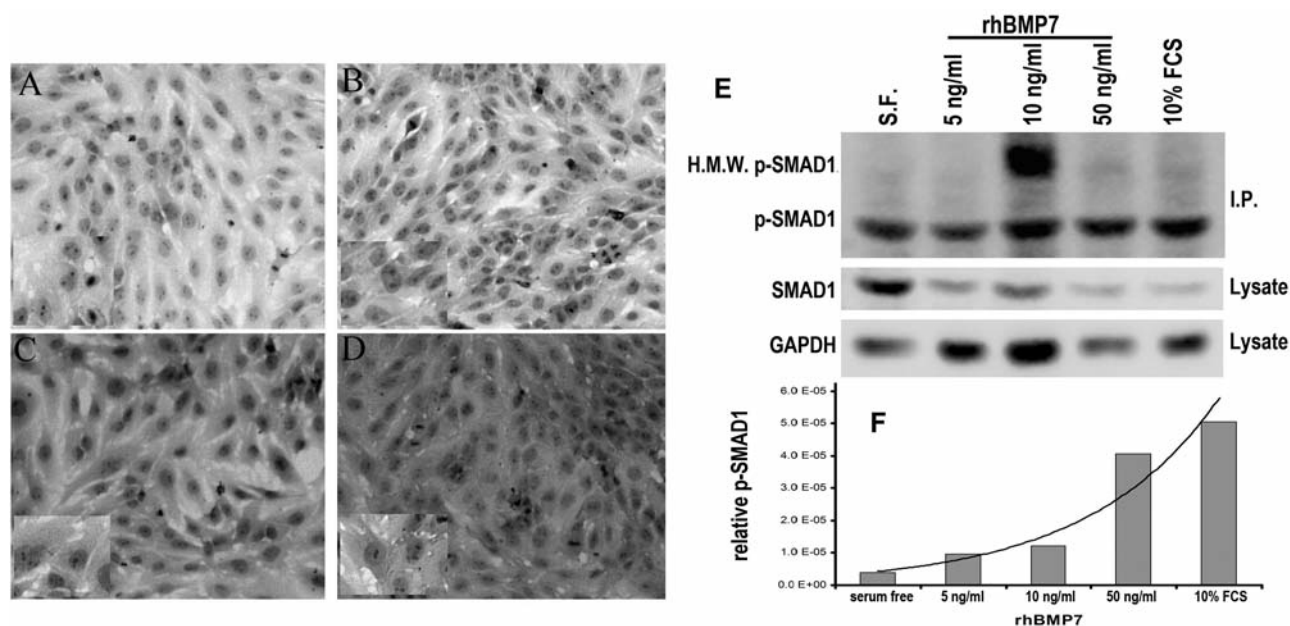
Figure 3. *Induction of phosphorylation of SMAD-1 by BMP7 in lung cancer cells. A-D: Immunocytochemical staining of phosphorylated SMAD1 (x100 magnification main figure and x400 in the inset). With an increase in the concentration of exogenous BMP7, there was an increase in phosphorylation of SMAD1 in SK-MES1 cells. A: Control; B-D: BMP7 protein concentration at 5, 10, and 15 ng/ml, respectively. E-F: Western blotting analysis of p-SMAD-1. The phosphorylation of SMAD1 (p-SMAD1) was increased after incubation with rhBMP7 protein in SkMes1 cells as shown by immunoprecipation (IP) analysis. When the rhBMP7 concentration was 10 ng/ml, the level of p-SMAD1 expression was high. S.F.: Serum-free; FCS: foetal calf serum; H.M.W. p-SMAD1: high molecular weight p-SMAD1. The bands of p-SMAD1 were quantified and normalized against corresponding total SMAD1 and GAPDH (F).*

SK-MES1 cells when treated with rhBMP7 5 ng/ml and 10 ng/ml was similar to the control, 35.75±6.77 and 35.67±6.02, respectively *vs*. 38.5±7.18 ($p > 0.05$, Figure 2A). When the concentration of BMP7 was increased to 50 ng/ml, the number of adherent cells was significantly reduced (26.5±6.24) compared with no treatment ($p < 0.001$, Figure 2A). The number of motile cells when treated with rhBMP7 was only significantly reduced when BMP7 was used at 50 ng/ml (11.11±4.14), $p < 0.001$ *vs*. control, Figure 3B). In the invasion assay, BMP7 significantly reduced the numbers of invading cells at 10 ng/ml (22.3±16.36) and 50 ng/ml (22.2±15.58), in comparison with control cells (41.65±18.51) ($p < 0.05$, Figure 2C).

It is interesting to observe that rhBMP7 had no effect on the growth of lung cancer cells (Figure 2D).

Phosphorylation of SMAD1 in response to BMP7. To assess the phosphorylation status of SMAD1, we utilized immunocytochemical and immunoprecipitation method. As shown in Figure 3A, there was almost no staining of p-SMAD1 in control cells and the cells exposed to lower concentrations of rhBMP7. A strong staining of p-SMAD1 was seen in the cytoplasm of cells treated with 50 ng/ml of rhBMP7 (Figure 3D). Western blotting also revealed an increased in p-SMAD-1 after treatment with rhBMP7 (Figure 3E/F).

BMP7 gene transcript expression in lung cancer. BMP7 transcripts in lung cancer specimens (tumour, n=70; matched paired normal tissue, n=70) were quantified using real-time quantitative PCR (shown in Figure 4. as *BMP7* transcript copies/µl). There was no significant difference in transcript expression between normal lung tissues and lung cancer tissues ($p = 0.074$) (Figure 4A). In addition, there were no differences in different T stage tumours ($p = 0.770$) (Figure 4B). Tumours from patients with and without bone metastasis also failed to show any difference in *BMP7* transcript levels (Figure 4C).

Perhaps the most interesting finding is the relationship between *BMP7* and nodal involvement. In the cohort, 29 tumours were without nodal involvement (N0), 16 were at N1 stage and 25 at N2 (N1+N2=41). N0 tumours had significantly higher levels of BMP7 transcript compared with tumours with N1 lymph node and N2 lymph node metastasis ($p = 0.031$) (Figure 4D). When N1 and N2 were combined, tumours with no lymph node metastasis (N0) had significantly high levels of *BMP7* compared with tumours with lymph node metastasis ($p = 0.008$) (Figure 4E).

After 48 months' follow-up, patients were analysed regarding the survival time based on BMP7 expression level. The mean time of follow-up for the cohort (n =70) was 18.49 months (range 1-48 months). The mean (SD) survival time was 18.49±10.07 months. Cumulative survival curves were
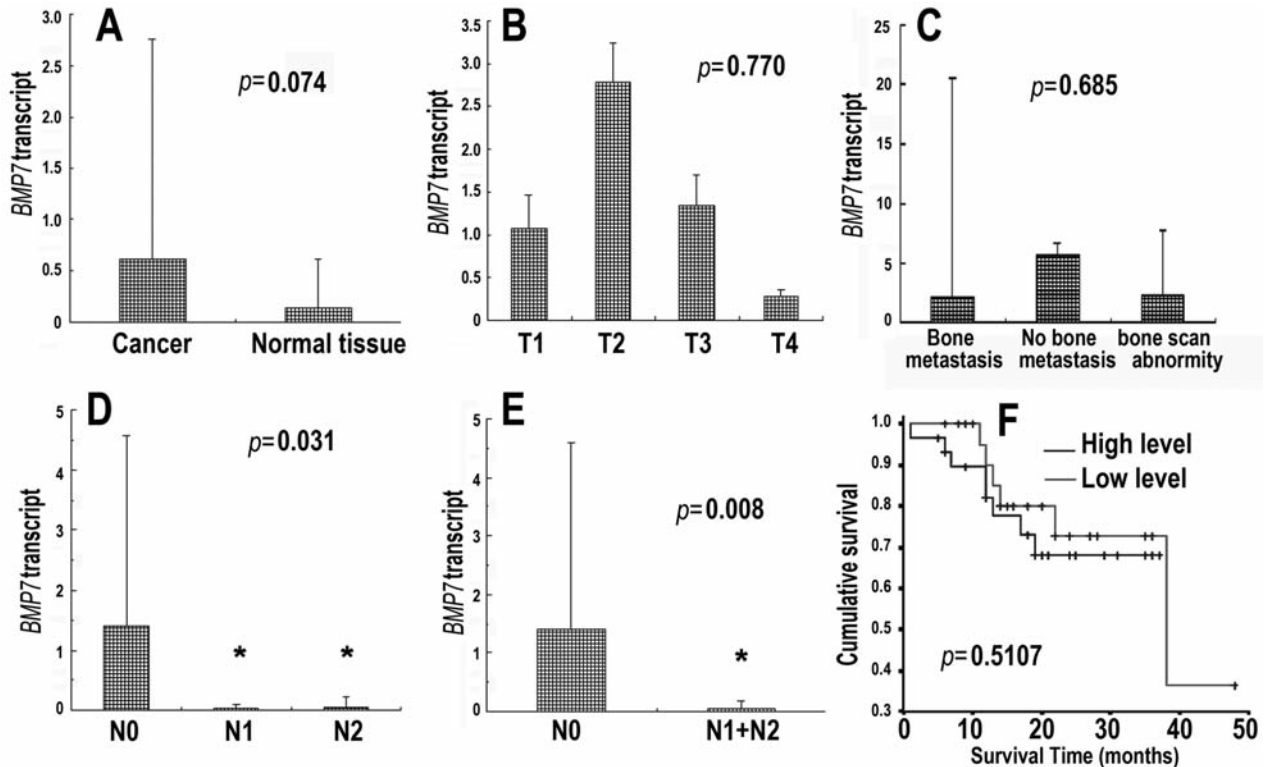
Figure 4. *Quantitative PCR analysis of BMP7 expression in lung cancer tissue. A: The level of BMP7 expression in cancer tissue was not significantly different from that of the normal tissue (p>0.05). B: Patients with different tumour stage had similar levels of BMP7 expression (p>0.05). C: Patients with bone metastasis also had similar levels of BMP7 expression to the patients with bone metastasis or bone scan abnormality (p>0.05). D: Patients with lymph node local metastasis (N1) and distant metastasis (N2) had significantly lower levels of BMP7 expression in their tumour compared with the tumours of patients with no lymph node metastasis (N0) (p=0.031). E: When N1 and N2 patients were combined as one group, N1+N2, the difference between this group and the N0 group was more significant (p=0.008). F: Kaplan-Meier analysis of overall survival of lung cancer patients does not appear to depend on the expression level of BMP7 mRNA (p>0.05).*

calculated using the Kaplan-Meier method. In the patients with a low level expression of BMP7, the mean survival time was 35.37 (95%CI: 27.71-43.04) months. In the patients with a high expression level of BMP7, the mean survival time was 28.90 (95%CI: 24.15-33.65) months. The levels of BMP7 mRNA expression were not correlated with cumulative survival time (p=0.5107, Figure 4F).

Discussion

Aberrant expression of BMP7 has been reported in a few cancer types, including colorectal cancer, breast cancer, melanoma and prostate cancer. BMP7 has also shown an influence on proliferation, migration and invasion, and has been indicated as a useful clinical marker (35-38). The present study has demonstrated that BMP7 also has a significant influence on the aggressiveness of lung cancer cells. Lung cancer cell lines had almost no BMP7 expression and different levels of BMP receptor expression.

A few studies have shown a possible inhibitory effect of BMP7 on cell growth, for example, Notting *et al*. showed that

BMP7 inhibits tumour growth of human uveal melanoma (39). In the present study, we did not find any effect of exogenous rhBMP7 on the growth of the lung cancer cell line, indicating that the impact of BMP7 on cell growth is cell type dependent. However, the most demonstrable effects of BMP7 on lung cancer cells are the inhibitory effects on matrix adhesion, motility and *in vitro* invasiveness, an effect seen at a relatively higher concentration (50 ng/ml). Transforming growth factor beta (TGF-ß) is a well characterized inducer of epithelial-mesenchymal transition (EMT). As members of the TGF-ß superfamily, BMPs can affect cancer cell adherence and migration which have important roles in cancer pathogenesis. Tada *et al*. reported that BMP2 can reduce microfilament organisation restoration in A549 lung cancer cells and reduce cancer cell migration (40). In breast cancer cells, however, exogenous BMP7 increases cell migration and invasion process (41).

BMP signalling requires serine-threonine kinase receptors, BMPRIA, BMPRIB and BMPRII, to transmit their signals (42). These receptors phosphorylate intracellular SMADs (43), which are transcription factors for gene transcription (44).

Here, we report that BMP7 is able to induce SMAD1 phosphoration. This is interesting, given that SK-MES1 cells expressed only BMPR1B, but not BMPR2. This suggests that BMP7 requires other type II BMP receptors.

Our results on the expression pattern of BMP7 in clinical lung cancer is also interesting. The study has shown that node positive tumours have significantly lower BMP7 levels in comparison with node-negative tumours. In contrast, levels of BMP7 transcripts do not have a clear correlation with tumour staging, nor with the long-term survival of the patients. This result probably reflects the possibility that BMP7 does not influence the growth of lung cancer. Buijs *et al*. reported that BMP7 can affect bone metastasis formation in breast and prostate cancer animal models (45, 46), a link not demonstrated in lung cancer as shown here.

In summary, we have demonstrated that BMP7 has an important role in controlling lung cancer cell motility. It can regulate the invasion, motility and adhesion of lung cancer cells by activating the intracelluar signalling pathway through the phosphorylation of SMAD1 without affecting the growth process. A lack of BMP7 expression is a good indicator of lymph node involvement. BMP7 may have a therapeutic role in lung cancer, an area which warrants further investigations.

Acknowledgements

Conflict of Interest

None identified.

References

1 Parkin DM, Bray F, Ferlay J and Pisani P: Estimating the world cancer burden: Globocan 2000. Int J Cancer *94*: 153-156, 2001.

2 Le Chevalier T and Lynch T: Adjuvant treatment of lung cancer: current status and potential applications of new regimens. Lung Cancer *46(Suppl 2)*: S33-S39, 2004.

3 Weaver M, Yingling JM, Dunn NR, Bellusci S and Hogan BL: BMP signaling regulates proximal-distal differentiation of endoderm in mouse lung development. Development *126*: 4005-4015, 1999.

4 Rosendahl A, Pardali E, Speletas M, Ten Dijke P, Heldin CH and Sideras P: Activation of bone morphogenetic protein/SMAD signaling in bronchial epithelial cells during airway inflammation. Am J Respir Cell Mol Biol *27*: 160-169, 2002.

5 Urist MR, Iwata H, Ceccotti PL, Dorfman RL, Boyd SD, McDowell RM and Chien C: Bone morphogenesis in implants of insoluble bone gelatin. Proc Natl Acad Sci USA *70*: 3511-3515, 1973.

6 Urist MR, Mikulski A and Lietze A: Solubilized and insolubilized bone morphogenetic protein. Proc Natl Acad Sci USA *76*: 1828-1832, 1979.

7 Massague J and Chen YG: Controlling TGF-beta signaling. Genes Dev 14: 627-644, 2000.

8 Vukicevic S, Helder MN and Luyten FP: Developing human lung and kidney are major sites for synthesis of bone morphogenetic protein-3 (osteogenin). J Histochem Cytochem *42*: 869-875, 1994.

9 Bellusci S, Henderson R, Winnier G, Oikawa T and Hogan BL: Evidence from normal expression and targeted misexpression that bone morphogenetic protein (Bmp-4) plays a role in mouse embryonic lung morphogenesis. Development *122*: 1693-1702, 1996.

10 Shi W, Chen H, Sun J, Chen C, Zhao J, Wang YL, Anderson KD and Warburton D: Overexpression of Smurf1 negatively regulates mouse embryonic lung branching morphogenesis by specifically reducing SMAD1 and SMAD5 proteins. Am J Physiol Lung Cell Mol Physiol *286*: L293-300, 2004.

11 Koli K, Wempe F, Sterner-Kock A, Kantola A, Komor M, Hofmann WK, von Melchner H and Keski-Oja J: Disruption of LTBP-4 function reduces TGF-beta activation and enhances BMP-4 signaling in the lung. J Cell Biol *167*: 123-133, 2004.

12 Feeley BT, Liu NQ, Conduah AH, Krenek L, Roth K, Dougall WC, Huard J, Dubinett S and Lieberman JR: Mixed metastatic lung cancer lesions in bone are inhibited by noggin overexpression and Rank:Fc administration. J Bone Miner Res *21*: 1571-1580, 2006.

13 Dai J, Kitagawa Y, Zhang J, Yao Z, Mizokami A, Cheng S, Nör J, McCauley LK, Taichman RS and Keller ET: Vascular endothelial growth factor contributes to the prostate cancer-induced osteoblast differentiation mediated by bone morphogenetic protein. Cancer Res *64*: 994-999, 2004.

14 Langenfeld EM, Calvano SE, Abou-Nukta F, Lowry SF, Amenta P and Langenfeld J: The mature bone morphogenetic protein-2 is aberrantly expressed in non-small cell lung carcinomas and stimulates tumor growth of A549 cells. Carcinogenesis *24*: 1445-1454, 2003.

15 Langenfeld EM, Bojnowski J, Perone J and Langenfeld J: Expression of bone morphogenetic proteins in human lung carcinomas. Ann Thorac Surg *80*: 1028-1032, 2005.

16 Nakamura Y, Ozaki T, Koseki H, Nakagawara A and Sakiyamas: Accumulation of p27 KIP1 is associated with BMP2-induced growth arrest and neuronal differentiation of human neuroblastoma-derived cell lines. Biochem Biophys Res Commun *307*: 206-213, 2003.

17 Kraunz KS, Nelson HH, Liu M, Wiencke JK and Kelsey KT: Interaction between the bone morphogenetic proteins and Ras/MAP-kinase signalling pathways in lung cancer. Br J Cancer *93*: 949-952, 2005.

18 Dai Z, Popkie AP, Zhu WG, Timmers CD, Raval A, Tannehill-Gregg S, Morrison CD, Auer H, Kratzke RA, Niehans G, Amatschek S, Sommergruber W, Leone GW, Rosol T and Otterson GA: Bone morphogenetic protein 3B silencing in non-small-cell lung cancer. Oncogene *23*: 3521-3519, 2004.

19 Dai Z, Lakshmanan RR, Zhu WG, Smiraglia DJ, Rush LJ, Frühwald MC, Brena RM, Li B, Wright FA, Ross P, Otterson GA and Plass C: Global methylation profiling of lung cancer identifies novel methylated genes. Neoplasia *3*: 314-323, 2001.

20 Buckley S, Shi W, Driscoll B, Ferrario A, Anderson K and Warburton D: BMP4 signaling induces senescence and modulates the oncogenic phenotype of A549 lung adenocarcinoma cells. Am J Physiol Lung Cell Mol Physiol *286*: L81-86, 2004.

21 Alarmo EL, Rauta J, Kauraniemi P, Karhu R, Kuukasjärvi T and Kallionie A: Bone morphogenetic protein 7 is widely overexpressed in primary breast cancer. Genes Chromosomes Cancer *45*: 411-419, 2006.

22 Alarmo EL, Kuukasjärvi T, Karhu R and Kallioniemi OP: A comprehensive expression survey of bone morphogenetic proteins in breast cancer highlights the importance of BMP4 and BMP7. Breast Cancer Res Treat *103*: 239-246, 2007.

23 Rothhammer T, Poser I, Soncin F, Bataille F, Moser M and Bosserhof AK: Bone morphogenic proteins are overexpressed in malignant melanoma and promote cell invasion and migration. Cancer Res *65*: 448-456, 2005.

24 Wang N, Lin KK, Lu Z, Lam KS, Newton R, Xu X, Yu Z, Gill GN and Anderse B: The LIM-only factor LMO4 regulates expression of the *BMP7* gene through an HDAC2-dependent mechanism, and controls cell proliferation and apoptosis of mammary epithelial cells. Oncogene *26*: 6431-6441, 2007.

25 Yamashita S, Tsujino Y, Moriguchi K, Tatematsu M and Ushijima T: Chemical genomic screening for methylation-silenced genes in gastric cancer cell lines using 5-aza-2'-deoxycytidine treatment and oligonucleotide microarray. Cancer Sci *97*: 64-71, 2006.

26 Yang S, Zhong C, Frenkel B, Reddi AH and Roy-Burman P: Diverse biological effect and SMAD signaling of bone morphogenetic protein 7 in prostate tumor cells. Cancer Res *65*: 5769-5777, 2005.

27 Yang S, Lim M, Pham LK, Kendall SE, Reddi AH, Altieri DC and Roy-Burm P: Bone morphogenetic protein 7 protects prostate cancer cells from stress-induced apoptosis *via* both SMAD and c-Jun NH-2-terminal kinase pathways. Cancer Res *66*: 4285-4290, 2006.

28 Sobin LH and Fleming ID: TNM Classification of Malignant Tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. Cancer *80*: 1803-1804, 1997.

29 Jiang WG, Davies G, Martin TA, Parr C, Watkins G, Mason MD, Mokbel K and Mansel RE: Targeting matrilysin and its impact on tumor growth *in vivo*: the potential implications in breast cancer therapy. Clin Cancer Res *11*: 6012-6019, 2005.

30 Jiang WG, Hiscox S, Hallett MB, Horrobin DF, Mansel RE and Puntis MCA: Regulation of the expression of E-cadherin on human cancer cells by gamma-linolenic acid (GLA). Cancer Res *55*: 5043-5048, 1995.

31 Jiang WG, Hiscox S, Hallett MB, Scott C, Horrobin DF and Puntis MCA: Inhibition of hepatocyte growth factor-induced motility and *in vitro* invasion of human colon cancer cells by gamma-linolenic acid. Br J Cancer *71*: 744-752, 1995.

32 Rosen EM, Meromsky L, Setter E, Vinter DW and Goldberg ID: Smooth muscle-derived factor stimulates mobility of human tumor cells. Invasion Metastasis *10*: 49-64, 1990.

33 Jiang WG, Watkins G, Lane J, Cunnick GH, Douglas-Jones A, Mokbel K and Mansel RE: Prognostic value of rho GTPases and rho guanine nucleotide dissociation inhibitors in human breast cancers. Clin Cancer Res *9*: 6432-6440, 2003.

34 Zhang N, Sanders AJ, Ye L, Kynaston HG and Jiang WG: Expression of Vascular endothelial growth inhibitor (VEGI) in human urothelial cancer of the bladder and its impacts on the adhesion and migration of bladder cancer cells *in vitro*. Anticancer Res *30*: 87-96, 2010

35 Motoyama K, Tanaka F, Kosaka Y, Mimori K, Uetake H, Inoue H, Sugihara K and Mori M: Clinical significance of BMP7 in human colorectal cancer. Ann Surg Oncol *15*: 1530-1537, 2008.

36 Ye L, Kynaston H and Jiang WG: Bone morphogenetic protein-10 suppresses the growth and aggressiveness of prostate cancer cells through a SMAD independent pathway. J Urol *181*: 2749-2759, 2009.

37 Rothhammer T, Wild PJ, Meyer S, Bataille F, Pauer A, Klinkhammer-Schalke M, Hein R, Hofstaedter F and Bosserhoff AK: Bone morphogenetic protein 7 (BMP7) expression is a potential novel prognostic marker for recurrence in patients with primary melanoma. Cancer Biomark *3*: 111-117, 2007.

38 Ye L, Lewis-Russell JM, Kynaston H and Jiang WG: Endogenous bone morphogenetic protein-7 controls the motility of prostate cancer cells through regulation of bone morphogenetic protein antagonists. J Urol *178*: 1086-1091, 2007.

39 Notting I, Buijs J, Mintardjo R, van der Horst G, Vukicevic S, Lowik C, Schalij-Delfos N, Keunen J and van der Pluijm G: Bone morphogenetic protein 7 inhibits tumor growth of human uveal melanoma *in vivo*. Invest Ophthalmol Vis Sci *48*: 4882-4889, 2007.

40 Tada A, Nishihara T and Kato H: Bone morphogenetic protein 2 suppresses the transformed phenotype and restores actin microfilaments of human lung carcinoma A549 cells. Oncol Rep *5*: 1137-1140, 1998.

41 Alarmo EL, Pärssinen J, Ketolainen JM, Savinainen K, Karhu R and Kallioniemi A: BMP7 influences proliferation, migration, and invasion of breast cancer cells. Cancer Lett *275*: 35-43, 2009.

42 Chen D, Zhao M and Mundy GR: Bone morphogenetic proteins. Growth Factors *22*: 233-241, 2004.

43 ten Dijke P, Korchynskyi O, Valdimarsdottir G and Goumans MJ: Controlling cell fate by bone morphogenetic protein receptors. Mol Cell Endocrinol *211*: 105-113, 2003.

44 Balemans W and Van Hul W: Extracellular regulation of BMP signaling in vertebrates: a cocktail of modulators. Dev Biol *250*: 231-250, 2002.

45 Buijs JT, Henriquez NV, van Overveld PG, van der Horst G, Que I, Schwaninger R, Rentsch C, Ten Dijke P, Cleton-Jansen AM, Driouch K, Lidereau R, Bachelier R, Vukicevic S, Clézardin P, Papapoulos SE, Cecchini MG, Löwik CW and van der Pluijm G: Bone morphogenetic protein 7 in the development and treatment of bone metastases from breast cancer. Cancer Res *67*: 8742-8751, 2007.

46 Buijs JT, Rentsch CA, van der Horst G, van Overveld PG, Wetterwald A, Schwaninger R, Henriquez NV, Ten Dijke P, Borovecki F, Markwalder R, Thalmann GN, Papapoulos SE, Pelger RC, Vukicevic S, Cecchini MG, Löwik CW and van der Pluijm G: BMP7, a putative regulator of epithelial homeostasis in the human prostate, is a potent inhibitor of prostate cancer bone metastasis *in vivo*. Am J Pathol *171*: 1047-1057, 2007.