

## Original Article

# Human beta-defensin 2 promotes the proliferation of lung cancer cells through ATP-binding cassette transporter G2

Cun Gao, Weiming Yue, Hui Tian, Lin Li, Shuhai Li, Libo Si

Department of Thoracic Surgery, Shandong University Qilu Hospital, Jinan, Shandong, People's Republic of China

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**Abstract:** Objective: The aim of this study was to investigate the effect of human beta-defensin 2 (hBD2) and ATP-binding cassette transporter G2 (ABCG2) on the proliferation of lung cancer cells. Methods: One hundred and thirty patients with non-small cell lung cancer were recruited. All the patients underwent pulmonary lobectomy and the specimens (lung cancer tissues and corresponding non-tumor normal tissues of 130 patients) were collected. All the specimens were divided into two groups: NSCLC group (lung cancer tissues) and Control group (corresponding non-tumor normal tissues). Protein hBD2 and ABCG2 expressions were detected by immunohistochemistry. An *in vitro* study was also performed using A549 cells. A549 cells were treated with hBD2, proliferation was analyzed using MTT and ABCG2 expression was detected by western blotting. Next, A549 cells were pretreated with Fumitremorgin C, a selective ABCG2 inhibitor, and cell proliferation and ABCG2 expression were investigated. Results: Protein hBD2 and ABCG2 expressions in the NSCLC group were both higher compared with the control group (both  $P < 0.01$ ). Furthermore, hBD2 induced ABCG2 expression and A549 proliferation in a dose- and time-dependent manner (both  $P < 0.01$ ), while these effects were inhibited by the Fumitremorgin C treatment (both  $P < 0.01$ ). Conclusion: hBD2 induced the proliferation of lung cancer cells through ABCG2, thus playing an important role in the development of NSCLC.

**Keywords:** Human beta-defensin 2, non-small cell lung cancer, ATP-binding cassette transporter G2, cell proliferation

## Introduction

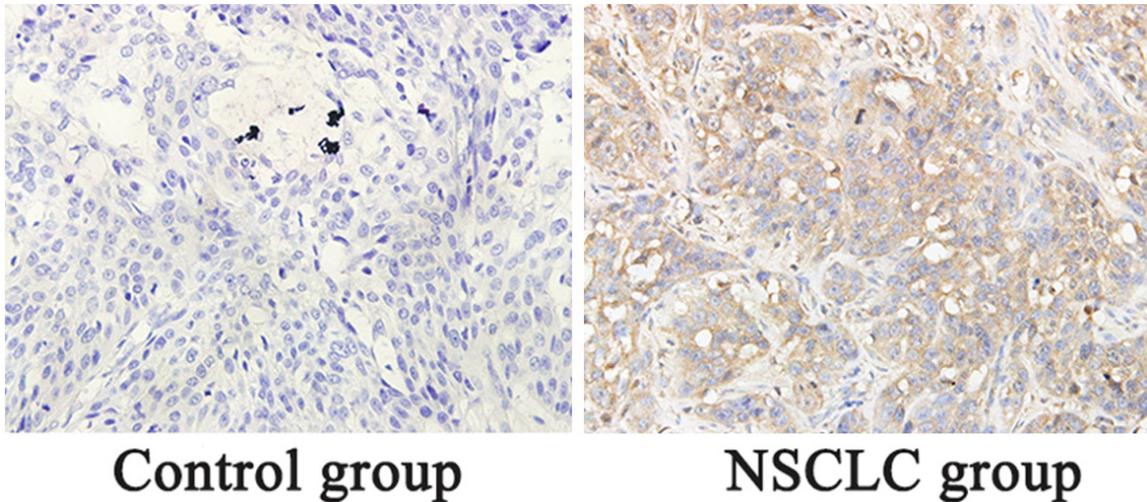
The respiratory epithelium is the first defensive barrier against the air pathogenic particles from the air. As a critical part of the human innate defensive system, the respiratory epithelium participates in the immunity by producing various bioactive materials. Human beta-defensin 2 (hBD2) is an important member of endogenous antimicrobial peptides rich in cysteine produced by the airway epithelium, playing an important role in protecting human body from microbes and regulating the immunity and inflammatory reactions [1]. Recent studies have reported that hBD2 is closely associated with a variety of respiratory disorders such as community acquired pneumonia (CAP), tuberculosis, chronic obstructive pulmonary disease (COPD), asthma, interstitial lung disease, Wegener's granulomatosis, and cystic fibrosis [2-4]. Lung cancer is one of the most important

disorders, and its development may be associated with hBD2 expression [5-7]. Indeed, hBD2 level in the lung tissue and serum is elevated in patients with lung cancer. However, the detailed relationship between lung cancer and hBD2 has not been clearly elucidated. The ATP-binding cassette transporter G2 (ABCG2) is a crucial member of ATP-binding cassette superfamily involving tumor drug resistance [8]. Both hBD2 and ABCG2 are epithelial biomarkers playing critical roles in the survival and proliferation of epithelial cells. Therefore, this study aimed to explore the effect of hBD2 and ABCG2 on lung cancer cells proliferation.

## Materials and methods

### Subjects

130 patients with non-small cell lung cancer undergoing lung resection were recruited for



**Figure 1.** hBD2 immunohistochemical staining in lung tissues. hBD2 expression was higher in NSCLC group than that in control group.

this study: 130 specimens of lung cancer tissues (NSCLC group) and 130 corresponding non-tumor normal tissues (Control group). Patients with comorbidities, such as interstitial lung diseases, chronic obstructive pulmonary disease, asthma and respiratory tract infection were excluded from this study. Lung cancer tissue and the corresponding non-tumor normal tissue specimens from NSCLC group and control group, respectively, were collected. The study was approved by the Ethics Committee of Shandong University, and written informed consent was given by each participant.

#### *hBD2 and ABCG2 immunostaining*

Lung tissues were formalin-fixed, paraffin-embedded and 4  $\mu\text{m}$  thick serial sections were cut and used for immunohistochemical staining. Sections were immunostained with primary antibodies anti-hBD2 and ABCG2 and were visualized using the avidin-biotin-peroxidase (ABC) complex (ZhongShan Biotech, Beijing, China) method. Color development was performed using a DAB color development kit (ZhongShan Biotech). Images were taken using an OLYMPUS IX81 light microscope (Olympus, Tokyo, Japan) fitted with a SPOT camera. Image analysis was performed using Image Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA). All slides were analyzed in a single batch by a single experienced quality assurance observer with quality assurance on ran-

domly selected slides provided by a professional academic pathologist.

#### *Cell culture and treatment*

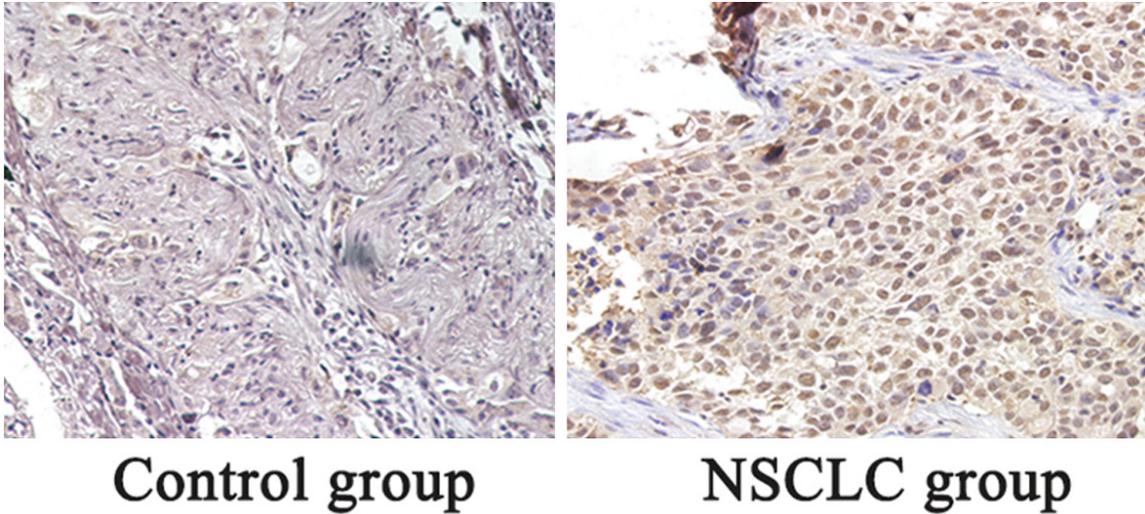
The human lung cancer A549 cells were obtained from the American Type Culture Collection (ATCC). The cell line was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 mg/ml streptomycin supplemented. Cells were incubated at 37°C in a humidified 5% CO<sub>2</sub> atmosphere. After achieving 80% confluence, the A549 cells were serum starved for 24 h before treatment with hBD2 in the concentration gradient (0, 0.5  $\mu\text{g}/\text{ml}$ , 1  $\mu\text{g}/\text{ml}$ , 2  $\mu\text{g}/\text{ml}$ , 5  $\mu\text{g}/\text{ml}$ , 10  $\mu\text{g}/\text{ml}$ ) at different incubation periods (0, 0.5 h, 1 h, 4 h, 12 h) with and without pretreatment with Fumitremorgin C.

#### *A549 cells proliferation*

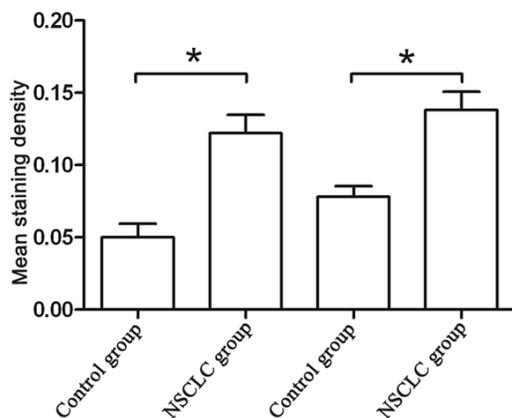
A549 cells proliferation was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, USA) assay as previously described.

#### *Western blotting*

ABCG2 expression in A549 cells following treatment with hBD2 with and without pretreatment with Fumitremorgin C was performed using western blotting. Total cell protein was extracted and an aliquot of 20  $\mu\text{g}$  was separated by



**Figure 2.** ABCG2 immunohistochemical staining in lung tissues. ABCG2 expression was higher in NSCLC group than that in Control group.



**Figure 3.** Quantitative analysis of HBD2 and ABCG2 expression in lung tissue (\* $P < 0.05$ ).

10% SDS-polyacrylamide gel electrophoresis, transferred to a PVDF membrane, and then immunoblotted with primary antibody specific for ABCG2. The chemical signal was detected following incubation with HRP-conjugated secondary antibody and chemiluminescence (Cell Signaling Technology, USA).

#### Statistical analysis

Results were expressed as mean  $\pm$  S.E.M. The Kruskal-Wallis and Mann-Whitney U were used for nonparametric variables. Comparisons between multiple treatment groups *in vitro* were performed by one-way ANOVA. A value of  $P < 0.05$  was considered statistically significant.

Statistical analysis was performed using SPSS 18.0 software.

#### Results

##### *hBD2 and ABCG2 expression in lung cancer tissue*

hBD2 and ABCG2 expression was higher in NSCLC group than that in control group (**Figures 1-3**).

##### *A549 cells proliferation induced by HBD*

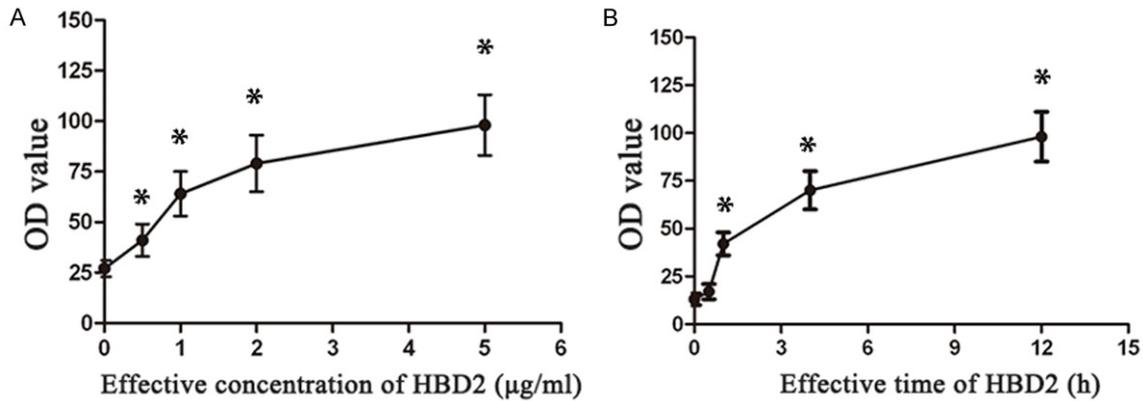
The results showed that hBD2 induced A549 cells proliferation in a dose- and time-dependent manner (**Figure 4**).

##### *Fumitremorgin C effect on A549 cells proliferation induced by hBD2*

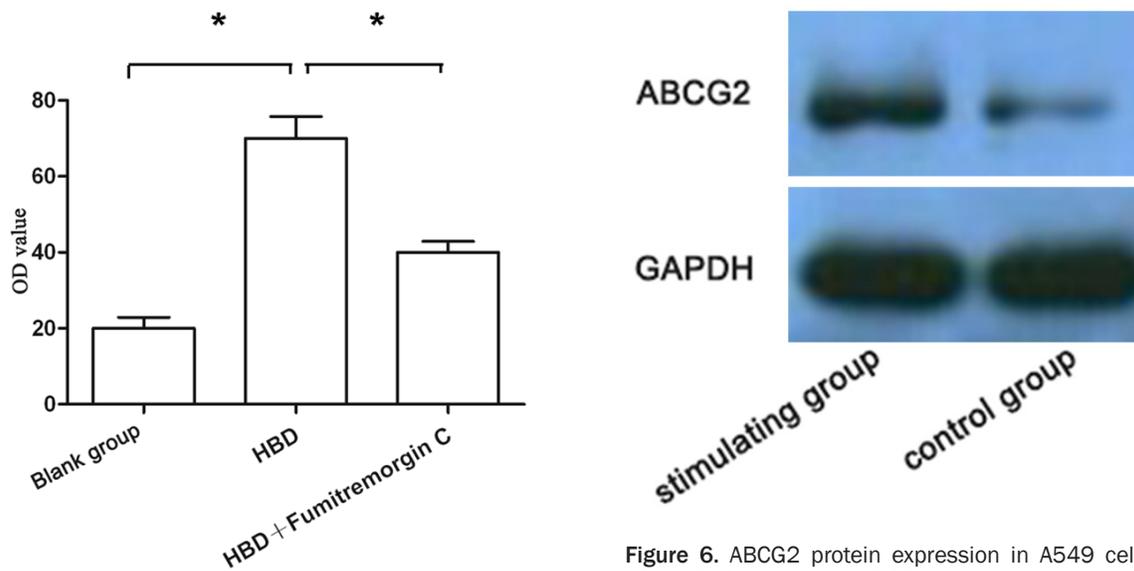
The Fumitremorgin C, an ABCG2 inhibitor, partially inhibited A549 cells proliferation induced by HBD2 incompletely. When pretreated with the inhibitor of ABCG2, However, this inhibition was statistically significant ( $P < 0.01$ ) (**Figure 5**).

##### *ABCG2 expression after hBD2 treatment*

The results showed that hBD2 induced ABCG2 expression in A549 cells. After hBD2 stimulation, ABCG2 expression was higher than the expression in the control group not stimulated with hBD2 (**Figure 6**).



**Figure 4.** Concentration- and time-dependent effect of hBD2 on A549 cells proliferation. Cells were treated with hBD2 at different concentrations and time. The MTT method was used to detect the A549 cells proliferation (\* $P < 0.05$ ).



**Figure 5.** Fumitremorgin C effect on A549 cells proliferation induced by hBD2. Cells were pretreated with Fumitremorgin C, and subsequently treated with hBD2. Cells proliferation was detected by MTT method, and the OD value was measured (\* $P < 0.01$ ).

**Figure 6.** ABCG2 protein expression in A549 cells after hBD2 treatment. A549 cells were treated with hBD2, and ABCG2 expression was evaluated by western blotting. ABCG2 expression was higher in the stimulated group (hBD2 treated group) than that in control group.

**Discussion**

Our study showed that hBD2 and ABCG2 expressions are both enhanced in lung cancer tissues, which hBD2 induced the proliferation of lung cancer A549 cells in vitro, and this induction was inhibited when ABCG2 was blocked.

As previously reported, defensins, host defense peptides, widely expressed in eukaryotic cells, protect humans from pathogenic microbes, thus playing an important role in regulating

inflammation in vivo. The defensin family included, but is not limited to, hBD1, hBD2 and hBD3. In the beta defensin family, hBD2 possesses the strongest antibacterial activity. In the human body the respiratory epithelium can synthesize and secrete hBD2 [9]. Indeed, the local airway, the epithelial lining fluid or bronchoalveolar lavage fluid can produce high levels of hBD2 under infection, COPD, asthma and other pathological conditions. From an evolutionary point of view, hBD2 is very conservative, and possesses the diversity in biological function. A recent study has shown that hBD2 possesses anti-microbial activity [10], and had the certain

bactericidal effects on bacteria, fungi and viruses. hBD2 also had an immunomodulatory activity, which could induce chemotaxis of T lymphocyte and immature dendritic cells. Besides, hBD2 induces various inflammatory factors such as IL-1, IL-6, IL-10 and TNF-alpha, and plays important roles in the natural immunity and acquired immunity [4, 11, 12]. In addition, hBD2 may also possess the ability to regulate the biological behavior of the tumors [13, 14]. Indeed, hBD2 could promote the proliferation of tumor cells, and play an important role in tumor progression and metastasis. The present study showed that the expression of hBD2 was upregulated in lung cancer tissues, and was able to induce alveolar epithelial A549 cells proliferation *in vitro*.

ABCG2 is an important member of the membrane transporter ABC superfamily which plays an important role in drug resistance. ABCG2 is associated with lung cancer progression and poor prognosis closely [15-17]. Our study indicated that the up regulation of ABCG2 expression in lung cancer tissues was consistent with most of the previous studies. Some studies suggesting that in the epithelial tissues ABCG2 and hBD2 could both be used as molecular markers and participate in the diagnosis and evaluation of disease [5, 18]. Our study detected for the first time, the expression of both ABCG2 and hBD2 in lung cancer tissue and A549 cells, and discussed their function in the proliferation of lung cancer cells. This study also showed that the proliferation of A549 lung cancer cells induced by hBD2 was significantly inhibited when ABCG2 was blocked, indicating that the inductive effect of hBD2 on lung cancer cells proliferation was at least partially mediated by ABCG2.

In summary, our study found that HBD2 and ABCG2 expression were upregulated simultaneously in lung cancer tissues, that hBD2 induced the proliferation of lung cancer cells, and that this inductive effect was at least partially mediated by ABCG2. This study showed that hBD2 could regulate the proliferation of lung cancer cells through a pathway involving ABCG2, thus playing a role in lung cancer progression and poor prognosis.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Hui Tian, Department of Thoracic Surgery, Shandong University Qilu Hospital, 107 Wenhuxi Road, Jinan 250012, Shandong, People's Republic of China. Tel: +86-18560086938; Fax: +86-531-82166662; E-mail: tianhui1967@126.com

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