Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

# Expression and function of Annexin Ⅱ in lung cancer tissue

Jun-Wei Cui, Yong-Liang Wang*

*First Affiliated Hospital of Xinxiang Medical University, Xinxiang 453100, Henan, China*

ARTICLE INFO

*Article history:*
Received 15 November 2012
Received in revised form 27 December 2012
Accepted 28 January 2013
Available online 28 February 2013

*Keywords:*
Annexin Ⅱ
Lung cancer
Cytokines

ABSTRACT

**Objective:** To explore the expression of Annexin Ⅱ and its relationship with the cell differentiation, proliferation in lung cancer. **Methods:** RT-PCR and Western blot assays were used to detect the expression of Annexin Ⅱ in lung cancer tissues and cell lines. **Results:** Annexin Ⅱ was significantly up-regulated in lung cancer tissues, and in lung cancer cell lines, Annexin Ⅱ had higher mRNA and protein expressions. **Conclusions:** Annexin Ⅱ is up-regulated in lung cancer, suggesting that the Annexin Ⅱ has a potential value in the human lung cancer.

## 1. Introduction

In some western countries and large cities of China, lung cancer has the highest morbidity of all malignancies in men. It accounted for 1.2 million new cases and 1 million deaths every year[1]. Non-small cell lung cancer (NSCLC) accounts for about 80% of all lung cancers, and is always diagnosed at an advanced stage. Despite advances in surgical therapies, radiotherapies and chemotherapies, the prognosis of NSCLC patients is still poor, with a 5-year overall survival rate at less than 20%[2]. Therefore, clinicians and scientists need to find novel strategies for more effective treatments. Recent research has determined that the change of multiple key molecules or signal pathways can promote the occurrence and the development of lung cancer via affect lung cancer cell proliferation, migration and invasion.

Annexin Ⅱ protein has multiple functions in the cytoplasm and nucleus, its abnormal expression is correlated to tumorigenesis and development. The purpose of this article was to explore the relationship between Annexin Ⅱ and lung cancer.

## 2. Materials and methods

### 2.1. Materials

Bronchial epithelial cells BEAS-2B, human lung adenocarcinoma A549, human lung squamous cell carcinoma line EBC-1 and control group cells were obtained from Cell Resource Center of Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). RPMI 1640 medium and trypsinogen were purchased from Gibco GRL (Grand Island, NY, USA).Trizol was purchased from Invitrogen (Carlsbad, California, USA).

### 2.2. Clinical sample preparation

Tumor specimens ($n$=75) were obtained from patients [male $n$=42, female $n$=53, mean age: (61.0±7.1) years old] with lung cancer who underwent surgical resection and

*Corresponding author: Yong-Liang Wang, Associate Chief Physician, First Affiliated Hospital of Xinxiang Medical University, Xinxiang 453100, Henan, China.
Tel: 13937335809
E-mail: hbedit@163.com

diagnosed by pathology in XX hospital. The carcinomas and their pericarcinous tissues (1.0 cm×0.5 cm×0.5 cm) were immediately frozen in liquid nitrogen and stored at −80 ℃ refrigerator.

### 2.3. Cell culture

Different differentiated human lung cancer cell lines (human lung adenocarcinoma A549, human lung squamous cell carcinoma line EBC-1) and bronchial epithelial cells BEAS-2B were maintained under a humidified atmosphere of 5% $CO_2$ at 37 ℃ in RPMI 1640 medium (Invitrogen), supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin. Cells were digested by 0.25% trypsin which contains 0.02% EDTA.

### 2.4. Reverse transcription and PCR analysis

Total RNA from tissue or cells was isolated with Trizol, and 2 μL RNA was used for cDNA synthesis. Using β-actin as internal standard control, 2 μL cDNA was used as a template for PCR amplification to detect the target gene mRNA expression levels. According the kites instruction, 20 μL PCR system was established: 2 μL cDNA, 10 μL SYBR Green Mix (Applied Biosystems, Foster City, CA), 0.5 μL upstream primer and downstream primer (10 μmol/L). PCR amplification was performed with 5 min at 95 ℃, 45 cycles of 30 sec at 94 ℃, 30 sec at 60 ℃. Before the experiment, primers were designed by Primer 5.0, and tested for specificity by Blast. The primer is as follows: Annexin Ⅱ primers: 5′-CAAATTCACCGAGATCCTGT-3′ and 5′-TGCTGGAGTGCTGTACGAAA-3′, β-actin: upstream primer 5′-ACCACAGTCCATGCCATCAC-3′, downstream primer 5′-TCCACCACCCTGTTGCTGTA-3′. Electrophoresis images were analyzed by GelprO4 gel documentation analysis software. The numbers were mean optical density, which stand for the expression level of the gene.

### 2.5. West-blot

Equal amounts (60 μg) of protein were separated on 12% SDS-polyacrylamide gels and transferred to polyvinylidene fluoride membranes. After blocking in 5% non-fat milk in TBST buffer for 1 hour at room temperature, the membranes were incubated overnight at 4 ħ with primary anti- bodies to target protein or β-actin, then washed with TPBS 3 times and incubated in horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. The bands were detected by method of scan the absorbance after chemiluminescence.

### 2.6. Stastical analysis

All data are presented as mean±SD. The difference between means was analyzed using *t*-test or one-way ANOVA. All statistical analyses were performed using SPSS 11.5 software. *P* values <0.05 were considered statistically significant.

## 3. Results

### 3.1. Expression of Annexin Ⅱ in lung cancer and their pericarcinous tissues

In order to clarify the function of Annexin Ⅱ in the development of lung cancer, we investigate the expression of Annexin Ⅱ in lung cancer and their pericarcinous tissues by RT-PCR, Western blot and immunohistochemistry. The results showed that, compared with pericarcinous tissues, the expression of Annexin Ⅱ mRNA in lung cancer was significantly increased ($P<0.05$) (Figure 1, Table 1). Western blotting revealed that Annexin Ⅱ proteins increased dramatically in lung cancer, compared with pericarcinous tissues ($P<0.05$) (Figure 2, Table 1).

**Table 1**
Expression of Annexin Ⅱ in different tissues.

| Number | RT-PCR | Western blot |
|---|---|---|
| 1 | 0.38 | 1.42 |
| 2 | 0.37 | 1.32 |
| 3 | 0.42 | 1.59 |
| 4 | 0.41 | 1.49 |
| 5 | 0.36 | 1.65 |
| 6 | 0.96* | 2.21* |
| 7 | 1.06* | 2.23* |
| 8 | 0.80* | 2.89* |
| 9 | 0.91* | 2.81* |
| 10 | 0.85* | 2.78* |

Compared with pericarcinous tissues: *$P<0.05$.

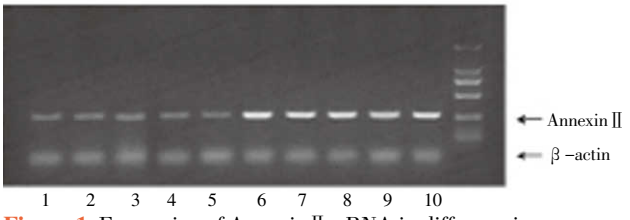


**Figure 1.** Expression of Annexin Ⅱ mRNA in different tissues. 1-5: pericarcinous tissues; 6-10: lung cancer.

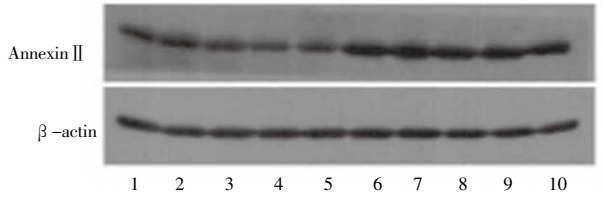


**Figure 2.** Expression of Annexin Ⅱ proteins in different tissues.

### 3.2. Expression of Annexin Ⅱ in lung cell lines

In order to reveal the relationship between Annexin Ⅱ and the development of lung cancer, We further observed

Annexin Ⅱ gene expression and correlation of lung cancer. The result of PCR and western blot showed that the expression of Annexin Ⅱ mRNA or Annexin Ⅱ protein varied from normal cells to lung cancer cells. Compared with normal bronchial epithelial cells, the expression of Annexin Ⅱ in adenocarcinoma and squamous cancer cells were significant increase ($P$<0.05) (Table 2, Figure 3, Figure 4).

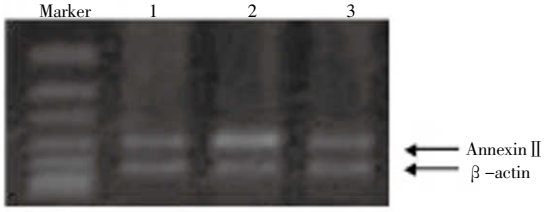


**Figure 3.** Expression of Annexin Ⅱ mRNA between different cell lines.
1: BEAS-2B, 2: A549, 3: EBC-1.

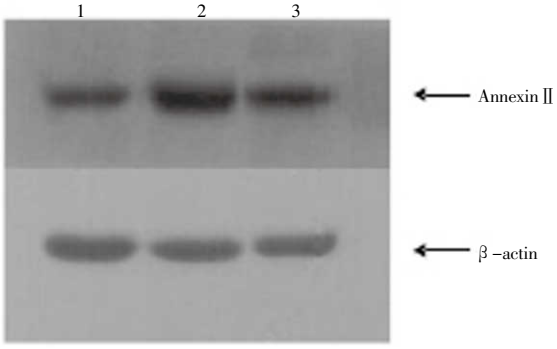


**Figure 4.** Expression of Annexin Ⅱ protein between different cell lines.
1: BEAS-2B, 2: A549, 3: EBC-1.

**Table 2**

Expression of Annexin Ⅱ mRNA or protein between different cell lines.

| Cell line | RT-PCR | Western blot |
|---|---|---|
| control | 0.772 | 0.32 |
| EBC-1 | 1.612* | 0.74* |
| A549 | 2.402* | 1.23* |

Compared with control group, *$P$<0.05.

## 4. Discussion

The incidence of Lung cancer continues to increase in the world and larger cities of China. The abnormal expression of the genies is one of the mechanisms of tumorigenesis.

Annexin family are $Ca^{2+}$ phospholipid proteins, which exist in cytoplasmic. They are expressed at high level, accounting for 1%-2% of the total protein. Annexin exists in two forms: soluble form and combining form-combining with cytoskeletal protein or extracellular matrix proteins[3]. The gene and protein molecular structure of Annexin Ⅱ is roughly same with membrane group protein family, which is a long repeats composed of 70 amino acids. A number of long repeats compose a homologous region, and every homologous region form five alpha helix. These areas are located in a plane, compose two symmetrical disk structures. In spite of the different $N$-terminal, Annexin family proteins are all binds to phospholipids and membranes in a $Ca^{2+}$-dependent manner[4]. Research shows that several diseases associate with the abnormal expression of Annexin family proteins. The members of Annexin family are related to tumor proliferation, differentiation, invasion, migration, and clinical stages. Annexin family proteins are getting more and more importance in the tumor development, and become the focus fields of cancer research recently[5,6].

A great deal of research show that the change of the expression of effects tumor development, drug resistance, and metastasis. The expression lever of Annexin Ⅱ vary from tumor type, and play an important role in DNA synthetizing. Zhang *et al* found that Annexin Ⅱ and S100A10 are over expressed in human breast cancer cell. Hyperproteolysis of Annexin Ⅱ or escape the degradation by proteasome might be a reason which leads to breast cancer[7]. Research shows that Annexin A5 is related to the differentiation of uterine squamous cell carcinoma. Hellman *et al*[8] considered that Annexin Ⅱ are also aberrantly expressed in cervical cancer.

Above all, we find that the expression lever of Annexin Ⅱ is related to the proliferation and differentiation of Lung cancer. Our results confirm the significantly high expression of Annexin Ⅱ in lung cancer tissue and cells. Based on these findings, We can assume that Annexin Ⅱ might be a negative marker to tumor progression. Although the exact mechanism is not very clear and needs more investigations, these results have already suggested that Annexin Ⅱ play an important role in the lung cancer development as an oncogene. And further research is needed to study whether Annexin Ⅱ -siRNAs could silent the genes and inhibit the differentiation and proliferation of tumor cells.

## Conflict of interest statement

We declare that we have no conflict of interest.

## References

[1] Juergens RA. Adjuvant treatment in non-small cell lung cancer: where are we now? *J Natl Compr Canc Netw* 2006; **4**: 595.
[2] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics. *CA Cancer J Clin* 2005; **55**(2): 74.
[3] Jung EJ, Moon HG, Park ST, Cho BI, Lee SM, Jeong CY, et al. Decreased annexin A3 expression correlates with tumor progression in papillary thyroid cancer. *Proteomics Clin Appl* 2010; **4**(5): 528-537. Epub 2010 Feb 26.
[4] Hou ML, Xing TY, Deng SS. Advance in relationship between Annexin A2 and breast cancer Annexin A2. *Chin J Clinicians* 2011; **14**(5): 4206-4208.
[5] Baine MJ, Chakraborty S, Smith LM, Mallya K, Sasson AR, Brand RE, et al. Transcriptional profiling of peripheral blood mononuclear cells in pancreatic cancer patients identifies novel genes with potential diagnostic utility. *PLoS One* 2011; 6(2): e17014.
[6] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**(2): 69-90. Epub 2011 Feb 4.
[7] Ge X, Yuan ZF. Advance in Annexin A2 and cancer. *Oncol Progr* 2009; **7**: 304-307.
[8] Hellman K, Alaiya AA, Becker S, Lomnytska M, Schedvins K, Steinberg W, et al. Differential tissue-specific protein markers of vaginal arcinoma. *Br J Cancer* 2009; **100**: 1303-1314.