# Co-expression of immunoglobulin-like transcript 4 and angiopoietin-like proteins in human non-small cell lung cancer

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Received December 17, 2013; Accepted November 5, 2014

DOI: 10.3892/mmr_xxxxxxxx

**Abstract.** The development of strategies for the inhibition of non-small cell lung cancer (NSCLC) progression and metastasis have been mainly unsuccessful, in part due to insufficient mechanistic understanding of the disease. In the current study, the critical role of the co-expression of immunoglobulin-like transcript 4 (ILT4) and its ligands, angiopoietin-like proteins (ANGPTLs), in the development of NSCLC was demonstrated. ILT4 and ANGPTL2 or ANGPTL5 were found to be co-expressed in the five NSCLC cell lines that were investigated at the mRNA and protein level. Upon up- or downregulation of ILT4, the expression of ANGPTL2 was increased or reduced, respectively, while the expression of ANGPTL5 was unaffected. The co-expression of ILT4 and ANGPTL2/ANGPTL5 was detected in human primary NSCLC tissues using immunohistochemical analysis. In total, 114 lung cancer specimens were included in the study; high expression of ILT4, ANGPTL2 and ANGPTL5 was observed in 58.8, 45.6 and 55.3%, respectively. The expression of ILT4 was found to be significantly correlated with a high expression level of ANGPTL2 (R=0.466, P=0.004); however, it was not correlated with the expression of ANGPTL5 (R=0.142, P=0.131). In ILT4-positive samples, cases with ANGPTL2-positive expression levels presented greater levels of lymph node metastasis (P=0.011) and shorter overall survival times (P=0.045). In addition, cases with ANGPTL5-positive expression presented poor overall survival rates (P=0.040). By contrast, in the ILT4-negative cases, no statistically significant differences were identified in the overall survival rates between samples with high and low expression of ANGPTL2 or ANGPTL5. In conclusion, the present study demonstrated the presence of interaction among ILT4 and ANGPTLs, which may be important in NSCLC progression. Therefore, the blockade of ANGPTLs or ILT4 may be an effective therapeutic approach for NSCLC treatment.

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*Key words:* immunoglobulin-like transcript 4, angiopoietin-like protein 2, angiopoietin-like protein 5, non-small cell lung cancer

## Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, due to its high incidence, malignant characteristics and the lack of available effective treatment strategies. Non-small cell lung cancer (NSCLC) accounts for ~85% of lung cancer cases. Surgery can be performed to treat patients in the early stages of the disease; however, the rates of recurrence and metastasis remain high in the majority of lung cancer patients (1). Thus, understanding the molecular mechanisms underlying progression and metastasis is essential and may aid the identification of novel therapeutic targets by improving the treatment response of NSCLC.

Immunoglobulin (Ig)-like transcript 4 (ILT4) is a member of the Ig-like inhibitory receptor family and contains four Ig domains and three immunoreceptor tyrosine-based inhibitory motifs (2). ILT4 is predominantly expressed in lymphoid and myeloid cells (3,4), binding to major histocompatibility complex-I molecules, including human leukocyte antigen (HLA)-A2, HLA-G and HLA-F, or their viral homologue, UL18 (5-8). Inflammatory stimuli or cytokines, such as interleukin-10 or interferon-α, and growth factors (9) have been demonstrated to regulate the expression of ILT4. A previous study revealed that ILT4 expression may be induced in NSCLC cells and was found to be associated with lymph node metastasis (10). However, the precise role of ILT4 in the progression and metastasis of NSCLC is poorly understood.

ILT4 and its mouse ortholog, paired Ig-like receptor (PirB), were found to be expressed in human and mouse hematopoietic stem cells (HSCs), respectively (11). ILT4 and PirB are receptors of several angiopoietin-like proteins (ANGPTLs) (11). The binding of ANGPTLs to ILT4 maintains the stemness of normal adult stem cells and supports acute myeloid leukemia (AML) development (11). Among the

ANGPTLs, ANGPTL2 and 5 have been shown to possess the highest affinities for ILT4 (11).

ANGPTLs constitute a family of seven secreted glycoproteins and play an important role in expansion, lipid metabolism, angiogenesis and inflammation in HSCs (12-15). ANGPTLs 1 to 7 possess an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain, which are characteristics of angiopoietins. ANGPTL2 is a causative mediator of chronic inflammation in obesity and its related metabolic abnormalities (12). In addition, ANGPTL2 has been demonstrated to be involved in inflammatory carcinogenesis (16). In patients with NSCLC, increased ANGPTL2 mRNA expression in tumor tissues is correlated with lymph node metastasis (17). Furthermore, the protein expression of ANGPTL2 has also been observed in a number of other tumor types, including ovarian cancer (18) and sarcoma (19). The tumor cell-derived protein, ANGPTL2, accelerates tumor metastasis by increasing tumor cell migration in an autocrine/paracrine manner, in addition to enhancing tumor angiogenesis (20). ANGPTL5, which is mainly expressed in adult human heart tissues, regulates the lipoprotein metabolism and supports the efficient expansion of HSCs without compromising their repopulating potential (21-23). However, the role of ANGPTL5 in cancer has not yet been explored.

To the best of our knowledge, no previous studies exist regarding the function and co-expression of ILT4 and ANGPTLs in solid tumor cells. Since ANGPTL2 and 5 bind to ILT4-expressing cells more efficiently compared with other ANGPTLs (11), the present study focused on assessing the co-expression of ANGPTL2/ANGPTL5 and ILT4 in the NSCLC cell lines and tissues. In addition, the correlation between ANGPTL2/ANGPTL5 and ILT4 was analyzed in cases where ILT4 expression was up- or downregulated. Furthermore, the association of ILT4/ANGPTL2 or ILT4/ANGPTL5 co-expression with clinicopathological features and the survival time of patients were determined using NSCLC specimens. The present study may aid future studies on the interaction among ILT4 and ANGPTLs in human NSCLC.

## Materials and methods

*Cell culture*. The following NSCLC cell lines were used: H1650, H226, H1299, H1975 and A549 (Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China). The cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (GE Healthcare Life Sciences, Logan, UT, USA), 100 U/l penicillin (Beyotime Institute of Biotechnology, Shanghai, China) and 100 mg/l streptomycin (Beyotime Institute of Biotechnology).

*Cell transfection*. The following plasmids were obtained from Genechem Co., Ltd. (Shanghai, China): Pez-lv105-ILT4 (ILT4 vector), pGPU6/GFP/Neo-shILT4-1 [short hairpin (sh)ILT4-1] and pGPU6/GFP/Neo-shILT4-2 (shILT4-2). The plasmids were transfected into H1650 cells using the X-tremeGENE HP DNA Transfection Reagent (Roche Diagnostics, Basel, Switzerland), according to the manufacturer's instructions. A nontargeting plasmid was used as a negative control (NC). The shRNA sequences were as follows: shILT4-1, 5'-GAAGAA GAACACCCACAATGC-3'; shILT4-2, 5'-GCTATGGTTATG ACTTGAACT-3'; and NC, 5'-GTTCTCCGAACGTGTCAC GT-3'. The transfected cells were collected at 72 h and the gene expression was assessed prior to further experiments.

*Reverse transcription-polymerase chain reaction (RT-PCR) analysis*. Total RNA was isolated using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), according to the manufacturer's protocol. cDNAs were synthesized from the total RNA (2 $\mu$g) using random primers with a First Strand cDNA Synthesis kit (Fermentas, Ontario, Canada). RT-PCR was performed using the following primers: ILT4 forward, 5'-GCA TCTTGGATTACACGGATACG-3', and reverse, 5'-CTGACA GCCATATCGCCCTG-3'; ANGPTL2 forward, 5'-CGCATC TCATCTCCAAACTACA-3', and reverse, 5'-CCAAACATC CAACATCTCACAC-3'; ANGPTL5 forward, 5'-CTGTAT GTGGCTTTGGAATCTG-3', and reverse, 5'-CGGTCTTGT TATGGAGGTGACT-3'); GAPDH forward, 5'-AGAAGGCTG GGGCTCATTTG-3', and reverse, 5'-AGGGGCCATCCA CAGTCTTC-3'. The reaction was incubated for 35 cycles at 94˚C for 20 sec, 55.2˚C for 20 sec and 72˚C for 45 sec. GAPDH was amplified simultaneously as an internal control.

*Western blot analysis*. Total protein (30 $\mu$g) was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (EMD Millipore, Boston, MA, USA). Unspecific binding was blocked with 5% skimmed milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h at room temperature. Next, the blotted membranes were incubated overnight at 4˚C with specific antibodies. The following primary antibodies were used: Mouse monoclonal (mAb) anti-human ILT4 (1:400; Abgent, Inc., San Diego, CA, USA), rabbit polyclonal anti-human ANGPTL2 (1:1,000 Proteintech Group, Inc., Wuhan, China), rabbit polyclonal anti-human ANGPTL5 (1:1,000; Proteintech Group, Inc.) and rabbit mAb anti-human GAPDH (1:10,000; Proteintech Group, Inc.). Detection was performed using horseradish peroxidase-conjugated goat anti-mouse/rabbit secondary antibodies (1:5,000; Proteintech Group, Inc.). Finally, western blots were developed using an enhanced chemiluminescence reagent (Beyotime Institute of Biotechnology, Nantong, China) and exposed to a Kodak X-ray film (XAR-5; Kodak, Rochester, NY, USA). The intensities of the bands were calculated by densitometric analysis using the Image lab 4.1 software (Bio-Rad, Co., Hercules, CA, USA).

*Patients and samples*. Tumor specimens were obtained following surgical resection for NSCLC at the Jinan Central Hospital Affiliated to Shandong University (Jinan, China). The study was approved by the Review Board and Ethics Committee of the Jinan Central Hospital Affiliated to Shandong University, and written informed consent was obtained from all patients. A total of 114 NSCLC patients (male, 86; female, 28; mean age at diagnosis, 61.6 years) who were not subjected to any preoperative therapy were included in the current study. Among these 114 tumors, 53 were adenocarcinomas, 48 were squamous cell carcinomas and 13 were other tumor types, while 6 were well-differentiated, 74 were moderately-differentiated and 34 were poorly-differentiated. The patients were classified according to the TNM classification of the International Union Against Cancer (24). Following classification, which indicated

the prognosis of patient, with stage IV being the worse prognosis, 34 patients were determined to be at stage I, 44 at stage II, 25 at stage III and 11 at stage IV.

*Immunohistochemical analysis*. The resected tissue specimens were fixed in formalin (ComWin Biotech Co., Beijing, China) overnight and embedded in paraffin (ComWin Biotech Co.). A series of 4-$\mu$m sections were prepared for immunohistological staining. The sections were deparaffinized and rehydrated, and antigens were retrieved in Tris buffer by boiling in a microwave oven (600 W) for 10 min. The endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 10 min. Subsequently, the sections were incubated overnight at 4˚C with the primary antibodies [anti-ILT4 mAb (1:200), anti-ANGPTL2 mAb (1:50) and anti-ANGPTL5 mAb (1:100)]. In order to detect primary antibody binding, the sections were incubated with Elivision™ plus Polyer HRP (mouse/rabbit) IHC kit (Maixin, Fuzhou, China) for 30 min at room temperature and with streptavidin-conjugated peroxidase (ComWin Biotech Co.) for further 30 min. The sections were visualized using a 3,3'-diaminobenzidine solution (MaiXin) and counterstained with hematoxylin. Negative controls were prepared using normal mouse and rabbit IgG (Proteintech Group, Inc.) instead of the primary antibody. Immunohistochemical assays were performed simultaneously by two independent investigators. The percentage of stained cells was recorded at a magnification of x400 using biological microscopes (bx43; Olympus Co., Tokyo, Japan), in at least five fields, in randomly selected tumor areas. Brown staining of >10% of cells was considered as positive.

*Statistical analysis*. SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. The association among the expression levels of ILT4/ANGPTL2/ANGPTL5 and clinicopathological variables were analyzed using $\chi^2$ test. Spearman's correlation analysis was used to analyze the correlation between the expression levels of ILT4 and ANGPTL2/ANGPTL5. The overall survival time was measured from the date of initial diagnosis to mortality or the last day of the follow-up evaluation. Survival curves were constructed using the Kaplan-Meier method and compared using the log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Co-expression of ILT4 and ANGPTL2/ANGPTL5 in NSCLC cell lines*. The mRNA co-expression levels of ILT4 with ANGPTL2 or ANGPTL5 in the five NSCLC cell lines were determined using RT-PCR (Fig. 1A). In addition, the protein co-expression levels of ILT4 and ANGPTL2/ANGPTL5 were determined using western blot analysis in all cell lines (Fig. 1B). The results showed that ILT4 and ANGPTL2/ANGPTL5 were found to be co-expressed in all five NSCLC cell lines at the mRNA as well as the protein level.

*Effect of ILT4 expression on the regulation of ANGPTL2 and ANGPTL5 in NSCLC cell lines*. To determine the effect of ILT4 expression on ANGPTLs, ILT4 expression was down- and upregulated using ILT4 shRNA (shILT4-1 and shILT4-2)

Table I. Correlation between the expression levels of ILT4 and ANGPTL2/ANGPTL5 in primary human NSCLC tissues.

| | ANGPTL2[a] (No. of cases) | | ANGPTL5[b] (No. of cases) | |
|---|---|---|---|---|
| ILT4 | + | – | + | – |
| + | 38 | 29 | 41 | 26 |
| – | 14 | 33 | 22 | 25 |

[a]R=0.466; P=0.004; [b]R=0.142, P=0.131. ILT4, immunoglobulin-like transcript 4; ANGPTL, angiopoietin-like protein; +, positive expression; -, negative expression.

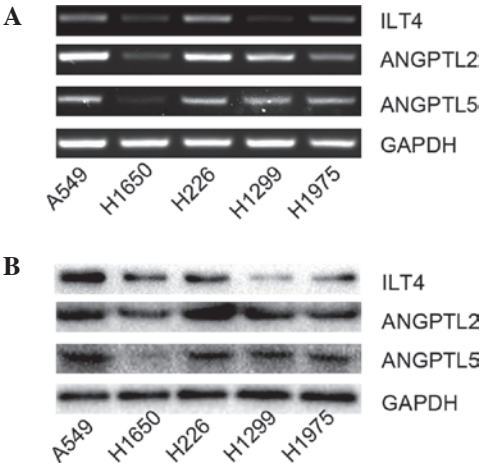


Figure 1. Co-expression of ILT4 and ANGPTL2/ANGPTL5 in five NSCLC cell lines, demonstrated by (A) RT-PCR at the mRNA level and (B) western blot analysis at the protein level. ILT4, immunoglobulin-like transcript 4; ANGPTL, angiopoietin-like protein; NSCLC, non-small cell lung cancer; RT-PCR, reverse transcription-polymerase chain reaction.

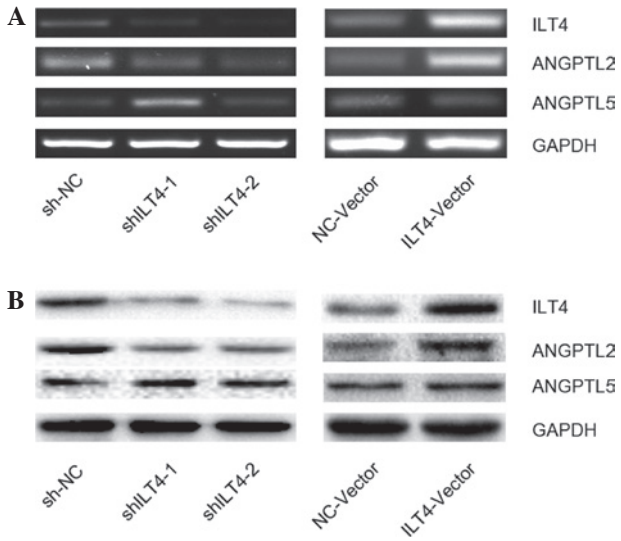


Figure 2. Effect of ILT4 expression on the regulation of ANGPTL2/ANGPTL5 in NSCLC cell line H1650. ILT4 were down- and upregulated using ILT4 shRNA (shILT4-1 and shILT4-2) and ILT4 expression plasmids (ILT4-vector). Next, the expression of ANGPTL2/ANGPTL5 was assayed at the (A) mRNA and (B) protein levels. sh-NC and NC-vector were used as the negative control. ILT4, immunoglobulin-like transcript 4; ANGPTL, angiopoietin-like protein; NSCLC, non-small cell lung cancer; NC, negative control.

Table II. Correlation between ILT4/ANGPTL2 co-expression and clinicopathological factors in primary human NSCLC tissues.

| | ILT4 (+) (no. of cases) | | | ILT4 (-) (no. of cases) | | |
|---|---|---|---|---|---|---|
| Variable | ANGPTL2 (+) | ANGPTL2 (-) | P-value | ANGPTL2 (+) | ANGPTL2 (-) | P-value |
| Age (years) | | | | | | |
| ≤60 | 14 | 8 | 0.185 | 11 | 15 | 0.205 |
| >60 | 24 | 21 | | 3 | 18 | |
| Gender | | | | | | |
| Male | 32 | 19 | 0.075 | 10 | 25 | 0.731 |
| Female | 6 | 10 | | 4 | 8 | |
| Smoking history (years) | | | | | | |
| <30 | 8 | 8 | 0.534 | 3 | 15 | 0.191 |
| ≥30 | 30 | 21 | | 11 | 18 | |
| Histology | | | | | | |
| ADC | 14 | 15 | 0.119 | 7 | 17 | 0.836 |
| SQCC | 21 | 9 | | 6 | 12 | |
| Others | 3 | 5 | | 1 | 4 | |
| Differentiation | | | | | | |
| W/M | 25 | 23 | 0.224 | 9 | 23 | 0.742 |
| P | 13 | 6 | | 5 | 10 | |
| Primary tumor size (cm) | | | | | | |
| ≤5 | 28 | 26 | 0.127 | 12 | 20 | 0.17 |
| >5 | 10 | 3 | | 2 | 13 | |
| Lymph node metastasis | | | | | | |
| Yes | 35 | 19 | 0.011[a] | 7 | 14 | 0.633 |
| No | 3 | 10 | | 7 | 19 | |
| TNM stage groupings | | | | | | |
| I, II | 23 | 15 | 0.471 | 8 | 32 | 0.002[a] |
| III, IV | 15 | 14 | | 6 | 1 | |

[a]P<0.05. ILT4 , immunoglobulin-like transcript 4; ANGPTL2 , angiopoietin-like protein 2; (+), positive expression; (-), negative expression; ADC, adenocarcinoma; SQCC, squamous cell carcinoma; W/M, well/moderately differentiated; P, poorly differentiated; NSCLC, non-small cell lung cancer.

and ILT4 plasmids (ILT4-vector), respectively. The cell line H1650 was selected to be used for all subsequent experiments as the expression levels of ILT4 were neither the highest or the lowest out of the cell lines examined. Next, the expression levels of ANGPTL2 and ANGPTL5 were assayed at the mRNA (Fig. 2A) and protein levels (Fig. 2B). Downregulation of ILT4 resulted in a reduced expression of ANGPTL2, whereas upregulation of ILT4 was associated with an increased ANGPTL2 expression. However, the results demonstrated that silencing or promoting ILT4 did not have a marked effect on the expression of ANGPTL5 in H1650 cells compared with the NC group (Fig. 2).

*Co-expression of ILT4 and ANGPTL2/ANGPTL5 in primary human NSCLC tissues*. Positive expression of ILT4 was observed in the cell nucleus, membrane or cytoplasm using immunohistochemical staining (Fig. 3), while positive ANGPTL2 or ANGPTL5 expression was identified in the cytoplasm of primary NSCLC cells using brown staining. No staining of these molecules was observed in the normal bronchial epithelium. For the NSCLC tissue samples, the positive rates of ILT4, ANGPTL2 and ANGPTL5 expression were found to be 58.8 (67/114), 45.6 (52/114) and 55.3% (63/114), respectively.

ILT4 and ANGPTL2/ANGPTL5 were found to be co-expressed in primary NSCLC tissues. A significant association was observed between the expression levels of ILT4 and ANGPTL2 (R=0.466, P=0.004; Table I). However, no correlation was identified between the expression levels of ILT4 and ANGPTL5 (R=0.142, P=0.131).

*Correlation between the co-expression of ILT4-ANGPTL2 or ILT4-ANGPTL5 with clinicopathological factors in primary human NSCLC tissues*. As presented in Tables II and III, the tumors were classified as ILT4-positive or -negative. In the ILT4-positive group, ILT4 and ANGPTL2 co-expression (ILT4-positive and ANGPTL2-positive) was associated with lymph node metastasis patients (P=0.011). A positive

Table III. Correlation between ILT4/ANGPTL5 co-expression and clinicopathological factors in primary human NSCLC tissues.

| | ILT4 (+) (No. of cases) | | | ILT4 (-) (No. of cases) | | |
|---|---|---|---|---|---|---|
| Variable | ANGPTL5 (+) | ANGPTL5 (-) | P-value | ANGPTL5 (+) | ANGPTL5 (-) | P-value |
| Age (years) | | | | | | |
| ≤60 | 15 | 7 | 0.412 | 14 | 12 | 0.282 |
| >60 | 26 | 19 | | 8 | 13 | |
| Gender | | | | | | |
| Male | 30 | 21 | 0.565 | 18 | 17 | 0.331 |
| Female | 11 | 5 | | 4 | 8 | |
| Smoking history (years) | | | | | | |
| <30 | 10 | 6 | 0.902 | 7 | 11 | 0.391 |
| ≥30 | 31 | 20 | | 15 | 14 | |
| Histology | | | | | | |
| ADC | 19 | 10 | 0.781 | 10 | 14 | 0.269 |
| SQCC | 17 | 13 | | 8 | 10 | |
| Others | 5 | 3 | | 4 | 1 | |
| Differentiation | | | | | | |
| W/M | 23 | 25 | <0.001[a] | 14 | 18 | 0.539 |
| P | 18 | 1 | | 8 | 7 | |
| Primary tumor size (cm) | | | | | | |
| ≤5 | 32 | 22 | 0.752 | 14 | 18 | 0.539 |
| >5 | 9 | 4 | | 8 | 7 | |
| Lymph node metastasis | | | | | | |
| Yes | 32 | 22 | 0.752 | 12 | 9 | 0.202 |
| No | 9 | 4 | | 10 | 16 | |
| TNM stage groupings | | | | | | |
| I, II | 20 | 18 | 0.100 | 16 | 24 | 0.040[a] |
| III, IV | 21 | 8 | | 6 | 1 | |

[a]P<0.05. ILT4, immunoglobulin-like transcript 4; ANGPTL5 , angiopoietin-like protein 5; (+), positive expression; (-), negative expression; ADC, adenocarcinoma; SQCC, squamous cell carcinoma; W/M, well/moderately-differentiated; P, poorly-differentiated; NSCLC, non-small cell lung cancer.

correlation was observed between ANGPTL2 expression and TNM staging in the ILT4-negative group (P=0.002). In addition, ILT4 and ANGPTL5 co-expression (ILT4-positive and ANGPTL5-positive) presented a significant correlation with cell differentiation (P<0.001). In the ILT4-negative group, a positive correlation was observed between ANGPTL5 expression and TNM staging (P=0.040). No significant associations were identified among the ILT4-ANGPTL2 or ILT4-ANGPTL5 co-expression and the age, gender, smoking history, histological types and tumor sizes.

*Association between ILT4-ANGPTL2 or ILT4-ANGPTL5 co-expression and patient survival*. In order to investigate whether the co-expression of ILT4 and ANGPTL2/ANGPTL5 may be used to predict the prognosis of patients with NSCLC, overall survival curves were constructed using the Kaplan-Meier method and investigated using the log-rank test. In the ILT4-positive group, the overall patient survival rate was lower in the ANGPTL2-positive cases compared with the ANGPTL2-negative cases (P=0.045; Fig. 4A). However, the difference in the overall survival between ANGPTL2-positive and ANGPTL2-negative cases was not found to be statistically significant in the ILT4-negative group (P=0.459; Fig. 4B). In addition, survival rate analysis revealed that ANGPTL5 expression was associated with the overall survival rates of patients in the ILT4-positive group (P=0.040; Fig. 4C), whereas the overall survival rate difference was not found to be statistically significant in the ILT4-negative cases (P=0.195; Fig. 4D).

## Discussion

ANGPTLs constitute a group of growth factors known to induce the expansion of mouse and human HSCs (13). ANGPTLs are also crucial in lipid metabolism, inflammation and angiogenesis. A number of studies have indicated that the aberrant expression of ANGPTLs is involved in tumor progression, metastasis and tumor cell epithelial-to-mesenchymal transitions (16,25,26). These molecules were previously considered to be 'orphan

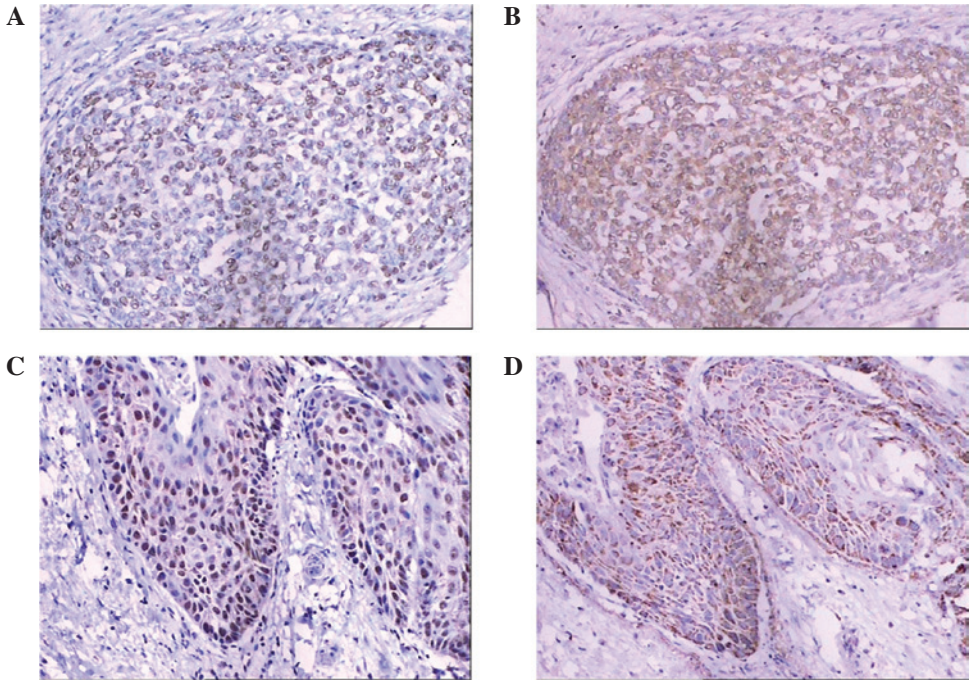


Figure 3. Co-expression of ILT4 and ANGPTL2/ANGPTL5 in primary NSCLC tissues. Co-expression of (A and B) ILT4 and ANGPTL2 and (C and D) ILT4 and ANGPTL5, in tumor cells of the same specimen. Expression of ILT4 was detected in the cell nucleus, membrane or cytoplasm by immunohistochemical analysis, while positive expression of ANGPTL2 and ANGPTL5 was identifiable by brown staining in the cytoplasm of primary NSCLC cells (magnification, x200). ILT4, immunoglobulin-like transcript 4; ANGPTL, angiopoietin-like protein; NSCLC, non-small cell lung cancer.

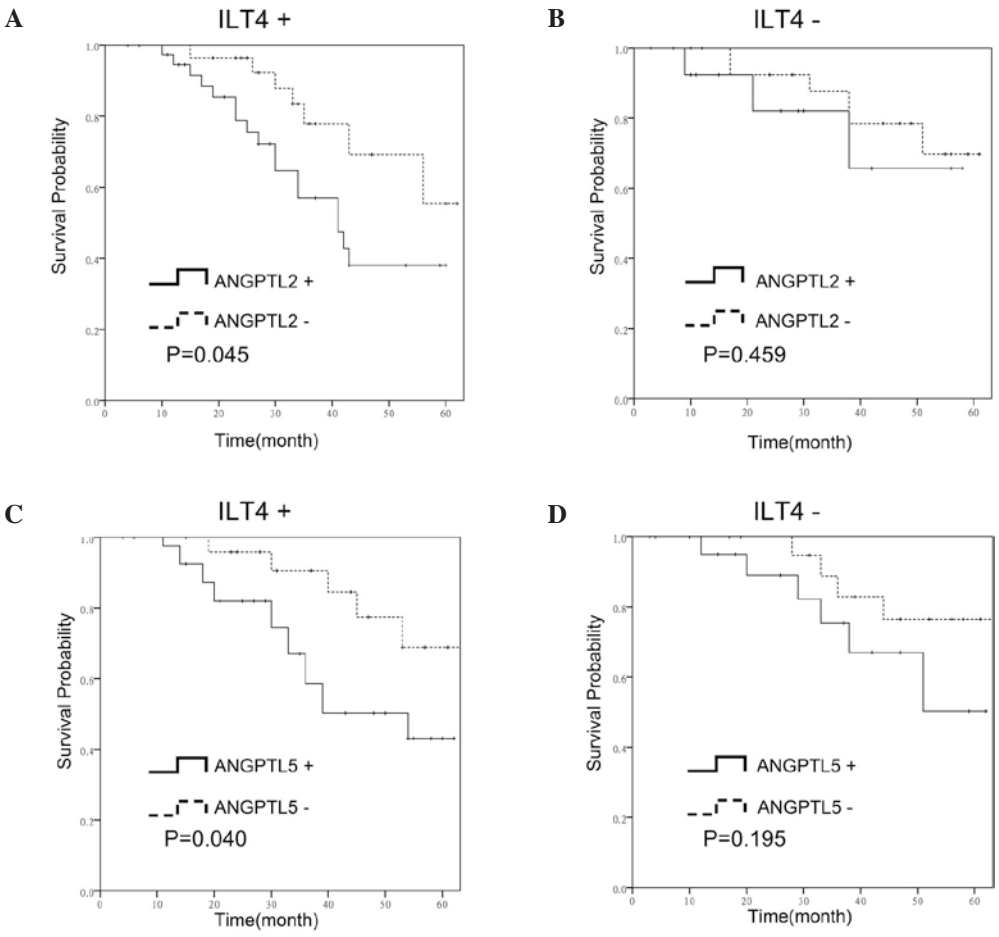


Figure 4. Association between ILT4-ANGPTL2 or ILT4-ANGPTL5 co-expression with patient survival. The overall survival rates of ANGPTL2+ and ANGPTL2- patients were analyzed in the (A) ILT4+ and (B) ILT4- group. The difference of the overall survival between ANGPTL5+ and ANGPTL5- cases was analyzed in the (C) ILT4+ and (D) ILT4- group. ILT4, immunoglobulin-like transcript 4; ANGPTL, angiopoietin-like protein; (+), positive expression; (-), negative expression.

ligands' since no receptor had been identified (27). However, a recent study has demonstrated that ILT4 and its mouse ortholog, Pirb, were receptors for ANGPTLs (11). ILT4 has also been shown to be overexpressed in NSCLC cells (10). Furthermore, lymph node metastasis was also more common in NSCLC cases with high ILT4 expression. However, the co-expression of ILT4 and ANGPTLs was rarely observed in cancer cells and their potential roles remain poorly understood.

The existence of the growth factor and its receptor in the same cancer cells is regarded as autocrine secretion. The term 'autocrine secretion' was proposed by Sporn and Roberts (28) and denotes the self-stimulation by which a cell secretes a hormone-like substance for which the cell itself has functional external receptors (28). In the current study, the presence of ILT4 and ANGPTL2/ANGPTL5 expression was determined in five NSCLC cell lines. The results indicated that ILT4 and ANGPTL2/ANGPTL5 were co-expressed in all the cell lines and primary human NSCLC tissues; thus, the interactions between them were investigated. The overexpression of ILT4 was found to enhance the expression of ANGPTL2 *in vitro*. This effect was abolished by the transfection of cells with shRNA targeted against ILT4. In addition, the expression of ILT4 was positively correlated with the expression of ANGPTL2 in clinical NSCLC samples (R=0.266, P=0.004). However, ILT4 was not found to induce the expression of ANGPTL5 in the NSCLC cell lines, and no relationship was observed between ILT4 and ANGPTL5 in the primary human NSCLC tissues. Therefore, these results imply that the ILT4-ANGPTL2 interaction in NSCLC cells may be self-stimulated whereby the NSCLC cells secrete ANGPTL2 for which the cells themselves have the functional external ILT4 receptors, while ANGPTL5 may be regulated through other pathways. Further studies are required to elucidate the detailed mechanisms underlying the interaction between ILT4 and ANGPTL2 in NSCLC cells. To the best of our knowledge, the present study is the first to describe the co-expression and correlation between ILT4 and ANGPTL2/ANGPTL5 in solid tumor cells.

Growth factors have been previously shown to be closely associated with oncogenes that directly code for growth factors or their receptors and amplify the mitogenic pathway signals produced by the growth factor through its receptor (28). In addition, the current study assessed the effect of the correlation between the expression of the growth factor, ANGPTL2, and its receptor, ILT4, on the prognosis of NSCLC. Among the ILT4-positive cases, high expression of ANGPTL2 was more common in the lymph node metastasis patients. Notably, patients with a high expression of ANGPTL2 had a significantly poorer prognosis. However, in the ILT4-negative cases, ANGPTL2 did not serve as a prognosis factor. Recently, Zheng *et al* (11) demonstrated that the binding of ANGPTLs to PirB promoted the development of leukemia by inhibiting the differentiation of AML cells, indicating a potential role of the autocrine mechanism of ILT4 and ANGPTL2 in the promotion of tumor growth and metastasis in NSCLC. Further studies on the role of the ILT4-ANGPTL2 interaction in the development of NSCLC are required to validate the findings of the present study.

Although no direct influence of ILTE on ANGPTL5 expression was identified, ILT4 and ANGPTL5 co-expression was found to be associated with low NSCLC differentiation and poor prognosis. Therefore, more comprehensive investigations should be performed to assess the underlying mechanistic interactions between ILT4 and ANGPTL5 in order to improve the understanding on the role of these interactions in the development of NSCLC.

In conclusion, the current study investigated the co-expression of ILT4 and ANGPTL2 and their potential autocrine mechanism in NSCLC cells. Co-expression of ILT4 and ANGPTL2/ANGPTL5 was found to be correlated with lower overall survival rates. Therefore, co-expression of ILT4 and ANGPTL2/ANGPTL5 may be crucial in the progression and development of NSCLC, and the identification of an ILT4 and ANGPTL pathway may be required for the prevention and treatment of NSCLC.

## Acknowledgements

The present study was supported by a grant from the Project of the National Natural Science Foundation of China (no. 81372334).

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