CHEST

Original Research

LUNG CANCER

Expression of Ig-Like Transcript 4 Inhibitory Receptor in Human Non-small Cell Lung Cancer*

Yuping Sun, PhD; Jie Liu, MD; Ping Gao, MD; Yunshan Wang, PhD; and Chuanyong Liu, MD

Background: **Human Ig-like transcript 4 (ILT-4) is a member of the inhibitory receptor family for immune function. Little is known about the expression levels of ILT-4 in tumor cells.**
Methods: **We have studied the expression levels of ILT-4 both *in vitro* in cancer cell lines and *in vivo* in tumor tissues from 70 patients with non-small cell lung cancer (NSCLC) by reverse transcriptase-polymerase chain reaction, fluorescence-activated cell sorting, and immunohistochemical analysis.**
Results: **Three cancer cell lines (H1299, A549, and U1810) express ILT-4 messenger RNA, and only two cell lines (H1299 and A549) express ILT-4 protein on the cell surface. Approximately 37.1% of 70 tumor tissue samples express ILT-4, which is localized in the cell membrane and cytoplasm. In addition, tumor cells and stromal and plasma cells also express ILT-4. The number of infiltrating lymphoid cells in the tumor tissues that express B7-H3 was much lower than those that did not, but there is no significant correlation between ILT-4 expression and disease progression including nodal metastasis.**
Conclusions: **These findings suggest that ILT-4 is frequently expressed in both tumor and stromal cells of NSCLC, and it might play an important role in regulation of the host immune system.**
(CHEST 2008; 134:783–788)

Key words: Ig-like transcript 4; immunohistochemistry; infiltrating lymphoid cells; non-small cell lung cancer

Abbreviations: HLA = histocompatibility leukocyte antigen; ILT = Ig-like transcript; ILT-3 = Ig-like transcript 3; ILT-4 = Ig-like transcript 4; NSCLC = non-small cell lung cancer; PBS = phosphate-buffered saline solution; TIL = infiltrating lymphoid cell

Costimulatory factors (cofactors) are defined as a group of molecules for efficient T-cell activation by submitting the second signal. According to molecular structures, cofactors are classified into two groups: Ig and the tumor necrosis factor superfamily. Both play positive and negative roles in regulation of the immune system. On receiving an antigen-specific signal, T cells may remain nonresponsive without activation of cofactors, whereas the same T-cell population may become overresponsive in the absence of inhibitory cofactors. In fact, the functional interplay between these molecules is far more complex than what we have expected. These molecules are involved in virtually all processes of immune responses and all types of immune cells, and they decide the direction and the degree of immune responses.

The concept of the immunologic surveillance against tumor cells was initially proposed by Erlich in 1909 and later elaborated by Burnet.[1] It is well known that cancer patients lacking an adequate immune response allow the tumor to grow unchallenged. The importance of cofactors in tumor immu-

*From Jinan Central Hospital, Shandong University, Jinan, People's Republic of China.
This work was performed at Jinan Central Hospital, Shandong University.
The authors have no conflicts of interest to disclose.
Manuscript received May 8, 2007; revision accepted April 29, 2008.

Correspondence to: Yuping Sun, PhD, Jinan Central Hospital, Shandong University, Jinan, Shandong 250013, People's Republic of China; e-mail: sunyupingsyp@hotmail.com
DOI: ;10.1378/chest.07–1100

nity has been known for a long time. It has been noticed that B7–1/B7–2 (CD80/CD86) is not expressed on tumor cells, suggesting that the absence of proper lymphocyte costimulation may affect the antitumor response. Subsequently, several tumor-derived inhibitory cofactors (PD-L1, B7-H1, and B7-H4) have been identified.[2–4] Expression of immunosuppressive molecules in tumor cells could be an alternative mechanism to induce immune tolerance.

Ig-like transcript 4 (ILT-4) [also known as LIR-2, CD85d, LILRB2, and MIR10] is a cell-surface receptor predominantly expressed in CD14+ monocytes and B cells. It is weakly expressed in dendritic cells,[5] natural-killer cells, endothelial cells,[6] placental trophoblasts,[7] and decidual macrophages.[8] ILT-4 belongs to a family of immune inhibitory receptors called Ig-like transcripts (ILTs). It is a member of the Ig-like superfamily and is structurally and functionally related to killer-cell inhibitory receptors.[9–12] A subset of ILT receptors including Ig-like transcript 3 (ILT-3) and ILT-4 displays a long cytoplasmic tail containing three immunoreceptor tyrosine-based inhibitory motifs and four extracellular Ig superfamily domains. ILT-4 binds to histocompatibility leukocyte antigen (HLA)-A, HLA-B, HLA-C, HLA-G, and HLA-F.[9,10,13–16] However, the functional consequences of its precise binding properties remain controversial. In general, the biological function of this molecule is believed to be an inhibitor of immune response. However, the expression and function of ILT-4 in tumor cells remain uncharacterized.

Lung cancer is the most common cancer type across the globe and has the highest mortality rate because of its refractory to conventional therapies. Adenocarcinoma and squamous cell carcinoma comprise of the majority of cases. The underlying mechanism by which these tumor cells escape the immune surveillance system remains unknown. However, accumulating evidence suggests that tumor-infiltrated T cells and monocytes are functionally defective. The aim of our present work is to study the expression and possible function of ILT-4 in lung cancer. Our data suggest that ILT-4 expressed in tumor cells may induce T-cell anergy *in situ*.

Materials and Methods

Cell Line and Maintenance

A549, H1299, H23, Wark (human adenocarcinoma), U1752 (human squamous carcinoma), U1810 (large cell), and U1906 (small cell lung cancer) cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum.

Patient Characteristics

Primary tumor specimens were obtained by surgery from 70 NSCLC patients (49 men and 21 women; mean age at diagnosis, 53.3 years) [Table 1] without any preoperative therapy at Clinical Hospital of Shandong University, China, between 2003 and 2004. All patients gave written informed consent, and the study was approved by the Institutional Review Board. Among these 70 tumors, 32 were adenocarcinomas and 38 were squamous cell carcinoma (9 well-differentiated, 49 moderately differentiated, and 12 poorly differentiated). Cell differentiation was determined using the current classification by World Health Organization as revised in 1999. Patients were staged according to surgical and pathologic findings based on the guidelines described in the American Joint Committee on Cancer Staging manual.[17] Thirteen patients were determined at stage I, 21 patients at stage II, and 36 patients at stage III. For all these patients, records of surgery, inpatient medical records, chest radiograph films, whole-body CT films, and bone scanning films were reviewed.

Reverse Transcriptase-Polymerase Chain Reaction

Cancer cells were harvest and lyzed in total RNA isolation reagent (Ultraspec-II RNA; Biotech; Stockholm, Sweden). First-strand complementary DNAs were prepared using random primers and following the manufacturer instructions (Fermentas China; Shenzhen, China). Synthesis of complementary DNAs

Table 1—*Relationship Between ILT-4 Expression and Clinical Parameters

Variables	ILT-4 Expression on Tumor Cells		p Value
	Negative	Positive	
Patients	44	26	
Age, yr			
< 60	29	19	
≥ 60	15	7	> 0.05†
Sex			
Male	30	19	
Female	14	7	> 0.05†
Smoking history			
Positive	18	13	
Negative	26	13	> 0.05†
Histology			
Adenocarcinoma	18	14	
Squamous cell carcinoma	26	12	> 0.05†
Differentiation			
Well	5	4	
Not well	39	22	> 0.05†
Pathologic stage			
I	8	5	
II + III	36	21	> 0.05†
Pathologic T factor			
T1 + T2	30	23	
T3 + T4	14	3	> 0.05†
Pathologic N factor			
N0	9	5	
N1–N3	35	21	> 0.05†
CD45+ cells (± SD)	37.11 ± 14.35	26.82 ± 20.22	< 0.05‡

*Data are presented as No. unless otherwise indicated.
†χ^2 test.
‡Student paired *t* test.