Analysis of CD137 and CD137L Expression in Human Primary Tumor Tissues

Qun Wang[1], Pin Zhang[1], Qixia Zhang[1], Xiaoyan Wang[1], Jianfeng Li[2], Chunhong Ma[1], Wensheng Sun[1], Lining Zhang[1]

[1]Department of Immunology, Shandong University School of Medicine, Jinan, People's Republic of China
[2]Central Laboratory, Shandong Provincial Hospital, Jinan, People's Republic of China

Aim To assess the expression of CD137 and CD137L in human primary tumor tissues and their potential role in tumor immunity.

Methods Expression of CD137 and CD137L was assessed by immunohistochemistry in frozen sections of 12 human normal tissues, 15 benign tumors of epithelial or mesenchymal origin (adenoma and leiomyoma), and 36 malignant tumors of epithelial origin (squamous cell carcinoma and adenocarcinoma). The expression of CD137L on 9 human tumor cell lines (3 hepatocarcinoma, 2 lung carcinoma, 2 colon carcinoma, 1 lymphoma, and 1 leukemia) was detected by reverse transcription polymerase chain reaction. To analyze the role of CD137L expressed on tumor cells, we co-cultured tumor cells expressing CD137L with activated T lymphocytes expressing CD137 or with Chinese hamster ovary cells expressing CD137 and then detected by ELISA the levels of cytokines (IL-8, IFN-γ) secreted by tumor cells or activated T cells.

Results The expression of CD137 and CD137L was observed only in human benign (2/15, 3/15) or malignant tumors (15/36, 21/36), but not in normal tissues (0/12, 0/12). CD137 was expressed on the vessel walls within tumor tissues, whereas CD137L was expressed on tumor cells. The expression of CD137 and CD137L was more common in malignant tumors, especially in moderate or low-differentiated tumors. Furthermore, CD137L expression found on tumor cell lines was functional because the ligation of CD137L on lung squamous carcinoma cells L78 with CD137 on T cells induced IFN-γ production by T cells, and ligation of CD137L on hepatocarcinoma cells HepG2.2.15 with CD137 triggered tumor cells to produce IL-8.

Conclusion CD137 and CD137L are expressed in different human primary tumor tissues, suggesting that they may influence the progression of tumors.

> **Correspondence to:**
Lining Zhang
Department of Immunology
Shandong University School of Medicine
44# Wenhua Xi Road, Jinan, 250012
People's Republic of China
immuno@sdu.edu.cn

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CD137, a member of the tumor necrosis factor receptor family, is expressed primarily on activated T lymphocytes and natural killer cells (1,2). In contrast, its ligand CD137L is mainly expressed on antigen-presenting cells, such as mature dendritic cells, activated B cells, and macrophages (3,4). The co-stimulation through CD137/CD137L enhances T cell activation, promotes the rejection of cardiac allografts and skin transplants, and eradicates experimentally induced tumors in mice (5-10).

However, expression of human CD137 and CD137L is not restricted to immune cells, and the functions of human CD137/CD137L pathway are more complex than that of mice. Expression of CD137 protein has been verified in chondrocytes (11) and on blood vessel walls in primary malignant tumors (12). Expression of CD137L has also been found on several human carcinoma cell lines and its function has been analyzed in vitro. On the one hand, CD137L expressed on carcinoma cells could function as a co-stimulatory molecule of T cell activation for the production of cytokines, most notably interferon-gamma (IFN-γ), in co-culture of T cells and tumor cells. On the other hand, incubation of tumor cells expressing CD137L with a CD137-Ig fusion protein leads to the production of interleukin-8 (IL-8) of tumor cells (13).

Up to now, however, no studies have reported the expression of CD137L in human primary tumors. Therefore, the aim of this study was to assess the expression of CD137 and CD137L in human primary tumor tissues and their potential role in tumor immunity.

Materials and methods

Cell lines

Human tumor cell lines – HepG2.2.15 (hepatocarcinoma integrated with whole hepatitis B virus genome), BEL7402 (hepatocarcinoma), HLE (hepatocarcinoma), HT29 (colon adenocarcinoma), L78 (lung squamous carcinoma), A2 (lung adenocarcinoma), U937 (histocytic lymphoma), HL60 (promyelocytic leukemia), and ECV304 (embryonic venous endotheliocytes) – were purchased from Medical Science Institute of Shandong Province. H6 (colon carcinoma) tumor cell line was kindly provided by Professor Jiayou Zhang from the University of Kentucky, USA.

To obtain Chinese hamster ovary (CHO) cells expressing membrane CD137, CD137-CHO cells (CHO transfected with human CD137 gene) were transfected as follows: the recombinant CD137-pCDNA3 plasmid (containing human CD137 cDNA sequence) and pSV2-dhfr plasmid were co-transfected into dhfr-CHO cells by lipid-mediated transfection. The positive clones were selected by 400 μg/mL G418. Expression of human membrane CD137 on dhfr-CHO cells was induced by raising the concentration of methotrexate. CD137 mRNA and protein were determined by reverse transcription polymerase chain reaction (RT-PCR), immunocytochemistry, and flow cytometry (14).

Cell culture

Tumor cell lines U937, HL60, HLE, HT29, A2, and L78 were routinely cultured in Roswell Park Memorial Institute medium (RPMI) 1640 supplemented with 10% fetal calf serum (FCS; Hangzhou Sijiqing, China) and 1% penicillin/streptomycin (Sigma, St. Louis, MO, USA); ECV304 and H6 were cultured in DMEM, and BEL7402 were cultured in MEM with the same supplements; HepG2.2.15 were cultured in MEM supplemented with 10% FCS and 380 ng/mL G418 (Sigma). CD137-CHO cells were cultured in F12 medium containing 10% FCS and G418. Human peripheral blood mononuclear cells were isolated from peripheral blood derived

from healthy donors by Ficoll density gradient centrifugation.

Surgical specimens

A total of 63 tissue specimens were obtained from patients who underwent operations at Qilu Hospital, Shandong University, China, from 2000 to 2004. Pathological diagnosis including tumor differentiation grade was made according to the recent World Health Organization criteria for different tumors by clinical pathologists (15-17). Malignant tumors were divided into three differentiation grades as follows: high differentiation, moderate differentiation, and low differentiation. Twelve specimens of normal tissue were excised from patients who were initially suspected to have a tumor but were later diagnosed as healthy. The tissue types and tumor differentiation grades are presented in Table 1. This study was conducted in full compliance with the national legislation and the ethical standards of the Chinese Medical Association. Informed consent was obtained from all patients before the biopsy.

Table 1. Tissue types and differentiation grades of the tumors (n = 63)*

	No. of specimens		
Tissue type	Total	HD (n = 20)	MLD (n = 16)
Malignant tumors (n = 36):			
Squamous cell carcinoma:	14	8	6
lung	3	0	3
nasal cavity	1	0	1
esophagus	6	5	1
cervix	4	3	1
Adenocarcinoma:	22	12	10
stomach	3	3	0
colon	4	2	2
rectum	4	2	2
gallbladder	4	2	2
pancreas	3	1	2
breast	4	2	2
Benign tumors (n = 15):			
lung adenoma	3		
ovarian serous cystadenoma	2		
uterine leiomyoma	6		
thyroid adenoma	4		
Normal tissues (n = 12):			
lung	3		
stomach	2		
colon	4		
breast	3		

*Abbreviations: HD – high differentiation; MLD – moderate or low differentiation.

Determination of CD137 or CD137L expression by immunohistochemistry

Fresh tissue specimens were frozen for 1 hour after the biopsy, then 4-6 μm-thick frozen sections were prepared at -20°C and fixed in acetone for 10 minutes at 4°C. Immunohistochemical staining for CD137 or CD137L expression was performed using an ABC kit (Boshide Company, Wuhan, China) according to the manufacturer's instructions. The sections were exposed to 0.3% hydrogen peroxide-methanol solution to quench endogenous peroxidase activity. After a 30-minute pre-incubation with normal goat serum, sections were incubated with anti-CD137 mAbs (5 μg/mL, BD Pharmingen Inc., San Diego, CA, USA) or anti-CD137L mAbs (4 μg/ml, BD Pharmigen Inc.) for 30 minutes, followed by biotinylated goat anti-mouse IgG, and avidin-biotin-peroxidase complex; diaminobenzidine (Boshide Company) was used as a peroxidase substrate. All incubations were performed at room temperature. As negative control, primary mAb was replaced with an isotype-matched antibody with irrelevant specificity. Three random sections were examined.

Expression of CD137 or CD137L was assessed by two independent pathologists who were blinded to the patient's outcome. The immunoreactivity results were presented as either "negative" (percentage of CD137 or CD137L staining was 0%-10%) or "positive" (percentage of CD137 or CD137L staining was >10%).

Assessment of CD137L expression by RT-PCR

CD137L mRNA expression in various cells was detected using RT-PCR. Briefly, total RNA was extracted from the cells using TRIZOL (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was synthesized using Superscript preamplification system (Promega, Madison

WI, USA) and PCR amplification was performed using the following primers (designed based on literature and synthesized by Shanghai Shenggong, China): human β-actin sense primer: 5'ACA CTG TGC CCA TCT ACG AGG GG 3'; anti-sense primer: 5'ATG ATG GAG TTG AAG GTA GTT TCG TGG AT 3', human CD137L sense primer: 5'GTT TCA CTT GCG CTG CAC CTG CAG CCA CTG 3'; anti-sense primer: 5'GGC TCT AGA TAT CAA GGT CCA ACT TGG GA AGG 3'. After denaturation at 94°C for 5 minutes, 30 PCR cycles were performed using human CD137L specific primers, each consisting of a denaturation step (94°C, 1 minute), an annealing step (52°C, 2 minutes), and an elongation step (72°C, 2 minutes), followed by an extension at 72°C for 5 minutes. The products were detected by electrophoresis on 2% agarose gel containing ethidium bromide and analyzed using Imagemaster Gel Analysis Software (Amersham Pharmacia Biotech, Uppsala, Sweden). The expression levels of CD137L mRNA were compared after normalizing OD value to β-actin, which was defined as the ratio of $OD_{CD137L}/OD_{\beta\text{-actin}}$.

Measurement of cytokines by ELISA

T cells were purified from peripheral blood using Rosettesep™ Antibody Cocktail (Senxiong Company, Shanghai, China). Briefly, 250 μL of RosetteSep™ cocktail was added per 5 mL of peripheral blood, mixed, and incubated at room temperature for 20 minutes. Next, the sample was diluted with phosphate buffer saline containing 2% FCS. The diluted sample was then layered on top of Ficoll-Paque at a 1:2 dilution and then centrifuged for 20 minutes at 2000 r/min at room temperature. Enriched T cells were harvested from the plasma-Ficoll interface and washed twice with phosphate buffer saline containing 2% FCS. The purity of T cells detected by flow cytometry was >95%.

Tumor cells L78 or HepG2.2.15 were cultured in 96-well plates at 37°C, 5% CO_2 in a volume of 200 μL with different concentration (1×10^6/mL, 5×10^5/mL, 1×10^5/mL), respectively. After 48 hours the supernatant was discarded, purified T cells (5×10^5/mL) were added in a volume of 200 μL in the presence or absence of anti-CD3 mAb (10 μg/mL, BD Pharmingen), or with anti-CD137L mAb (5 μg/mL, BD Pharmingen). After incubation for 48 hours, the supernatant was collected and IFN-γ was detected by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Senxiong Company). Briefly, the supernatant was added into 96-well plates coated with anti-IFN-γ antibody in duplicate and incubated at 37°C for 2 hours. Human recombinant IFN-γ was included as standard. After six washes, biotinylated anti-IFN-γ antibody was added and incubated at 37°C for 1 hour; avidin-conjugated horseradish peroxidase (HRP) was added for an additional 1 hour at 37°C, followed by the substrate working solution until color development. Six phosphate buffer saline washes were performed between steps, and plates were read at OD 492 nm using an automated microtiter plate reader (Bio-Rad, Hercules, CA, USA).

CD137-CHO cells expressing human CD137 (5×10^4/mL) were cultured in 96-well plate in a volume of 200 μL. After 24 hours the supernatant was discarded and 200 μL of tumor cells A2 and HepG2.2.15 (5×10^4/mL) was added. After incubation for 16-18 hours, the supernatant was collected and IL-8 was determined by ELISA according to the manufacturer's guidelines (Senxiong Company).

Statistical analysis

The *t* test and ANOVA (Turkey-Kramer multiple comparisons test) were used to assess the statistical significance. $P<0.050$ was considered significant. All calculations were per-

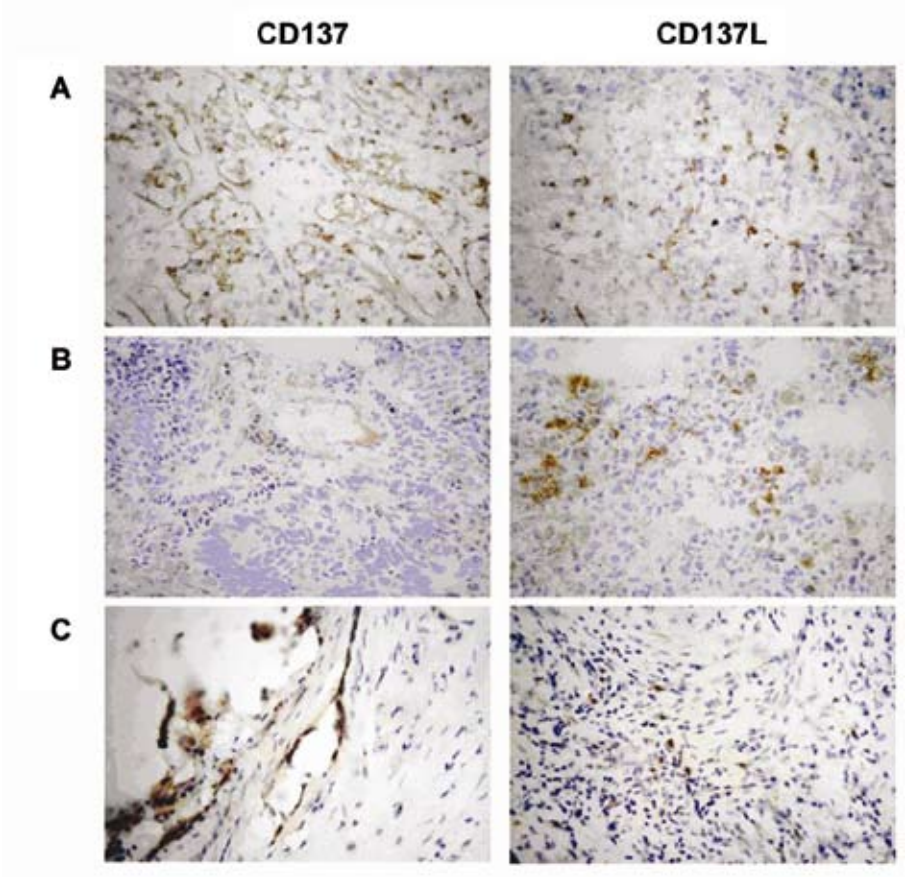

Figure 1. Expression of CD137 or CD137L in human primary tumor tissues by immunohistochemistry. Within tumor tissues, CD137 was only expressed on the vessel walls including endothelial cells and smooth muscle cells, whereas CD137L was only expressed on the tumor cells. (**A**) Expression of CD137 and CD137L in thyroid adenoma. (**B**) Expression of CD137 and CD137L in rectal cancer. (**C**) Expression of CD137 and CD137L in breast cancer.

formed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA).

Results

Human primary tumor tissues express CD137 and CD137L at different locations

Both CD137 and CD137L expression was detected in human benign or malignant tumor tissues but not in normal tissues (Table 2). CD137L was expressed on tumor cells, whereas CD137 was detected on endothelial cells and smooth muscle cells of tumor vessel walls (Figure 1). In addition, tumors expressing CD137 also expressed CD137L, whereas some tumors expressing CD137L did not show detectable CD137 (Table 2). Though the samples were derived from many different tissue types, expression of CD137 or CD137L was more often found in malignant tumors, especially in moderate or low-differentiated tumors. Only a few benign tumors expressed CD137 and CD137L, as opposed to about a half of malignant tumors (Table 3). Among malignant tumors, CD137 or CD137L expression in moderate or low-differentiated malignant tumors was higher than in highly differentiated tumors.

Human tumor cell lines express high levels of CD137L

Our results showed that tumor cells in human primary tumor tissues expressed CD137L. We also detected CD137L expression in human

Table 2. Status of CD137 and CD137L expression in benign and malignant tumors*

Tumor	Differentiation grade	CD137/CD137L pattern +/+	+/−	−/+	−/−	No. of specimens
Lung adenoma	/	0	0	0	3	3
Ovarian serous cystadenoma	/	0	0	0	2	2
Uterine leiomyoma	/	1	0	0	5	6
Thyroid adenoma	/	1	0	1	2	4
Lung squamous cell carcinoma	HD	0	0	0	0	0
	MLD	3	0	0	0	3
Nasal cavity squamous cell carcinoma	HD	0	0	0	0	0
	MLD	1	0	0	0	1
Esophageal squamous cell carcinoma	HD	1	0	1	3	5
	MLD	1	0	0	0	1
Cervical squamous cell carcinoma	HD	1	0	1	1	3
	MLD	0	0	0	1	1
Stomach adenocarcinoma	HD	0	0	1	2	3
	MLD	0	0	0	0	0
Colonic adenocarcinoma	HD	1	0	0	1	2
	MLD	1	0	0	1	2
Rectal adenocarcinoma	HD	0	0	1	1	2
	MLD	2	0	0	0	2
Gallbladder adenocarcinoma	HD	0	0	0	2	2
	MLD	1	0	1	0	2
Pancreatic adenocarcinoma	HD	0	0	1	0	1
	MLD	1	0	0	1	2
Breast adenocarcinoma	HD	1	0	0	1	2
	MLD	1	0	0	1	2

*Abbreviations: HD – high differentiation; MLD – moderate or low differentiation.

Table 3. Summary of CD137 and CD137L expression in normal or tumor specimens

Tissue specimens	No. of specimens Total	CD137	CD137L
Normal tissues	12	0	0
Benign tumors	15	2	3
Malignant tumors:	36	15	21
high differentiation	20	4	9
moderate or low differentiation	16	11	12

tumor cell lines derived from different primary tissues by RT-PCR. Normal unstimulated peripheral blood mononuclear cells did not express CD137L mRNA but all investigated tumor cell lines (HepG2.2.15, BEL7402, HLE, HT29, L78, A2, U937, HL60, and H6) showed high levels of CD137L expression (Figure 2). Unexpectedly, non-tumor cell line, embryonic venous endotheliocytes ECV304, also expressed CD137L. These results indicated that CD137L was not only expressed in primary tumors but also in tumor cell lines and some non-tumor cell lines, suggesting CD137L expression may be related to rapid cell growth.

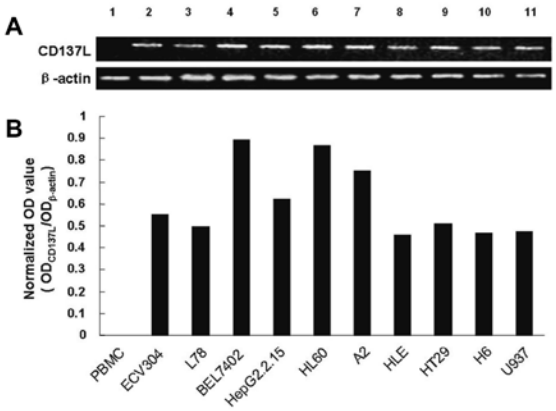

Figure 2. Expression of CD137L mRNA on tumor cell lines. (**A**) CD137L mRNA expression on normal unstimulated peripheral blood mononuclear cells (PBMC), normal embryonic cell line (ECV304), and various tumor cell lines was detected by reverse transcription polymerase chain reaction. Lanes: 1 – PBMC; 2 – ECV 304; 3 – L78; 4 – BEL7402; 5 – HepG2.2.15; 6 – HL60; 7 – A2; 8 – HLE; 9 – HT29; 10 – H6; 11 – U937. (**B**) CD137L mRNA levels were normalized to β-actin mRNA.

CD137L expressed on tumor cells is functional

To investigate the role of CD137L on tumor cells, we co-cultured tumor cells L78 or HepG2.2.15 expressing CD137L with activated human T cells in the presence of anti-CD3 mAb to stimulate T cell to express CD137. We detected IFN-γ secreted by T cells using ELISA. In the presence of anti-CD3 mAb, CD137-expressing T cells co-cultured with L78 cells produced higher levels of IFN-γ than CD137-lacking T cells (in the absence of anti-CD3 mAb) ($P<0.001$), depending on the tumor cell number (data not shown). After blocking CD137/CD137L pathway using anti-CD137L mAb, the levels of IFN-γ significantly decreased ($P<0.001$), compared with control IgG. However, there was no obvious change of IFN-γ secretion when T cells were incubated with or without HepG2.2.15 cells (in the presence or absence of anti-CD3 mAb) ($P = 0.375$) (Figure 3). This suggests that CD137L on L78 cells could conduct a co-stimulatory signal into T cells and this action could be specifically blocked by anti-CD137L mAb.

To confirm whether CD137L expressed on tumor cells could also signal back into

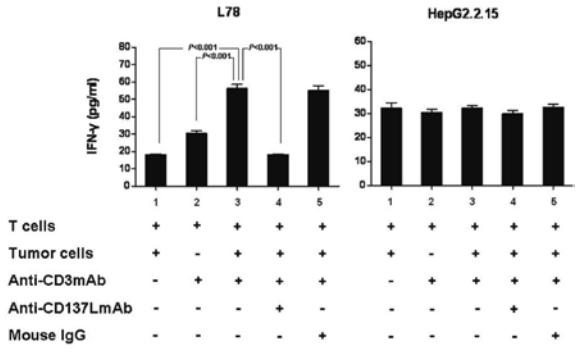

Figure 3. Effect of CD137L on tumor cells on interferon-gamma (IFN-γ) secretion by T cells. Tumor cells L78 or HepG2.2.15 (1×10^6/mL) were cultured in 96-well plates at 37°C, 5% CO_2 in a volume of 200 μL. After 48 hours the supernatant was discarded, purified T cells (5×10^5/ml) were added in a volume of 200 μL in the presence or absence of anti-CD3 mAb, or with anti-CD137L mAb. After incubation for 48 hours, the supernatant was collected and IFN-γ was detected by enzyme-linked immunosorbent assay (ELISA). The results are shown as means ± standard error of the mean of four independent experiments. Plus indicates that the culture system contains the indicated cells or antibodies. Minus indicates that the culture system lacks the indicated cells or antibodies.

tumor cells, HepG2.2.15, or A2 cells were co-cultured with fixed CD137-CHO cells expressing CD137, respectively, and IL-8 in the supernatant was detected by ELISA. Ligation of CD137L on tumor cells by CD137 expressed on CD137-CHO cells elevated the levels of IL-8 produced by HepG2.2.15 cells ($P = 0.038$). However, the action of CD137L on A2 cells was weak (Figure 4).

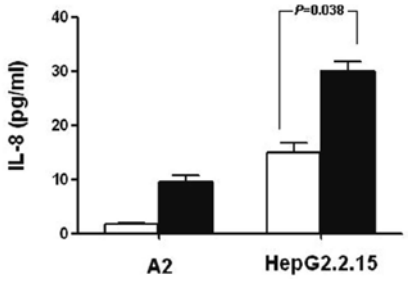

Figure 4. Effect of CD137L on tumor cells on IL-8 secretion by tumor cells themselves. CD137-CHO cells expressing human CD137 (5×10^4/mL) were cultured in 96-well plate in a volume of 200 μL, after 24 hours the supernatant was discarded and 200 μL of tumor cells A2, HepG2.2.15 (5×10^4/mL) were added, respectively. After incubation for 16-18 hours, the supernatant was collected and IL-8 was determined by enzyme-linked immunosorbent assay. The results were shown as means ± standard error of the mean of three independent experiments. Open bars – tumor cells; closed bars – tumor cells + CD137-CHO cells.

Discussion

We showed that both CD137 and CD137L were simultaneously expressed in human primary tumor tissues, but on different locations. CD137 was expressed on the vessel walls in tumor tissues, whereas CD137L was expressed on tumor cells. The expression of CD137 and CD137L was more common in malignant tumors, especially in moderate or low-differentiated tumors. Furthermore, we found that CD137L expressed on tumor cells was functional in several ways. On the one hand, ligation of CD137L on lung squamous carcinoma cells L78 with CD137 on T cells caused T cells to produce high levels of IFN-γ. On the other hand, ligation of CD137L on hepatocarcinoma cells HepG2.2.15 with CD137 caused tumor cells to produce IL-8.

The CD137/CD137L pathway provides an important co-stimulatory signal for the activation and proliferation of T cells as well as a reverse signal for IL-8 production by macrophages or tumor cells (6,18-22). Ligation of the CD137 molecule with CD137L or anti-CD137 antibody can stimulate the activation and proliferation of $CD4^+$ and $CD8^+$ T cells in vitro. In mice model, administration of anti-CD137 antibody eradicates experimentally induced tumors and CD137L on the tumor cell line or CD137L fusion proteins stimulates an effective anti-tumor immunity, suggesting that CD137L may be a target molecule for tumor therapy (8,9,23,24). To the best of our knowledge, our results are the first demonstration that human primary tumor tissues express CD137L and that low-differentiated malignant tumor tissues show a high rate of CD137L, while normal tissues do not. Our results and Salih's report (13) also indicate that CD137L is constitutively expressed on carcinoma cell line. Although CD137L on tumor cells L78 could enhance IFN-γ secretion by T cells, its action was weak. Nota-

bly, CD137L on tumor cells HepG2.2.15 also triggered them to produce IL-8. As a multifunctional cytokine, IL-8 is initially characterized for its leukocyte chemotactic activity but now is known to possess tumorigenic and proangiogenic properties as well. IL-8 is critical to the development and progression of numerous malignancies through angiogenesis (25). Therefore, whether human CD137L can be a target molecule for tumor therapy and what is the influence of CD137L on T cell-tumor cell interaction in vivo remains to be clarified.

Although CD137 and CD137L were often simultaneously expressed in the same tumor tissue samples, their expression locations were different. CD137L was expressed on tumor cells, while CD137 was expressed on endothelial cells or smooth muscle cells of the vessel walls within tumor tissues, which is consistent with the previous report by Broll et al (12). However, the significance of CD137 in tumor tissues is unknown. Broll et al (12) speculated that CD137 on the endothelium could cause monocyte activation and may contribute to monocyte extravasation into the tissue. We propose the hypothesis that the interaction of CD137 on the endothelium with CD137L on tumor cells may promote tumor progression based on the following evidence. First, CD137L on some tumor cells could trigger tumor cells to produce IL-8, which is critical for the development and progression of numerous malignancies (25). Second, CD137 and CD137L were only expressed in benign or malignant tumors simultaneously but not in normal tissues, and their expressions were more common in malignant tumors, especially in moderate or low-differentiated tumors. However, more investigation remains to be done.

Taken together, we demonstrated that the expression of CD137 and CD137L was observed only in human benign or malignant tumors but not in normal tissues. CD137 was expressed on the vessel walls within tumor tissues, whereas CD137L was expressed on tumor cells. The expression of CD137 and CD137L was more common in malignant tumors, especially in moderate or low-differentiated tumors. Furthermore, we found that CD137L expressed on tumor cells was functional. These data suggest that CD137 and CD137L expressed in human tumor tissues may influence tumor immunity during its progression.

Acknowledgments

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References

1 Sica G, Chen L. Biochemical and immunological characteristics of 4-1BB (CD137) receptor and ligand and potential applications in cancer therapy. Arch Immunol Ther Exp (Warsz). 1999;47:275-9. Medline:10604232

2 Taraban VY, Rowley TF, O'Brien L, Chan HT, Haswell LE, Green MH, et al. Expression and costimulatory effects of the TNF receptor superfamily members CD134 (OX40) and CD137 (4-1BB), and their role in the generation of anti-tumor immune responses. Eur J Immunol. 2002;32:3617-27. Medline:12516549 doi:10.1002/1521-4141(200212)32:12<3617::AID-IMMU3617>3.0.CO;2-M

3 Vinay DS, Kwon BS. Role of 4-1BB in immune responses. Semin Immunol. 1998;10:481-9. Medline:9826581 doi:10.1006/smim.1998.0157

4 Laderach D, Wesa A, Galy A. 4-1BB-ligand is regulated on human dendritic cells and induces the production of IL-12. Cell Immunol. 2003;226:37-44. Medline:14746806 doi:10.1016/j.cellimm.2003.11.003

5 Cannons JL, Lau P, Ghumman B, DeBenedette MA, Yagita H, Okumura K, et al. 4-1BB ligand induces cell division, sustains survival, and enhances effector function of CD4 and CD8 T cells with similar efficacy. J Immunol. 2001;167:1313-24. Medline:11466348

6 Wen T, Bukczynski J, Watts TH. 4-1BB ligand-mediated costimulation of human T cells induces CD4 and CD8 T cell expansion, cytokine production, and the development of cytolytic effector function. J Immunol. 2002;168:4897-906. Medline:11994439

7 Saoulli K, Lee SY, Cannons JL, Yeh WC, Santana A, Goldstein MD, et al. CD28-independent, TRAF2-dependent costimulation of resting T cells by 4-1BB ligand. J Exp Med. 1998;187:1849-62. Medline:9607925 doi:10.1084/jem.187.11.1849

8 May KF Jr, Chen L, Zheng P, Liu Y. Anti-4-1BB monoclonal antibody enhances rejection of large tumor burden by promoting survival but not clonal expansion of tumor-specific CD8+ T cells. Cancer Res. 2002;62:3459-65. Medline:12067989

9 McMillin DW, Hewes B, Gangadharan B, Archer DR, Mittler RS, Spencer HT. Complete regression of large solid tumors using engineered drug-resistant hematopoietic cells and anti-CD137 immunotherapy. Hum Gene Ther. 2006;17:798-806. Medline:16942440 doi:10.1089/hum.2006.17.798

10 Cho HR, Kwon B, Yagita H, La S, Lee EA, Kim JE, et al. Blockade of 4-1BB (CD137)/4-1BB ligand interactions increases allograft survival. Transpl Int. 2004;17:351-61. Medline:15349720 doi:10.1111/j.1432-2277.2004.tb00454.x

11 Schwarz H, Valbracht J, Tuckwell J, von Kempis J, Lotz M. ILA, the human 4-1BB homologue, is inducible in lymphoid and other cell lineages. Blood. 1995;85:1043-52. Medline:7849293

12 Broll K, Richter G, Pauly S, Hofstaedter F, Schwarz H. CD137 expression in tumor vessel walls. High correlation with malignant tumors. Am J Clin Pathol. 2001;115:543-9. Medline:11293902 doi:10.1309/E343-KMYX-W3Y2-10KY

13 Salih HR, Kosowski SG, Haluska VF, Starling GC, Loo DT, Lee F, et al. Constitutive expression of functional 4-1BB (CD137) ligand on carcinoma cells. J Immunol. 2000;165:2903-10. Medline:10946324

14 Wan B, Zhang LN, Ma CH, Song J, Cao YL, Li CF, et al. Expression on dhfr-CHO cells and activity study of CD137 (human 4-1BB) [in Chinese]. Chinese Journal of Immunology. 2002;18:678-81.

15 Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC, editors. World Health Organization classification of tumours: pathology and genetics of tumours of the lung, pleura, thymus and heart. Lyon (France): IARC Press; 2004.

16 Tavassoli FA, Devilee P, editors. World Health Organization classification of tumours: pathology and genetics of tumours of the breast and female genital organs. Lyon (France): IARC Press; 2003.

17 Hamilton SR, Aaltonen LA, editors. World Health Organization Classification of Tumours: Pathology and genetics of tumours of the digestive system. Lyon (France): IARC Press; 2000.

18 Hurtado JC, Kim SH, Pollok KE, Lee ZH, Kwon BS. Potential role of 4-1BB in T cell activation. Comparison with the costimulatory molecule CD28. J Immunol. 1995; 155:3360-7. Medline:7561030

19 Langstein J, Michel J, Fritsche J, Kreutz M, Andreesen R, Schwarz H. CD137 (ILA/4-1BB), a member of the TNF receptor family, induces monocyte activation via bidirectional signaling. J Immunol. 1998;160:2488-94. Medline:9498794

20 Schwarz H. Biological activities of reverse signal transduction through CD137 ligand. J Leukoc Biol. 2005;77:281-6. Medline:15618293

21 Watts TH. TNF/TNFR family members in costimulation of T cell responses. Annu Rev Immunol. 2005;23:23-68. Medline:15771565 doi:10.1146/annurev.immunol.23.021704.115839

22 Zhang H, Snyder KM, Suhoski MM, Maus MV, Kapoor V, June CH, et al. 4-1BB is superior to CD28 costimulation for generating CD8+ cytotoxic lymphocytes for adoptive immunotherapy. J Immunol. 2007;179:4910-8. Medline:17878391

23 Zhang N, Sadun RE, Arias RS, Flanagan ML, Sachsman SM, Nien YC, et al. Targeted and untargeted CD137L fusion proteins for the immunotherapy of experimental solid tumors. Clin Cancer Res. 2007;13:2758-67. Medline:17460060 doi:10.1158/1078-0432.CCR-06-2343

24 Johnson BD, Gershan JA, Natalia N, Zujewski H, Weber JJ, Yan X, et al. Neuroblastoma cells transiently transfected to simultaneously express the co-stimulatory molecules CD54, CD80, CD86, and CD137L generate antitumor immunity in mice. J Immunother. 2005;28:449-60. Medline:16113601 doi:10.1097/01.cji.0000171313.93299.74

25 Brat DJ, Bellail AC, Van Meir EG. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. Neuro-oncol. 2005;7:122-33. Medline:15831231 doi:10.1215/S1152851704001061