Blocking CD137-CD137L interactions inhibits proliferation and survival of cutaneous T-cell lymphoma cells via hampering several signaling pathways

Hiroaki Kamijo¹, Tomomitsu Miyagaki¹, Tomonori Oka¹, Naomi Takahashi¹,
Hiraku Suga¹, Makoto Sugaya^{1,2}, Shinichi Sato¹

1 Department of Dermatology, The University of Tokyo Graduate School of Medicine, Tokyo, Japan
2 Department of Dermatology, International University of Health and Welfare, Chiba, Japan

Introduction :

CD137 is expressed on activated T cells and natural killer (NK) cells. The interaction between CD137 and its ligand CD137L expressed on activated antigen-presenting cells is reported to enhance antitumor effects of T cells or NK cells. However, studies on CD137 and CD137L concerning T-cell lymphoma, whose tumor cells can express CD137, have been limited.

Objective :

To investigate expression and function of CD137 and CD137L in cutaneous T-cell lymphoma (CTCL)

Results :

Figure 1. CD137 and CD137L were expressed on malignant T cells in CTCL. (A, B) CD137 and CD137L expression was analyzed by flow cytometry in CD4⁺CD7⁻ T cells from Sézary syndrome (SS) patients, CD4⁺ T cells from healthy control (A), and CTCL cell lines Hut78, MyLa, SeAx, HH, and MJ cells (B). (C) CD137 and CD137L expression in lesional skin of CTCL was determined by IHC.

Figure 3. CD137 and CD137L mRNA expression was increased in CTCL lesional skin. Quantitative RT-PCR was performed to measure expression levels of CD137 and CD137L using mRNA extracted from CTCL lesional skin (n = 36) and normal skin (n = 10). *P < 0.05.

Figure 4. Anti-CD137 or anti-CD137L neutralizing antibody inhibited proliferation and induced apoptosis in CTCL cell lines. (A) Hut78 and MyLa cells (1.0×10^5 /well) were cultured with anti-CD137 (5 μ g/ml) or anti-CD137L (5 μ g/ml) antibodies for 48 hours. Viable cells were counted by Trypan blue exclusion. (B) Hut78 and MyLa cells were cultured with anti-CD137L antibody (5 μ g/ml) for 24h, and Annexin V and 7-AAD staining for flow cytometer. Data are presented as mean $\pm$ SD. *P < 0.05 **P < 0.01

Figure 5. Recombinant CD137-Fc and CD137L promoted proliferation of CTCL cell lines. Hut78, MyLa, SeAx, and HH cells (5.0×10^4 /well) were cultured with plate-coated recombinant CD137-Fc (10 μ g/ml) or CD137L (10 μ g/ml) for 48 hours. Viable cells were counted by Trypan blue exclusion. Data are presented as mean $\pm$ SD. *P < 0.05 **P < 0.01.

Figure 6. Anti-CD137L neutralizing antibody attenuated p-AKT, p-ERK1/2, p-p38 MAPK, p-JNK, and anti-apoptotic proteins (Bcl-2 and p-Bad). Western blotting analysis was conducted on the lysates of Hut78 and MyLa cells treated with anti-CD137L antibody (5 μ g/ml) as indicated time point.

Figure 7. Anti-CD137L antibody inhibited tumor growth of HH cells *in vivo*. HH cells (8.0×10^6) were inoculated into shaved abdomen of SCID/beige mice with anti-CD137L antibody (50 μ g/ml) or Isotype IgG in 100 μ l of PBS. Each reagent was injected on days 0, 4, 7, and 11. The tumor volume was calculated using the equation: $V = \pi (L1 \times L2^2)/6$, where V = volume (mm^3), L1 = longest diameter (mm), and L2 = shortest diameter (mm). **P < 0.01.

Summary :

Blocking CD137-CD137L interaction between malignant T cells inhibited proliferation, cell survival, and *in vivo* tumor growth in CTCL.

COI disclosure :

The authors have no conflicts of interest to declare concerning the presentation.