Galectin-9 induces apoptosis of tumor cells through caspase pathways in cutaneous T-cell lymphoma.

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Introduction :

Galectin-9 (Gal-9) is a member of the tandem-repeat galectin family, which is widely distributed in human tissues. Gal-9 has the capacity to regulate multiple physiological and pathological processes including antitumor immunity. T cell immunoglobulin and mucin domain 3 (Tim-3), one of the receptors of Gal-9, is expressed on IFN- γ -producing Th1 cells and CD8 $^{+}$ T cells, and Gal-9 induces apoptosis of these cells. Actually, anti-Tim-3 antibody promotes IFN- γ -mediated antitumor immunity. On the other hand, Gal-9 is known to induce apoptosis of malignant cells, such as chronic myeloid leukemia cells and malignant melanoma cells. Thus, Gal-9 has the capacity to both suppress antitumor immunity and promote apoptosis of tumor cells.

Objective :

To elucidate Gal-9 involvement in cutaneous T-cell lymphoma (CTCL).

Material and Method :

Patients and samples

Skin samples were collected from 36 patients with CTCL and 5 healthy control subjects. Blood samples were collected from 51 patients with CTCL and 16 healthy control subjects. Informed consent was obtained.

CTCL cell lines

The human CTCL cell lines, Hut78, MyLa, SeAx, HH, and the mouse CTCL cell line EL-4 were used.

Apoptosis assay

Cultured cells were incubated with Gal-9 (1 or 2 μ g/ml) for 24hours. Viable cells were counted by Trypan blue exclusion.

In vivo animal experiments

EL-4 (1x10 7) were injected subcutaneously into the back of C57BL/6 mice. On days 0,4,7 Gal-9 was injected, or on days 4,7,11 anti-Tim-3 antibody was injected. HH (1x10 7) were injected subcutaneously into the back of SCID-beige mice with Gal-9 every 3 days.

Results :

Figure 1. Immunohistochemistry of Gal-9 in CTCL lesional skin and healthy control subjects. Immunohistochemistry revealed increased Gal-9 expression in tumor cells of CTCL lesional skin.

Figure 2. Gal-9 mRNA expression and serum levels of Gal-9 of CTCL and healthy control subjects. (a) Gal-9 mRNA expression in lesional skin of CTCL and normal skin was assessed by RT-PCR. *p<0.05. (b) Serum Gal-9 levels in patients with CTCL and healthy controls. **p<0.01. (c) Correlations between serum Gal-9 levels and serum lactate dehydrogenase (LDH) levels. n=46. (d) Correlations between serum Gal-9 levels and serum soluble IL-2 receptor (sIL-2R) levels. n=38.

Figure 3. Gal-9 induced cell death of CTCL cell lines. CTCL cell lines were cultured with Gal-9 (1 or 2 μ g/ml) or medium for 24 hours and cell numbers were counted. *p<0.05, **p<0.01.

Figure 4. Anti-Tim-3 Ab failed to inhibit Gal-9-induced cell death of CTCL cell lines. (a) Tim-3 surface expression was analyzed by flow cytometry. (b) CTCL cell lines were incubated with or without anti-Tim-3 antibody for 1hour, followed by Gal-9 (2 μ g/ml) for 24 hours. Cell numbers were counted. *p<0.05. **p<0.01.

Figure 5. Gal-9 induced cell death through caspase pathways. Cells were incubated with or without caspase Inhibitors (caspase inhibitor 1, 3, 8, 9) for 1hour, followed by Gal-9 for 24hours. Cell numbers were counted. * p<0.05.

Figure 6. Gal-9 induced apoptosis in vivo. (a) HH was injected into shaved back of SCID-Beige mice with Gal-9 or PBS every three days. n=4~7. *p<0.05. (b) Gal-9 induced cell death of EL-4. (c) EL-4 was injected into shaved back of WT mice with Gal-9 or anti-Tim-3. Gal-9 injected days 0, 4, 7, 11. Anti-Tim-3 injected days 4, 7, 11. n=8~13. *p<0.05. **p<0.01.

Discussion :

- Gal-9 was expressed on tumor cells in lesional skin of CTCL. Gal-9 mRNA expression levels were increased in lesional skin of CTCL, especially that of advanced CTCL. Serum Gal-9 levels were elevated in advanced CTCL patients and correlated with disease severity markers.
- These results suggest that Gal-9 is associated with development of CTCL, maybe through suppressing anti-tumor immunity.
- Gal-9 induced cell death of CTCL cell lines through caspase pathways, independent of Tim-3 expression.
- Gal-9 suppressed the growth of HH and EL-4 cells in vivo. Furthermore, combination of Gal-9 and anti-Tim-3 antibody achieves greater inhibition of tumor growth, compared to single administrations.
- These results suggest that combination of Gal-9 and anti-Tim-3 antibody can be a new therapeutic strategy for CTCL through not only inducing direct cell death of CTCL tumor cells, but also augmenting anti-tumor immunity.