Cytotoxicity of bispecific antibodies is dependent on their T-cell activation ability

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1. Introduction

T-lymphocytes are one type of cells from the immune system whose function is targeting and damaging invaders. They can also target and eliminate cancer cells, and bispecific antibodies such as diabodies, boost the potential of T-lymphocytes to eliminate cancer cells by artificially activating the cells. In this study, we demonstrated that the cytotoxicity of diabody is dependent on their T-cell activation ability. Thus, it can be used as an alternative way to treat cancer by targeting therapy.

2. Experiments

Luciferase assays 25 µL of diabodies and 25 µL of Jurkat cells were added together following by 5hr of incubation (37°C, 5% of CO_2). After 10 min at room temperature, 50 µL of luciferase's substrate (Promega) was added. Detection time was 10 min. When TFK-1 (bile duct cancer cell line) were required, 100 µL were seeded (4×10^4 cells/well) and left incubating overnight.

Cytokine study TFK-1 cells were seeded (1×10^4 cells/well) for 20 hr before adding the diabodies and T-LAK cells. Then 75 µL of antibodies and 75 µL of T-LAK (5×10^4 cells/well) were added together and left incubating. Incubation times are 6 hr, 24 hr and 48 hr. To run the test, the samples were centrifugated at 3000 rpm for 10min. The supernatant was then removed and transferred to another plate before adding them to the ELISA plate.

3. Results and discussion

Luciferase assay. Here, 7 diabodies (LH5, LH18, LH21, LH22, LH33, HL21) with two Fv fragments for EGFR expressed on TFK-1 cells and CD3 expressed on T-lymphocytes were prepared, and they were incubated with Jurkat cells only. However, no activation signal was observed. The experiments were also conducted in the presence of sEGFR (500nM, 100nM and 0nM), but Jurkat cells were not activated. These results implied that the antibody might need to form a bridge between cancer cells and T-cells to activate them. The experiment was done once more with LH18, LH21, HL21 and OKT3 by incubating TFK-1 and Jurkat cells. The results showed a possible correlation between cytotoxicity and activation signal. The correlation was confirmed in the second experiment where more samples were used (Fig. 1), and the dependency of the activation signal on the valence of the antibody was also observed. By comparing those results with the previous study on the inhibitory concentration (IC_{50}) of those antibodies (Table 1)[i], we concluded that the T-cell activation ability had a significant impact on their cytotoxicity.

Cytokine study 12 recombinant antibodies (LH5, LH18, LH21, LH22, LH33, HL21, OKT3 and BiBian) were applied at two concentrations (100nM and 10nM) for four different tests (IL-2, TNF-α, IFN-γ, GM-CSF) at three different times: 6 hr, 24hr and 48 hr. The results showed no signal for the GM-CSF independently of the time, whereas INF-γ showed an increasing and high concentration. Thus, INF-γ has a really important role in the activation of T-cells. TNF-α and IL-2 showed intermediate concentrations mostly decreasing with time. This means that those cytokines are produced at the beginning of the immune response and then degraded. They also seem to have an important part in the activation of T-cells. Their role and how they are helping the activation are not clear yet.

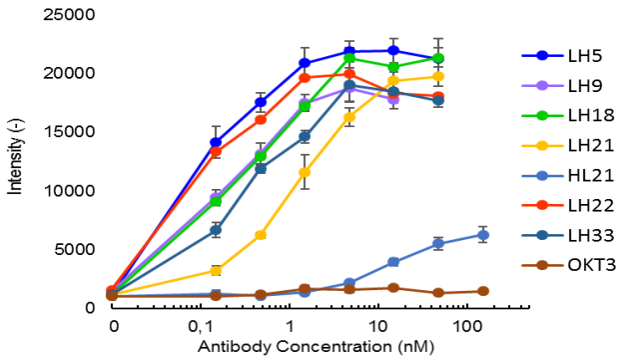

Figure 1 : Luciferase assay done with 8 kinds of antibodies, TFK-1 and Jurkat cells. 10 min of detection time.

	IC50 (pM)	CD3 binding fragment		EGFR binding fragment	
LH5	≦0.5	L2K	(100 nM)	DL11	(1.9 nM)
LH9	≦0.5	L2K	(100 nM)	11F8	(3.3 nM)
LH18	≦0.5	OKT3	(200 nM)	DL11	(1.9 nM)
LH22	≦0.5	OKT3	(200 nM)	528	(50 nM)
LH21	1.2	OKT3	(200 nM)	528	(50 nM)
LH33	20	UCHT1	(0.45 nM)	425	(113 nM)
HL21	100,000	OKT3	(200 nM)	528	(50 nM)

Table 1 : Median inhibitory concentrations (IC_{50}) of antibodies

[i] Sugiyama, A., Umetsu, M. *et al.*, *Sci Rep*, 6; 7(1):2862 (2017)