Generation of a bispecific antibody (EphA10/CD3) as a novel biodrug candidate against breast cancer

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ABSTRACT SUMMARY

Recently, we discovered that the Eph receptor A10 (EphA10), a subtype of transmembrane receptor of ephrins, was often positive in HER2-negative breast tumors, indicating it might be a suitable drug target for breast cancer patients. In this study, we created a bispecific antibody (BsAb) targeting both EphA10 and CD3, and then evaluated its usefulness as a novel biodrug for breast cancer. Using in vitro and in vivo experiments, we show that BsAb (EphA10/CD3) induces specific tumor killing in EphA10-positive tumors, probably by redirecting T-cells. These findings suggest that the BsAb (EphA10/CD3) could be a potentially valuable biodrug for EphA10-positive breast cancer.

INTRODUCTION

Monoclonal antibodies are widely used as cancer therapeutics in the treatment of various malignancies. Trastuzumab, a humanized mAb directed against HER2, was approved for clinical use for patients with HER2-overexpressing metastatic breast cancer. However, a significant proportion of patients who were either negative or display a low level of HER2 expression do not respond to trastuzumab. Therefore, it is crucially important to discover novel biomarkers for such HER2-negative breast cancer patients.

Previously, we identified Ephrin receptor A10 (EphA10) as a new biomarker of human breast cancer (1). An immunohistological study has revealed that expression of EphA10 occurred in 49% of breast cancer cases but not in all of the normal tissues except for testis. Given that most breast cancer patients are female, EphA10 is a promising target for this disease.

Recently, immune cell-mediated antitumor therapy was found to be achieved more effectively using bispecific antibody (BsAb), which binds to a surface target antigen on both cancer and immune cells. In particular, bispecific T-cell engager (BiTE), which has the potential to redirect tumor resident and cytotoxic T cells (CTL), show significant cytotoxicity against tumor cells (2).

In this study, we report the construction of novel BsAb targets EphA10 and CD3, resembling BiTE technology. We also show the specificity and potent efficacy to redirected target cell lysis and an antitumor activity of BsAb (EphA10/CD3) both in vitro and in vivo.

EXPERIMENTAL METHODS

Production of BsAb (EphA10/CD3); The BsAb (EphA10/CD3) expression vector was constructed as described previously (2). BsAb (EphA10/CD3) was expressed transiently in CHO-S cells and the recombinant C-terminal His tagged protein was purified by metal ion affinity chromatography (IMAC) followed by gel-filtration chromatography.

Binding specificity of BsAb (EphA10/CD3) for human EphA10 and CD3; Binding activities of BsAb (EphA10/CD3) were examined by flow cytometric analysis using MDA-MB-435 parental cells, MDA-MB-435EphA10 cells and Jurkat cells.

Redirected target cell lysis of BsAb (EphA10/CD3) with PBMC; The cytotoxic effect of BsAb (EphA10/CD3) from PBMC was analyzed using a lactate dehydrogenase (LDH) release assay. Target cells were co-cultured respectively with human PBMC from healthy donors at E/T ratios of 5 with each antibody (10⁻²-10⁻² μg/mL). After 2 days of incubation, the amount of released LDH in the supernatant was measured and calculated as a percentage of specific lysis.

In vivo efficacy of BsAb (EphA10/CD3); In vivo efficacy of dimer BsAb (EphA10/CD3) was evaluated using a xenograft model that consisted of BALB/c nu/nu mice that received a s.c. engraftment of 5 x 10⁵ MDA-MB-435EphA10 cells together with 1 x 10⁴ non-stimulated PBMC and each antibody (anti-EphA10 IgG or dimer BsAb (EphA10/CD3)). Tumor growth was measured at the indicated days with calipers in two perpendicular dimensions; tumor volume (mm³) was calculated using the formula: (width² x length) / 2.

RESULTS AND DISCUSSION
BsAb (EphA10/CD3) was purified from culture supernatants of BsAb gene-transfected CHO-S cells using the IMAC purification method followed by gel-filtration chromatography. After purification, two main peaks were observed as the result of chromatography while reduced SDS-PAGE and western blot analysis showed only single band of 53 kDa protein. This observation suggested that BsAb (EphA10/CD3) may exist as a monomer or dimer in the supernatant. The two fractions obtained by gel-filtration chromatography were examined to determine the binding activity of each form of BsAb (EphA10/CD3). The result of flow cytometric analysis revealed that binding activities of each fraction of BsAb (EphA10/CD3) against both antigens were similar to the format of scFv, which is the source of BsAb.

The efficacy of T-cell mediated redirected lysis with BsAb (EphA10/CD3) was examined against human EphA10-transfected cells (MDA-MB-435EphA10 cells) and the parental cells using the LDH cytotoxicity assay. As shown in Fig. 1, dimer BsAb (EphA10/CD3) showed a potent cytotoxic activity against MDA-MB-435EphA10 cells in a dose-dependent manner compared with full IgG format (anti-EphA10 IgG, anti-CD3 IgG).

Finally, we evaluated the antitumor activity of dimer BsAb (EphA10/CD3) in xenograft model mice. Mean values of tumor growth curves are shown for mice non-treated (□) or only PBMC-treated (◇), PBMC treated with anti-EphA10 IgG (▲) and PBMC-treated with dimer BsAb (EphA10/CD3) (●). Values represent mean tumor size (in mm³) ± SEM (n = 6 per group).

CONCLUSION
We have created a novel BsAb that targets EphA10 and CD3 as a promising antibody drug for breast cancer. Our findings demonstrate that dimer BsAb (EphA10/CD3) displays a potent antitumor T-cell response in EphA10-positive breast cancer patients.

REFERENCES
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