Evaluation of a novel breast cancer-related protein, Eph receptor A10 for targeting therapy

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ABSTRACT

The aim of our work is to discover cancer-specific proteins and to develop new cancer targeting therapies. We previously used an original proteome-based analysis to identify Eph receptor A10 (EphA10), which is highly expressed in breast cancer tissues compared to normal breast tissues, as a novel cancer therapy target candidate. However, the detailed biological distribution of EphA10 was poorly understood. Here, we first evaluated EphA10 as a drug target by analyzing its protein expression profile. We also used anti-EphA10 antibody to treat EphA10 expressing tumor-bearing mice. Our results suggest that EphA10 is expressed in breast cancer tissues in refractory cases such as triple negative breast cancer patients, while the expression of EphA10 is normally specific to the testis of healthy individuals. Furthermore, an anti-tumor effect was observed by administration of our developed anti-EphA10 antibody. Therefore, EphA10 is a promising candidate protein for breast cancer targeting therapy.

INTRODUCTION

Her-2 targeting therapies using trastuzumab and hormone treatment such as tamoxifen are effective treatments for breast cancer patients. Indeed, these drugs have been shown to contribute to improved prognosis of breast cancer. However, Her-2 is only expressed in 20-30% of all breast cancer cases, which limits the therapeutic utility of anti-Her-2 antibody. Furthermore, in triple negative breast cancer (TNBC) patients who do not express Her-2, estrogen and androgen receptors (ER and PR, respectively), it is also difficult to apply hormone treatments as well as Her-2 targeting therapy. As a consequence, novel targets for breast cancer therapeutic agents are urgently needed. Against this background, we developed a novel “antibody proteomics system” that facilitates the efficient screening of useful target proteins. Using this approach, we established that EphA10 is expressed in many breast cancer tissues compared to normal breast tissues.1

The Eph type A receptor family are a group of tyrosine kinase receptors that comprise nine molecules (EphA1-A8 and EphA10). According to recent reports, the Eph receptors are also upregulated in several different types of cancer. As such, these Eph receptors may be promising targets for cancer therapy. In particular, one family member, EphA2, has been linked to various kinds of cancer. Clinical trials of an anti-EphA2 antibody drug conjugate are currently ongoing. As for EphA10, Aasheim et al in 2005 reported that the EphA10 mRNA is expressed in the testis by northern blot analysis.2 However, the protein expression profile of EphA10 has never been elucidated. Here, we have evaluated EphA10 as a drug target by analyzing its protein expression profile and by using anti-EphA10 antibody to treat EphA10 expressing tumor-bearing mice.

EXPERIMENTAL METHODS

Distribution analysis of EphA10; Expression profile of EphA10 was analyzed by immunostaining with anti-EphA10 antibody using paraffin-embedded tissue microarray (TMA) slides. The TMA slides were mounted with various kinds of normal tissue and breast cancer tissues derived from each patient containing different histological types.

Development of anti-EphA10 antibody; BALB/c mice were immunized with EphA10-Fc chimera recombinant protein (total of four times). After antibody titers for EphA10 were elevated, hybridoma cells were obtained by fusion of myeloma cells with immunized spleen cells in the usual manner. Positive clones for EphA10 binding were selected by flow cytometry methods.

Specificity evaluation of anti-EphA10 antibody by an ELISA method; Each human Eph receptor A family recombinant protein was immobilized overnight. After anti-EphA10 antibody incubation, the binding of anti-EphA10 antibody for each protein was detected using HRP conjugated anti-mouse IgG antibody.

Affinity evaluation of anti-EphA10 antibody by the surface plasmon resonance (SPR) method; Anti-EphA10 antibody at a series of concentrations was
mixed with EphA10-Fc chimera recombinant protein immobilized on a sensor chip CM5. The kinetic parameters of these interactions were calculated using a single-cycle kinetic analysis method.

Anti-EphA10 antibody treatment to EphA10-bearing mice in vivo: EphA10 expressing tumor-bearing mice were constructed by orthotopic transplantation of a EphA10 stably expressing MDA-MB-435 cell line. When the tumor size reached 100 mm³, anti-EphA10 antibody and isotype control antibody were i.p. administrated.

RESULTS AND DISCUSSION
In order to establish an effective and safe targeted cancer therapy, it is important that the target antigen is specifically expressed in cancer tissues. However, knowledge concerning the EphA10 expression profile was limited as mentioned in the Introduction. Therefore, we endeavored to analyze the EphA10 protein expression by immunohistochemistry (IHC)-staining of TMA, which contains many tissues. IHC staining of various normal tissues showed that EphA10 was only expressed in testis tissue, consistent with previous reported mRNA profiles. Thus, EphA10 could be a highly cancer-specific protein, making it a promising target for female breast cancer patients. TMA contains a lot of breast cancer tissues with information about molecular expression such as Her-2, ER and PR. Thus, we stained TMA with anti-EphA10 antibody. EphA10 was found to be expressed in not only Her-2 negative cases but also TNBC cases. Thus, EphA10 may be a target in refractory breast cancer cases, although further work is required to validate these findings.

Next, we developed anti-EphA10 antibody as a targeting tool for highly breast cancer-specific EphA10. This was achieved by selecting a clone that could bind to EphA10 expressed on cells from an established hybridoma cell line. In order to evaluate the utility of this antibody, we analyzed its specificity and affinity. Binding characteristics of the antibody for each Eph receptor A family showed that the antibody specifically binds EphA10 but not the other proteins. Furthermore, SPR analysis indicated that the Kd value of the antibody was 3.9 x 10⁻⁹ M (Fig. 1). These data suggested that our original anti-EphA10 antibody could have specificity and affinity for EphA10, equivalent to that of existing antibodies.

Finally, we attempted to examine the anti-tumor effect by the antibody, aiming for the development of a novel EphA10 targeting therapy. Administration of anti-EphA10 antibody showed that tumor volumes were significantly inhibited, compared to a group of isotype control antibody (Fig. 2).

CONCLUSION
Our results suggest that EphA10 is specifically expressed in breast cancer tissues and also in TNBC cases. Moreover, our original anti-EphA10 antibody bound to EphA10 with high specificity at nanomolar level affinity and showed an anti-tumor effect. Therefore, targeting EphA10 might be a promising new form of therapy. We are currently analyzing the function of EphA10 to elucidate its role in cancer. In addition, we are improving the antibody by humanization or drug conjugation, with the aim of developing a new targeting drug.

REFERENCES

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Fig. 1 Affinity binding analysis of anti-EphA10 antibody by a SPR method

Anti-EphA10 antibody was continuously injected from weak concentration samples. Kinetic parameters were determined using a single-cycle kinetic analysis method.

Fig. 2 anti-tumor effects by anti-EphA10 antibody in vivo

Isotype control antibody or anti-EphA10 antibody (1.0 mg/mouse) were i.p. administrated in xenograft model mice twice a week.
Mean tumor volumes at day 42 ± S.E.M., (n = 5), p < 0.01.