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Bispecific Antibody Goes to Hybrid

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Abstract

Bispecific antibody has being actively pursued in cancer immunotherapy. By binding to specific surface proteins on T cells or NK cells, bispecific antibodies recruit T cells or NK cells to close proximity of tumor cells and kill tumor cells. A number of bispecific antibodies with diverse formats have been developed and some of them already in clinical stages. In the study of "Her2-S-Fab bispecific antibody has potent cytotoxicity against Her2-expressing tumor cells", a novel format of bispecific antibody was reported. Here we briefly describe this study and its implications in cancer immunotherapy.

Keywords: Bispecific antibody; Her2-S-Fab; S-Fab; Single domain antibody; Her2; CD16

Introduction

Bispecific Antibodies

Bispecific antibody is a powerful approach to combat tumor by directly engaging immune cells to tumor cells. Different from conventional antibodies, a bispecific antibody can bind two different antigens simultaneously. By binding to specific surface proteins on T cells or NK cells, bispecific antibodies recruit T cells or NK cells to close proximity of tumor cells that are recognized by the other modules on bispecific antibodies. The recruited T cells or NK cells then exert potent cytotoxicity toward the tumor cells. Many bispecific antibodies with diverse formats are now at different stages of development, including two bispecific antibodies, Catumaxomab (anti-EpCAM and anti-CD3) and Blinatumomab (anti-CD19 and anti-CD3) already approved for patient care [1-6].

Her-S-Fab

In the study of "Her2-S-Fab bispecific antibody has potent cytotoxicity against Her2-expressing tumor cells" [7], a novel format of bispecific antibody was reported. Her2 (Human Epidermal Growth Factor Receptor 2), or ErbB2, or Her2/neu, is a member of trans membrane epithelial growth factor receptor family [8]. It is frequently overexpressed in invasive breast carcinomas, gastric and ovary carcinomas with increased metastatic potential and poor prognosis. Anti-Her2 antibodies, such as Trastuzumab (Herceptin®), have been approved in clinic, and achieved a significant impact on patient outcomes [9]. Nevertheless, resistance developed eventually. Thus, there is an urgent need to develop other approaches with longer-term effect and fewer side effects [10].

Her2-S-Fab was a genetic engineered hybrid protein with a single domain antibody anti-CD16 VHH [11] fused to the C-terminal of Trastuzumab Fab [12]. The heterodimer of Trastuzumab anti-Her2 VL-CL/VH-CH1-VHH (anti-CD16) can be expressed and purified from *E. coli* periplasm efficiently with Ni-NTA agarose affinity purification followed by Anti-IgG CH1 affinity matrix. Good yield (0.6 mg/L) could be obtained [7]. Gel filtration showed that the Her2-S-Fab appeared as a single peak at the expected molecular weight (about 65 kD). Flow cytometry analysis confirmed the specific binding of Her2-S-Fab to Her2 positive cells. Tumor cell killing was observed *in vitro* specifically for Her2 positive but not Her2 negative cell lines. In the presence of NK cell, Her2-S-Fab can efficiently kill the Her2 positive cells at the concentration of less than 1 μg/mL. Compared to Trastuzumab, enhanced tumor cell killing was observed even for Her2 weak positive cell MCF7. The cell killing by Her2-S-Fab was dependent on NK cells as no tumor cell killing was observed without NK cells. In an adoptive transfer model using SKOV3 (Her2+) cells, Her2-S-Fab had strong tumor growth inhibition, supporting Her2-S-Fab as a valid alternative to Her2 positive cancer therapy.

CEA-S-Fab

In another attempt to broaden the use of Fab (Fragment antigen-binding) and VHH (Variable domain of the Heavy-chain of Heavy chain antibody) as blocks of bispecific antibody, CEA-S-Fab was designed by linking an anti-CEA (Carcino-Embryonic Antigen) single domain antibody to the c-terminal of an anti-CD3 Fab [13]. Similar to the Her2-S-Fab, the CEA-S-Fab can also be expressed and purified from bacteria. The CEA-S-Fab can recruit T cells to tumor cells and kill CEA positive tumor cells specifically both *in vitro* and *in vivo* [13].

Advantages and Issues of S-Fab Bispecific Antibodies

Different from other bispecific constructs, both Her2-S-Fab and CEA-S-Fab used single domain antibodies (anti-CD16 in Her2-S-Fab and anti-CEA in CEA-S-Fab) instead of conventional ScFv (Single chain variable fragment). Comparing to ScFv, single domain antibodies (also named VHH) derived from Camelid [14,15] have a number of physical properties, such as high refolding efficiency, high solubility, less tendency for aggregation, and high expression level in *E. coli* [16]. These properties prompted us to combine the single domain antibodies with conventional Fab to make the hybrid S-Fab (Single

domain antibody-Fab). Indeed, the additional of single domain antibody has not changed the properties and functions of Fab. In our lab, we have found that the solubility of S-Fab expressed in *E. coli* is largely depended on Fab rather than single domain antibodies. Furthermore, the S-Fab bispecific antibodies have a suitable molecular weight (~65 kD) to ensure longer half-life that tandem ScFv bispecific antibodies (~50 kD), and more likely permeating into solid tumors than IgGs (~150 kD). Furthermore, S-Fab provides the flexibility to make bispecific antibodies. T cells or NK cells can be engaged using specific antibodies in the form of conventional Fab or VHH, and verse vice, for the tumor recognizing modules (Figure 1).

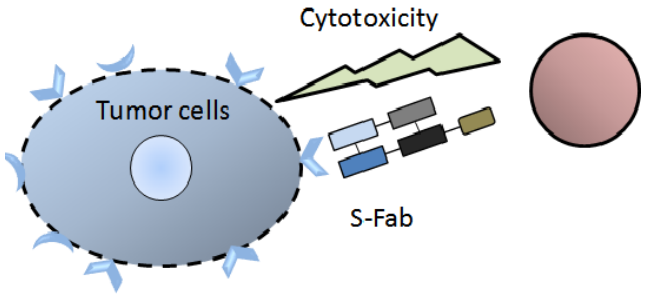

Figure 1: S-Fab bispecific antibody T cells or NK cells can be engaged using conventional Fab or VHH, and vice versa, tumor-recognizing modules can be either conventional Fab or VHH. Engaged T cells or NK cells will then have cytolytic activities against the tumor cells recognized by the bispecific antibodies.

One potential problem with S-Fab is whether the single-domain antibody will elicit immunogenicity in patients. As the sequences of single-domain antibodies are very similar to human VH (Variable domain of the heavy-chain) sequences, no or very minor immunogenicity is expected. Moreover, methods to humanize the single-domain antibodies have been developed to reduce the potential immunogenicity of VHH. In summary, similar to other bispecific antibodies, S-Fab is efficacious *in vitro* and *in vivo* at killing tumor cells. With a variety of bispecific formats have been studied in pre-clinical, the choice of specific format for specific type of tumor may depend on the combination of many factors, such as tumor type, the presence of specific tumor antigen, antibody half-life, drug penetrance, available antibodies, and the feasibility of manufacturing. With its unique properties, the hybrid bispecific antibody S-Fab warrant further development in immunotherapy.

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References

1. Chen S, Li J, Li Q, Wang Z (2016) Bispecific antibodies in cancer immunotherapy. Hum Vacc Immunother 1: 1-10.
2. Heiss MM, Murawa P, Koralewski P, Kutarska E, Kolesnik OO, et al. (2010) The trifunctional antibody catumaxomab for the treatment of malignant ascites due to epithelial cancer: Results of a prospective randomized phase II/III trial. Int J Cancer 127: 2209-2221.
3. Kontermann RE (2012) Dual targeting strategies with bispecific antibodies. MAbs 4: 182-197.
4. Kontermann RE, Brinkmann U (2015) Bispecific antibodies. Drug Discov Today 20: 838-847.
5. Newman MJ, Benani DJ (2016) A review of blinatumomab, a novel immunotherapy. J Oncology Pharm Practice 22: 639-645.
6. Przepiorka D, Ko CW, Deisseroth A, Yancey CL, Candau-Chacon R, et al. (2015) FDA Approval: Blinatumomab. Clin Cancer Res 21: 4035-4039.
7. Li A, Xing J, Li L, Zhou C, Dong B, et al. (2016) A single-domain antibody-linked Fab bispecific antibody Her2-S-Fab has potent cytotoxicity against Her2-expressing tumor cells. AMB Express 6: 32.
8. Elster N, Collins DM, Toomey S, Crown J, Eustace AJ, et al. (2015) HER2-family signalling mechanisms, clinical implications and targeting in breast cancer. Breast Cancer Res Tr 149: 5-15.
9. Albanell J, Baselga J (1999) Trastuzumab, a humanized anti-HER2 monoclonal antibody, for the treatment of breast cancer. Drugs Today 35: 931-946.
10. Hurvitz SA, Hu Y, O'Brien N, Finn RS (2013) Current approaches and future directions in the treatment of HER2-positive breast cancer. Cancer Tr Rev 39: 219-229.
11. Behar G, Siberil S, Groulet A, Chames P, Pugniere M, et al. (2008) Isolation and characterization of anti-FcgammaRIII (CD16) llama single-domain antibodies that activate natural killer cells. Protein Eng Des Sel 21: 1-10.
12. Akbari V, Mir Mohammad Sadeghi H, Jafrian-Dehkordi A, Abedi D, Chou CP (2014) Functional expression of a single-chain antibody fragment against human epidermal growth factor receptor 2 (HER2) in Escherichia coli. J Ind Microbiol Biotechnol 41: 947-956.
13. Li L, He P, Zhou C, Jing L, Dong B, et al. (2015) A novel bispecific antibody, S-Fab, induces potent cancer cell killing. J Immuno 38: 350-356.
14. Muyldermans S (2013) Nanobodies: natural single-domain antibodies. Annu Rev Biochem 82: 775-797.
15. van der Linden RH, Frenken LG, de Geus B, Harmsen MM, Ruuls RC, et al. (1999) Comparison of physical chemical properties of llama VHH antibody fragments and mouse monoclonal antibodies. Biochim Biophys Acta 1431: 37-46.
16. Harmsen MM, De Haard HJ (2007) Properties, production, and applications of camelid single-domain antibody fragments. Appl Microbiol Biotechnol 77: 13-22.