Bispecific Antibody Goes to Hybrid

Aifen Li1,2, Li Li1,2, Qing Li1,2 and Zhong Wang1,2*

1School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, China
2Center for Cellular and Structural Biology, Sun Yat-Sen University, Guangzhou, China

*Corresponding author: Zhong Wang, Center for Cellular and Structural Biology, Sun Yat-Sen University, Guangzhou, China, Tel: 020-39943453; E-mail: wangzh357@mail.sysu.edu.cn

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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 vice verse, 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 preclinical, 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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