Empowering scFv with effector cell functions for improved anticancer therapeutics

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Single chain variable fragment (scFv) constructs have been an important source of high affinity reagents to target cancer cells. Including an immunoglobulin Fc-binding domain can empower scFvs and other tumor antigen-binding proteins with immune effector cell functions and hence significantly increase their therapeutic potential.

Since 1988, the U.S. Food and Drug Administration (FDA) has approved 35 antibody-based therapeutics for clinical use. A remarkable effort in the search for the next generation of therapeutic antibodies has led to the engineering of antibody fragments. Single chain variable fragments (scFvs) are created by joining the variable heavy (VH) and light (VL) antibody chains using a short linker. Compared with their full-length counterparts, scFvs are much smaller in size and hence are expected to penetrate much better into solid tumors. With the advance of phage and yeast display technologies, scFv libraries can be quickly generated to obtain constructs that exhibit high affinity for their targets. It is not uncommon to improve affinity of the original antibody by 10–100 fold using scFv libraries. Novel scFv constructs can also be established by panning scFv libraries against a defined target.

In reality, scFvs are not real antibodies, as they are missing the constant fragment (Fc), the region that is critical for antibody-based anticancer therapeutics as it is responsible for engaging immune effector functions. In addition, the half-life of scFvs is much shorter than that of an intact, Fc-containing IgG molecule. These issues have limited the use of scFvs as therapeutic agents. So far, no scFv-based therapeutics are approved for clinical use. Although several scFv variants are being evaluated in the clinical studies, all of them have not yet reached Phase III trials, and most of them are being employed as toxin or cytokine conjugates. Some scFv constructs have been fused with Fc fragments to improve their half-life or with Fc receptor-binding proteins to activate effector functions. The clinical potential of these approaches remain to be demonstrated. Clearly, there are tremendous obstacles for scFvs and other small antigen-binding non-Fc proteins to advance toward therapeutic applications in cancer patients.

We have just reported a novel “Grababody” approach, empower a scFv construct with immune cell functions. This approach relies on the IgG-binding Z domain fused on the C-terminus to the scFv, allowing it to capture endogenous circulating IgGs. Using a scFv specific for the ERBB2/HER2 receptor as an example, we have shown that Grababodies can bind to their targets on tumor cells. Surface plasmon resonance experiments confirm that, upon antigen binding, the Grababody can in fact capture IgGs. As an improved form of scFv molecules, Grababodies activate complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) activity against antigen-expressing tumor cells, an activity that depends on the availability of circulating IgGs. Most importantly, the Grababody significantly reduced the growth of ERBB2/HER2-expressing malignant cells growing in compatible mice.

Another benefit of Grababodies is their extended half-life. scFvs and antigen-binding fragments (Fabs) are smaller than 70 kDa, the minimal size for a protein to avoid being lost by rapid renal elimination. The plasma β half-life of the 4D5 Fab was reported to be only 1.28 h. Binding to proteins abundant in the serum has been explored as a strategy to reduce the clearance of antibody fragments. When fused to a peptide tag interacting with albumin, the 4D5 Fab exhibits a 15-fold increased plasma half-life in mice. The β half-life of scFvs is even shorter than that of Fabs (0.6 h). In a very recent study, Ig-binding domains were shown to increase the plasma half-life of scFvs by as much as 34.7-fold. Although the exact half-life of Grababodies is yet to be determined, we expect it to be in the range of 12–18 h.

One concern for the Grababody is the origin of the Fc-binding domain sequence, which is derived from protein A (SpA), a component of the cell wall of Staphylococcus aureus. Based on current knowledge, we expect no major issues to stem from the small Fc-binding domain of the Grababody. Although SpA is not an endogenous protein, the prevalence of S. aureus infection and colonization in humans is very high. SpA has been
process whereby phagocytes become able to ingest and destruct pathogens upon the binding of antibodies or complement components. In one study, SpA was tested as a vaccine against *S. aureus* in mice.10

Although the effects of Grababodies in mice bearing xenografted tumors are encouraging, this animal model does not precisely reflect their full activity in humans, for multiple reasons. First, Grababodies have a much higher affinity for human than for murine IgGs (KD: human IgG, 1.3 nM; mouse IgG, 325 nM). As a result, the recruitment of circulating IgGs in mouse is less efficient than it would be in humans. Second, the β half-life of scFvs is linearly correlated with their affinity for host proteins.5 It is therefore possible that the half-life of Grababodies in humans could be longer than in mice. In summary, the activity of Grababodies could be even better in humans. Our studies have provided proof-of-concept for a novel approach that improves the half-life and activity of scFv constructs and other small tumor-targeting peptides in vivo.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

Figure 1. A simple approach to enable single chain variable fragments and antigen-binding proteins to induce immune effector cell functions. A small antigen-binding protein such as a single chain variable fragment (scFv) is fused with an immunoglobulin-binding domain, such as the Z domain of staphylococcal protein A (SpA) to create a Grababody. Grababodies are able to bind to antigens exposed on the surface of tumor cells and then recruit endogenous circulating antibodies, hence activating complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC).

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that of the full-length protein.8 The Fc-binding domain of SpA is not involved in the activation of Type I interferon (IFN) by *S. aureus*.9 Interesting, the Fc-binding activity of SpA may be beneficial for the host, as it is required for opsonization, the