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Notes & Tips

Development of a novel engineered antibody targeting human CD123

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ABSTRACT

Antibody engineering involves a range of custom modifications of immunoglobulins to improve their affinity, valency, and pharmacokinetics, ensuring a better target therapy achievement. A number of therapeutic antibodies have been used for cell surface receptor blockage, interfering with the ligand binding and inhibiting receptor-driven activation of cells. Here we describe the construction and characterization of a recombinant bivalent single-chain Fv (biscFv) that targets CD123. On conversion of anti-CD123 scFv to biscFv format, the recognition of the cognate ligand is not altered. Moreover, the increased overall efficacy of the anti-CD123 biscFv in binding and inhibition of CD123/IL-3 (interleukin-3) interactions in TF-1 cells is demonstrated.

Leukemic cancer stem cells (LSCs) characterized by aberrant overexpression of CD123 promote the initiation and development of acute myeloid leukemia (AML) and are regarded as the main cause of leukemia relapse and chemotherapy resistance [1]. Binding of CD123 to interleukin-3 (IL-3) results in increased cell survival and proliferation [2]. Therefore, antagonist agents targeting CD123 effectively eliminate LSCs, and in this context a number of targeting agents have been developed, some of which have been used in clinical trials [3].

Cell surface receptor blockade impeding receptor/ligand interaction and the consequent receptor activation has been long considered as an antibody-mediated therapeutic approach in various conditions [4]. Application of monoclonal antibodies (mAbs) for those purposes has been accompanied by undesirable Fc-induced effector functions, which leads to proposing the application of smaller recombinant antibody fragments such as single-chain Fv (scFv) as alternatives to mAbs [5]. Monovalent scFvs are limited in their binding affinities; therefore, to overcome this limitation, other types of recombinant antibodies such as bi/multivalent and bi/mutispecific scFv antibodies have been proposed [6]. The latter approaches have been used to improve antitumor efficacy of anti-CD123 scFv in other studies, for instance, anti-CD123–CD16 bispecific scFv [7], anti-CD16–CD33–CD123 [8], fusion of anti-CD123 scFv to a 38-kDa fragment of *Pseudomonas* exotoxin A [9], and CD123–CD3 bispecific scFv [10]. Application of these agents, except for the immunotoxin, enhances lysis of LSCs through induction of antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC).

A combination of different therapies such as surgery, radiation, chemotherapy, and targeted immunotherapy is favorable in the treatment of cancer. However, various immunosuppressive factors in cancer patients, including regulatory T cells, myeloid-derived suppressor cells, inhibitory cytokines, and the varying ability of cytotoxic T cells, interfere with the efficacy of immunotherapy [11]. Moreover, standard chemotherapy often affects different compartments of the immune system that further diminish the efficacy of antibody

Abbreviations used: LSC, leukemic cancer stem cell; AML, acute myeloid leukemia; IL-3, interleukin-3; mAb, monoclonal antibody; scFv, single-chain Fv; ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity; biscFv, bivalent scFv; PCR, polymerase chain reaction; dsDNA, double-stranded DNA; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.

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0003-2697/

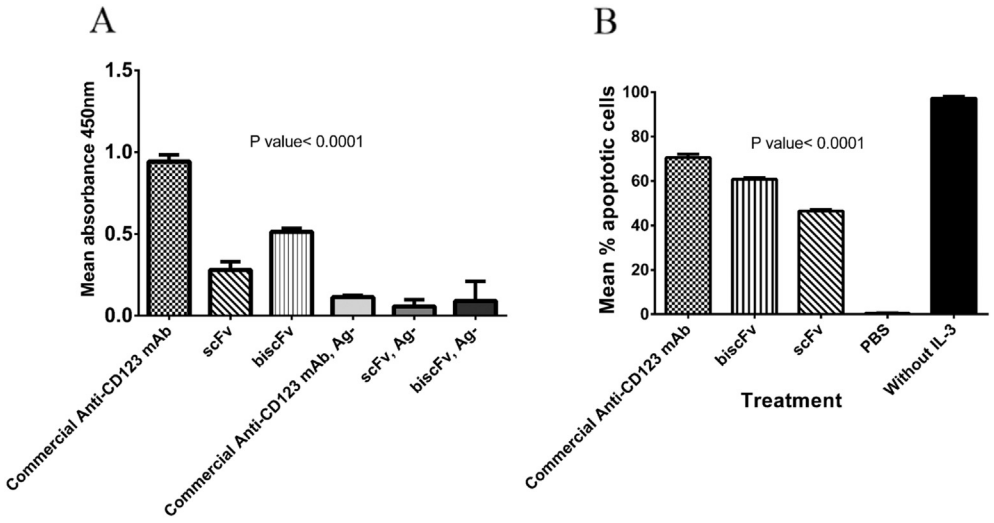

Fig.1. Functional ability of purified biscFv in CD123 binding and blocking CD123/IL-3 interactions illustrated by enzyme-linked immunosorbent assay (ELISA) and apoptosis assays by flow cytometry of TF-1 cells, respectively. (A) ELISA. The biscFv showed significant increase in binding (~2-fold) to CD123 compared with its scFv counterparts. Vertical error bars represent the standard deviations of means from triplicates in the given experiment. Results are with one-way analysis of variance (ANOVA) test with P value < 0.0001. (B) Mean percentage of total early apoptotic and late apoptotic percentages of cells together in triplicate experiment for each treatment. Vertical error bars represent the standard deviations of means from triplicates in the given experiment. One-way ANOVA reveals significant differences (P value < 0.0001).

therapy mediated by ADCC and CDC [12]. In addition, FcγR genotypes and cancerous cell expression of natural killer cell inhibitory proteins might have an impact on the ADCC function of antibodies in antibody therapy [13]. Thus, it seems that choosing the targeted therapy approaches that are minimally regulated by different components of the immune system is of great importance [11].

Targeting growth factor receptors on tumor cells with antagonist antibodies inhibits key survival mechanisms by antagonizing ligand/receptor signaling and, thereby, can effect tumor cell elimination through apoptotic processes beyond immune effector functions and increasing tumor cell susceptibility to cytotoxic therapy. This illustrates the versatility of this therapeutic approach and enhances their attractiveness as anticancer agents in combination with conventional chemotherapy [14]. Hence, with respect to the increasing demands of alternative therapies for AML that are independent of the host immune system, here we describe construction of a bivalent scFv (biscFv) directed against CD123. This approach could be a novel approach for IL-3 blockade by using CD123/IL-3-neutralizing compounds consisting of antibody fragment.

To generate the bivalent scFv, the scFv-110 construct encoding the murine anti-CD123 scFv previously isolated from our constructed scFv phage display library [15] was employed as a polymerase chain reaction (PCR) template for generation of biscFv-110 using the primers shown in Table 1 in Ref. [16]. The coding sequence of scFv1 of biscFv was generated by using scFv1-F and scFv1-R primers introducing *Nco*I and *Bam*HI restriction sites, respectively. Similarly, to yield the PCR fragment of scFv2, scFv2-F and scFv2-R primers embedded with *Eco*RI and *Not*I restriction sites were applied. To introduce the most commonly used 15-amino-acid linker $(Gly_4Ser)_3$ and generate a double-stranded DNA (dsDNA), the synthesized overlapping oligonucleotides named fragments L1 and L2 (see Table 1 in Ref. [16]) were annealed after heating for 10 min at 94 °C and cooling slowly to room temperature (1:1 M ratio). *Bam*HI and *Eco*RI restriction sites were introduced to the annealed L1–L2 dsDNA for cloning purposes without any digestion needed (see Fig. 1 in Ref. [16]). Fragment L, scFv1, and scFv2 coding sequences were ligated to linearized vector pET22-b (Merck–Millipore, Germany), respectively, in order to form the bivalent antibody expression cassette in a three-step cloning procedure. Following

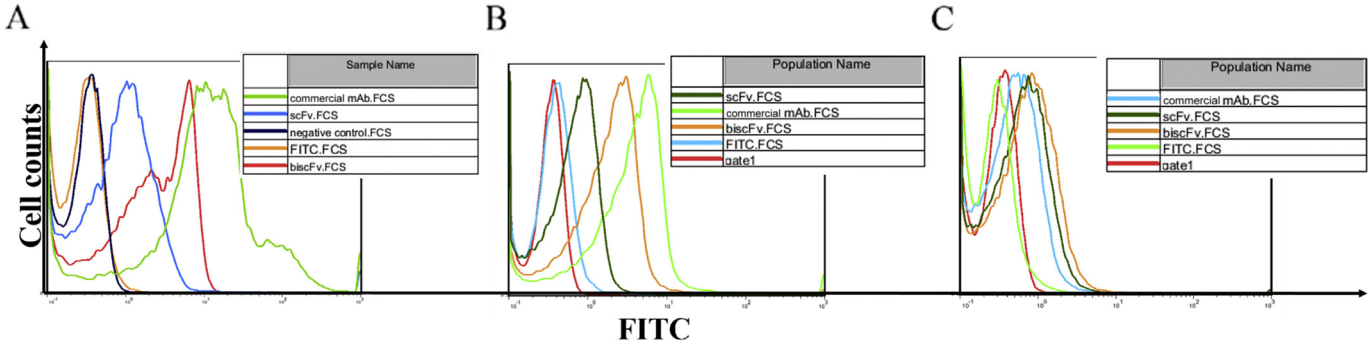

Fig.2. Cell binding assay of purified biscFv by flow cytometry. (A) Flow cytometry of purified biscFv and scFv on $CD123^+$ TF-1 cell line. As shown by the shift in fluorescence value, commercial anti-CD123 mAb, biscFv, and scFv bind preferentially compared with background staining in the absence of primary antibody. (B) Flow cytometry of purified biscFv and scFv on $CD123^+$ KG-1 cell line. As shown by the shift in fluorescence value, commercial anti-CD123 mAb, biscFv, and scFv bind preferentially compared with background staining in the absence of primary antibody. (C) Flow cytometry of purified biscFv and scFv on $CD123^-$ MOLT-4 cell line. As shown, no obvious shift in fluorescence value was observed compared with background staining in the absence of primary antibody, indicating no cross-reactivity with other cellular antigens. FITC, fluorescein isothiocyanate.