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Structural analysis of a humanized anti-human epidermal growth factor receptor antibody 528

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Introduction

The human epidermal growth factor receptor (hEGFR) is a cell-surface receptor involved in regulation of cell growth and proliferation. Overexpression of EGFR has been found in a number of tumors and its expression level is highly correlated with the malignancy of tumors. Thus EGFR is a promising target for cancer immunotherapy.

Previously, we have constructed an Ex3 bispecific diabody, composed of variable domains of anti-CD3 antibody OKT3 and anti-hEGFR antibody 528 [1]. The Ex3 diabody has shown potent anti-tumor activities *in vitro* and *in vivo*. To reduce the possible immunogenicity of murine antibody 528 in human, humanization has been performed by a CDR-grafting method. Thermodynamic analyses of the interactions between the variable domain fragments of antibody and the soluble extracellular domain of EGFR showed that humanization led to approximately 40-fold reduced affinity for the target [2].

Here we report the crystal structures of humanized and murine 528, and discuss the crucial factors for affinity reduction via humanization from structural aspects.

Experimental

The Fv fragment of humanized 528 (h528) was prepared by using an *Escherichia coli* secretory expression system. The Fab fragment of murine 528 (m528) was prepared by papain digestion of m528 IgG. The best crystals were grown in 3 M sodium chloride and 0.1 M Tris-HCl (pH 7.5) for h528Fv, and 1.4 M ammonium sulfate, 0.01 M cadmium chloride and 0.1 M Tris-HCl (pH 7.1) for m528Fab, respectively.

Data for h528Fv were collected at the beamline NW12A of Photon Factory (PF), and data for m528Fab were collected at the beamline BL-6A of PF. Collected data were processed with programs HKL2000 and SCALA (CCP4 program suite). The structures of h528Fv and m528Fab were solved by a molecular replacement (MR) method with the program MOLREP. For h528Fv, the resulting model from MR was first refined by rigid body refinement using the program REFMAC and simulated annealing using the program CNS. Further refinement of structure was carried out with programs XtalView and CNS. For m528Fab, the resulting model from MR was first refined by rigid body refinement and simulated annealing using the program CNS. Further refinement of structure was carried out with programs O and CNS. The atomic coordinates and structural factors have been deposited in the Protein Data Bank (ID codes 1WT5 and 2Z4Q for h528Fv and m528Fab, respectively).

Results and Discussion

The crystal structures of h528Fv and m528Fab demonstrated that the overall structures of the Fv portions were almost identical each other (Figure 1). Furthermore, the conformations of the complementarity-determining region (CDR) loops were almost completely conserved after humanization. Shape complementarity (SC) values between the variable domains of the heavy and light chains (VH and VL) of h528Fv and m528Fab were 0.735 and 0.754, respectively. These results suggested that no major conformational changes were introduced by humanization. However, when VH and VL of h528Fv were superposed onto those of m528Fab, the root mean square deviations of Cα atoms of the VL and VH of h528Fv were 1.03 and 1.56 Å, respectively, suggesting that slight but critical changes in the relative orientations of VH and VL were introduced by humanization. Recently, we have reported that appropriate association of variable domains was critical for humanization of murine antibodies with maintaining the function [3]. In the future, complex structures of the soluble EGFR and 528 would provide precise information of the 528-EGFR interactions.

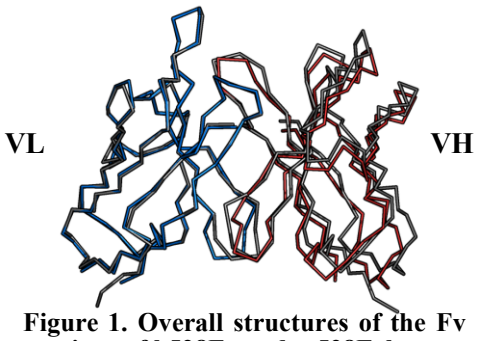

Figure 1. Overall structures of the Fv portions of h528Fv and m528Fab.
Gray, murine; color, humanized.

References

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