Epitope-directed *in silico* screening and *in vivo* application of anti-OX40 agonistic antibody

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Abstract

Antigen immunization induces animals to generate innumerable antigen-specific antibody clones targeting various epitopes. Therefore, identifying and developing agonistic antibodies has been always challenging. Here, we identified an agonistic antibody clone against OX40, a member of tumor necrosis factor receptor super family on T cells, by in silico screening and genetically modified it for in vivo use using nanobody-based technology. By clustering analysis and computational processing of biopanning data of a huge nanobody library, we identified a unique clone that shares the epitope with the native ligand gp34. The identified clone had a large negatively charged surface formed by a complementarity-determining region 3, which enabled to bind OX40 across multiple cysteine-rich domains similarly to gp34. Furthermore, conjugation of anti-serum albumin nanobody with trimerized nanobody showed a favorable pharmacokinetics and potent therapeutic efficacy in combination with chimeric antigen receptor T-cells. These findings highlight the potential of our novel methodology to further facilitate antibody-based therapies.