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Short communication

A large human domain antibody library combining heavy and light chain CDR3 diversity

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ABSTRACT

Domain antibodies (dAbs) are promising candidate therapeutics and diagnostics. Efficient selection of novel potent dAbs with potential for clinical utility is critically dependent on the library diversity and size, and the scaffold stability. We have previously constructed a large (size $\sim 2.5 \times 10^{10}$) dAb library by grafting human antibody heavy chain complementarity determining regions (CDRs) 2 and 3 (H2s, H3s) into their cognate positions in a human heavy chain variable domain (VH) scaffold and mutagenizing the CDR1 (H1). High-affinity binders against some antigens were selected from this library but panning against others was not very successful likely due to limited diversity. We have hypothesized that by grafting highly variable, both in length and composition, human CDRs into non-cognate positions, the dAb library diversity could be significantly increased and the library would allow for more efficient selection of high-affinity antibodies against some targets. To test this hypothesis we designed a novel type of dAb library containing CDRs in non-cognate positions. It is based on our previous library where H1 was replaced by a library of human light chain CDR3s (L3s) thus combining three most diversified fragments (L3, H3 and H2) in one VH scaffold. This large (size $\sim 10^{10}$) phage-displayed library was highly diversified as determined by analyzing the sequences of 126 randomly selected clones. Novel high-affinity dAbs against components of the human insulin-like growth factor (IGF) system were selected from the new library that could not be selected from the previously constructed one. Most of the newly identified dAbs were highly soluble, expressible, monomeric and may have potential as candidate cancer therapeutics. The new library could be used not only for the selection of such dAbs thus complementing existing libraries but also as a research tool for the exploration of the mechanisms determining folding and stability of human antibody domains.

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1. Introduction

Currently, almost all therapeutic antibodies (except ReoPro, Lucentis and Cimzia which are Fabs) approved by the U.S. Food and Drug Administration and the vast majority of those in clinical trials are full size antibodies mostly in IgG1 format of about 150 kDa size (Dimitrov and Marks, 2009). A fundamental problem for such large molecules is their poor penetration into tissues (e.g., solid tumors) and poor or absent binding to functionally important regions on the surface of some molecules (e.g., the human immunodeficiency virus envelope glycoprotein) which are accessible by molecules of smaller size. Decreasing the size of the molecule dramatically, non-linearly, increases its penetration in tissues (Yokota et al., 1992, 1993). Similarly, antibody size dependence of epitope accessibility can be highly non-linear and some protein surface-exposed structures can be completely obstructed for full size antibodies. Therefore, a large amount of work especially during the last decade has been aimed at developing novel scaffolds of much smaller size (Holt et al., 2003; Nygren and Skerra, 2004; Binz et al., 2005; Hey et al., 2005; Holliger and Hudson, 2005; Skerra, 2007; Kolmar and Skerra, 2008; Saerens et al., 2008; Dimitrov, 2009; Dimitrov and Marks, 2009). Several scaffolds are derived from single antibody domains which are about 10-fold smaller than full size antibodies (Holt et al., 2003; Saerens et al., 2008; Dimitrov, 2009). Such scaffolds are stable, soluble, and easy to format, manufacture and express in microbial cell cultures.

Abbreviations: VH, heavy chain variable domain; VL, light chain variable domain; dAb, domain antibody; Fab, antigen-binding fragment; CDR, complementarity determining region; L3, light chain CDR3; H2, heavy chain CDR2; H3, heavy chain CDR3; FR, framework region; KL, kappa light chain; LL, lambda light chain; IGF-2, human insulin-like growth factor 2; IGF-1R, IGF-1 receptor; HIV, human immunodeficiency virus; SPA, staphylococcal protein A; MW, molecular weight; PBS, phosphate-buffered saline; PBST, PBS containing 0.05% Tween-20.

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One of the most advanced antibody domain scaffold is based on the single heavy chain variable domain (VH) (Ward et al., 1989; Holt et al., 2003; Chen et al., 2008b). Binders derived from libraries based on mammalian VH or light chain variable domain (VL) scaffolds are called domain antibodies (dAbs). The human dAb, ART621 (targeting TNFα), is now in phase II clinical trials (www.arana.com). The efficient selection of high-affinity binders against various targets is critically dependent on the size and diversity of the antibody library. To minimize immunogenicity it is desirable to use fully human sequences for diversification. We have recently constructed a large (size, $\sim 2.5 \times 10^{10}$) phage-displayed dAb library by grafting naturally occurring human antibody heavy chain complementarity determining regions (CDRs) 2 and 3 (H2s, H3s) into a scaffold based on a newly identified fully human VH and randomly mutating four putative solvent-accessible residues in the CDR1 (H1) (Chen et al., 2008b, 2009). High-affinity dAbs were selected from this library against viral and human cancer-related antigens (Chen et al., 2008a, 2009).

In the absence of the VH–VL combinatorial diversity, the importance of constructing highly diversified libraries increases. The diversity of dAbs, however, is inherently limited using only three CDRs compared to six CDRs of a conventional antibody. Remarkably, camelidae (and other species) naturally produce functional antibodies which are composed solely of heavy chains, designated heavy chain antibodies or HCAbs (Hamers-Casterman et al., 1993). The antigen-binding site of the HCAbs contains a single variable domain (referred to as V_HH). Compared to human VHs, V_HHs underwent remarkable changes in sequence and structure during evolution (Nguyen et al., 2000). Most strikingly, an extra hypervariable region is present exclusively in the H1s of V_HHs and their H3s are, on average, longer than those of human VHs (17 residues versus 12 residues). These changes, together with others, dramatically increase the diversity of V_HH repertoire and enlarge the surface area interacting with antigens resulting in novel paratopes that are different from those of conventional antibodies (Nguyen et al., 2000). In our previously constructed library (Chen et al., 2008b) H1 was mutagenized but its length remained constant. To increase diversity, both in length and composition, we have hypothesized that H1s, which are relatively weakly diversified, could be replaced by more diverse non-cognate CDR3s, specifically those of the light chain (L3s), without significantly affecting the structural integrity of the scaffold. Here, we describe the generation and characterization of a fully human large (size, $\sim 10^{10}$) phage-displayed dAb library, which was constructed by combining naturally occurring human antibody H2s and H3s with L3s on the same scaffold. We identified novel dAbs against human cancer-related proteins, including components of the human insulin-like growth factor (IGF) system, that exhibit high solubility, affinity, specificity, and were not selected by panning of our previously constructed library based on mutated H1s.

2. Materials and methods

2.1. Amplification of L3 repertoire

Primers used for PCR amplification of gene fragments are described in Table 1. L3 repertoire was harvested from five different sources. Two of them were plasmid DNAs of a phage-displayed naive human Fab library (5×10^9 members) constructed from peripheral blood B cells of 10 healthy donors (Zhu et al., 2006) and a phage-displayed immune human Fab library ($\sim 10^9$ members) constructed from bone marrow of three long-term non-progressors whose sera exhibited the broadest and most potent human immunodeficiency virus 1 (HIV-1) neutralization among 37 HIV-infected individuals (Zhang et al., 2003), respectively. To reduce PCR amplification bias, cDNAs, which were commercially purchased or produced in our group, were directly used as the other three sources. These include (a) a cDNA mixture from bone marrow of 10 healthy donors and fetal spleen of 24 spontaneously aborted male/female Caucasian fetuses; (b) a cDNA mixture from cord blood of two healthy babies, from which two naive Fab libraries (6×10^8 and 7.2×10^8 members, respectively) have been recently constructed; and (c) a cDNA mixture from peripheral blood B cells of 22 healthy donors, spleens of 3 healthy donors, and lymph nodes of 34 healthy donors which has also been used for the construction of a large non-immune Fab library (1.5×10^{10} members). To increase the efficiency for amplification of L3, full-length kappa (KL) and lambda (LL) light chains were first amplified from the five

Table 1
Primers used for library construction.

Primer description	Name	Sequence	Target
L3 sense	L3F1	CTC TCC TGT GCA GCC TCT TAY TAC TGY	L3
	L3F2	CTC TCC TGT GCA GCC TCT TAY TAC RGY	L3
	L3F3	CTC TCC TGT GCA GCC TCT NAY TAC RGY	L3
KL3 antisense	KL3R1	CTG GCG GAC CCA GCT CAT GAA CGT CCA	KJ1[a]
	KL3R2	CTG GCG GAC CCA GCT CAT AAA AST GYA	KJ2
	KL3R3	CTG GCG GAC CCA GCT CAT GAA AGT GAA	KJ3
	KL3R4	CTG GCG GAC CCA GCT CAT GAA MGT GAG	KJ4
	KL3R5	CTG GCG GAC CCA GCT CAT GAA GGT GAT	KJ5
LL3 antisense	LL3R1	CTG GCG GAC CCA GCT CAT GAA GAC ATA	LJ1[b]
	LL3R2	CTG GCG GAC CCA GCT CAT GAA TAC CAC	LJ2, 3
	LL3R3	CTG GCG GAC CCA GCT CAT AAA TAC AAA	LJ4
	LL3R4	CTG GCG GAC CCA GCT CAT AAA CAC CCA	LJ5
	LL3R5	CTG GCG GAC CCA GCT CAT GAA CAC ATT	LJ6
	LL3R6	CTG GCG GAC CCA GCT CAT GAA CAC AGC	LJ7
FR1 sense	FR1F	TGG TTT CGC TAC CGT GGC CCA GGC GGC CCA GGT GCA GCT GGT G	FR1
FR1 antisense	FR1R	AGA GGC TGC ACA GGA GAG	FR1
FR2 sense	FR2F	ATG AGC TGG GTC CGC CAG	FR2
Extension 1	L3R	CTG GCG GAC CCA GCT CAT	5 end of L3 antisense primer
Extension 2	HISR	GTC GCC GTG GTG GTG GTG GTG GTG GCC GGC CTG GCC ACT TG	pComb3X vector

The sequences underlined enable amplification of human antibody L3 repertoire.
[a] Family of human antibody KL J gene.
[b] Family of human antibody LL J gene.