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Research review paper

Applications of single-chain variable fragment antibodies in therapeutics and diagnostics

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ABSTRACT

Antibodies (Abs) are some of the most powerful tools in therapy and diagnostics and are currently one of the fastest growing classes of therapeutic molecules. Recombinant antibody (rAb) fragments are becoming popular therapeutic alternatives to full length monoclonal Abs since they are smaller, possess different properties that are advantageous in certain medical applications, can be produced more economically and are easily amendable to genetic manipulation. Single-chain variable fragment (scFv) Abs are one of the most popular rAb format as they have been engineered into larger, multivalent, bi-specific and conjugated forms for many clinical applications. This review will show the tremendous versatility and importance of scFv fragments as they provide the basic antigen binding unit for a multitude of engineered Abs for use as human therapeutics and diagnostics.

Contents

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1. Introduction

Small rAb fragments are increasingly being used as alternatives to monoclonal antibodies (mAbs) in medical diagnostic and therapeutic applications. A variety of rAb formats have been tailored for specific applications including engineered modifications to antigen binding, valency, and molecular weight (MW). One of the most popular types of rAbs are scFvs as they have been successfully modified into a number of different Ab formats and are easily expressed by several expression systems.

scFvs contain the complete antigen binding site, which includes the variable heavy (V_H) and variable light (V_L) domains, of an Ab. The V_H domain is linked to a V_L domain by an introduced flexible polypeptide linker (*e.g.* $(GGGGS)_3$). However, these domains can also associate non-covalently (Fv fragment), by a disulfide bond (dsFv), or by both (sc-dsFv) to form monovalent Ab fragments that are similar to scFvs (Wörn and Plückthun, 2001). Monovalent rAbs, including scFvs, are limited in their binding affinity and *in vivo* persistence. Thus, to address this problem, multivalent and mutispecific molecules have been created of which scFv fragments have served as the basic antigen binding unit. scFv and scFv-based molecules have also been fused to a vast array of reagents including drugs, toxins, radionuclides for cancer treatment, viruses for gene therapy, liposomes for improved drug delivery and biosensors for real-time detection of target molecules (Holliger and Hudson, 2005). Several structural designs have improved *in vivo* pharmacokinetics, enhanced affinity, stability, and expression levels. Engineered scFv-based fragments are poised to provide the next wave of Ab-based reagents, and many are already in late-phase clinical trials (Holliger and Hudson, 2005).

This review will discuss the recent developments and applications of scFv, scFv-based fragments and scFv-based conjugates that have been engineered to target antigens associated with human disease. Wherever relevant, comparisons to their mAb counterparts are presented.

2. Natural and synthetic antibody fragments

2.1. Basic structure of immunoglobulins

Vertebrates express five classes (or isotypes) of antibodies called immunoglobulin (Igs): alpha (IgA), delta (IgD), epsilon, (IgE), gamma (IgG,) and mu (IgM). Structural differences among the isotypes, summarized in Table 1, include, *e.g.*, differences in MW and antigen binding sites (Lobo et al., 2004; Salfeld, 2007). IgGs are the most abundant Igs in human blood (*ca.* 85% of Ig in serum) and are the most widely used Igs for therapeutic and diagnostic applications (Maynard and Georgiou, 2000). Human IgG is a heterotetramer of two identical γ heavy chains and two identical light chains that are joined with a series of disulfide bonds. Any single human IgG molecule has two γ heavy chains of subclasses 1, 2, 3 or 4, and either two κ or λ light chains. Each light chain contains one variable domain (V_L) and one constant domain (C_L); heavy chains contain one variable domain (V_H) and three constant domains (*i.e.* C_H1, C_H2 and C_H3). The variable regions, at the N-terminus of the Fab fragment, determine the specificity, diversity and affinity of antigen binding and the constant domains are responsible for mediating Ab structure and effector functions and for determining IgG *in vivo* half-life (Fig. 1). V_H-region antigen specificity results from recombination of variable (V), diversity (D) and junctional (J) gene segments, whereas V_L region antigen specificity results from recombination of the VJ gene segments that occurs during B-cell maturation to generate the DNA encoding the unique Ab combining site.

Within each V_H and V_L domain are three hypervariable regions where sequence variability is concentrated and loops are formed. These hypervariable regions are primarily responsible for antigen recognition and because the antigen-binding site is complementary to the structure of the epitope, the hypervariable regions are also called complementary determining regions (CDRs). The remainder of the V_H and V_L domains exhibit far less variation; these portions are referred to as the frameworks regions which act as a scaffold to support the loops. The CDRs and framework regions in each the V_H and V_L

Table 1
Properties and biological activities of human immunoglobulin isotypes[a].

	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM	IgE	IgD
Molecular weight (kDa)	150	150	150	150	160, 400	160, 400	950, 1150	190	175
Molecular form	Monomer	Monomer[b]	Monomer	Monomer	Monomer, dimer, tetramer	Monomer, dimer, tetramer	Pentamer, hexamer	Monomer	Monomer
Valence	2	2, 4	2	2	2, 4	2, 4	10, 12	2	2
Heavy chain	γ	γ	γ	γ	α	α	μ	ε	δ
Light chain	K or λ	K or λ	K or λ	K or λ	K or λ	K or λ	K or λ	K or λ	K or λ
In vivo serum half-life (d)	36	37	29	16	6	6	5	2.5	3
Effector functions									
Binds C1q	++	−	+++	−	−	−	+++	−	−
FcγR1	+++	−	+++	++	−	−	−	−	−
FcγRII	+	±	+	?	−	−	−	−	−
FcγRIIIa/b	+	−	+	±	−	−	−	−	−

"+" denotes positive response or interaction, "−" denotes no response or interaction.
[a] Adapted in part from Lobo et al. (2004) and Salfeld (2007).
[b] IgG2 has been reported to form covalent dimers (Yoo et al., 2003).