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Single-chain fragment variable (scFv) with medical potential

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Today generation of single-chain fragment variable (scFv) by phage display has become an established technique and could be used to select a completely functional antigen-binding fragment. Detailed overviews about this in vitro selection technology are given in different reviews [1-3]. A scFv is a molecule of about ~30 kDa and consists of the variable heavy (V_H) and variable light (V_L) chain joined together by a flexible peptide linker of about 15 amino acids. It is half the size of the antigen-binding (Fab) fragment and retains the specificity of the parent immunoglobulin.

Most often *E. coli* is the bacterial host for expression of the scFvs and the molecule is secreted directly into the periplasm space. There, due to the oxidizing environment of this bacterial compartment the antibody domains are folded.

Over the last decades researchers have isolated scFvs from different type of libraries like immune, naive and synthetic libraries with a complexity of 10^8 to 10^{11} antibody genes encoding antibodies with unknown properties. While immune libraries are constructed from variable domains of B cell antibody genes of either immunized animals or humans, naïve libraries derive from non-immunized sources and are not biased. At least synthetic libraries derive as well from non-immunized sources and combine germ line gene sequences together with randomized complementary determining regions (CDRs). However, for medical therapies immune libraries are the preferred type of library because of the antigen driven in vivo selection and the fact that these libraries already contain affinity-matured antibodies.

ScFvs have been raised successfully against different bacteria, viruses, toxins as well as diverse cellular structures and proteins and it would burst this editorial to list them all. ScFvs were for example generated against *Salmonella typhimurium*, *Yersinia pestis*, botulinum and tetanus toxins, alphaviruses, influenza, herpes simplex and human immunodeficiency viruses (HIV) [4-13].

ScFvs can be applied for either research, identification or diagnosis. They are used for loss-off-function studies as well as for *in vitro* and *in vivo* inactivation studies [14-16]. However, only neutralizing antibodies are of medical interest. Most often they recognize surface molecules like outer membrane proteins, capsule antigens, glyco- or envelope proteins.

ScFvs can serve as a reasonable alternative to vaccination and treatment of a viral or bacterial infection. Moreover, many generated scFvs are applied in tumor therapy, treatment of autoimmune and cardiovascular diseases as well as neurogenerative diseases [14-17].

Every year several scFvs are evaluated in clinical studies of different phases and especially late-stage studies sponsored by commercial companies have substantially increased in recent years. 50 antibodies have been approved by the US Food and Drug Administration (FDA) or European Medicines Agency (EMA), several hundred are under clinical investigation, demonstrating the value of phage-display for the generation of therapeutic antibodies [18]. The article series 'Antibodies to watch" give each year a brief summary of antibody therapeutics and introduces those antibodies that have received their first marketing approvals in either the EU or the US [19,20]. In addition, several scientific reviews like those of Frenzel et al. and Ahmad et al. outline phage display-derived antibodies and antibody conjugates evaluated in clinical studies [14,16].

Compared to monoclonal antibodies, gained by hybridoma technology according to Kohler and Milstein, 1975, scFvs gained by phage-display offer several advantages. This is especially true for the generation of antibodies which are difficult to generate because the antigen is either nonimmunogenic or toxic. The small size of antibody fragments permits this type of molecules easier tissue and blood brain barrier penetration. Moreover, the low cost and ease of production is often an argument for screening of specific binders by phage display. However, besides high hopes and enthusiasm, half life, improper folding, aggregation of the peptide and a missing modification are often setbacks during the development of a therapeutic antibody.

Progress in recombinant DNA technology and antibody engineering allows researchers today to express antibodies not only in *E. coli* but also in diverse mammalian cells, yeast and plant.

Each expression system has its advantages and disadvantages and requires special vectors [21]. Depending on the scFv expression system, the ability to fold and secrete varies.

Continued effort has been made to express scFvs in different formats. Sometimes a constant (C) domain or fragment crystillizable region (Fc) of an IgG is added to the variable (V) regions of the scFv to generate either a Fab fragment [fragment, antigen-binding] or scFv-Fc-fusion. Other formats include disulfide-bond stabilized scFv (ds-scFv) as well as di- and multimeric antibody formats.

A trend seems to be emerging towards the use of human or humanized antibody formats (scFv-Fc-fusion) due to their compatibility with the human immune system. In addition, their application reduces the risk of serum sickness and anaphylactic shock.

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Moreover, the fragments or fusions can be genetically modified to enhance desirable pharmacokinetic properties like multivalency, slower blood clearance and higher affinity.

Outlook to the future

To my personal opinion antibody fragments like scFvs are going to be the next important class of protein-based therapeutics after monoclonal antibodies. Today, their special value for medical treatment is demonstrated by the high number of phage display-derived antibodies in clinical investigation. In addition, expiration of technology patents will open the market for this class of therapeutics.

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