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\textsubscript{H}) and variable light (V\textsubscript{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 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 crystallizable 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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