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Review

Therapeutic antibody engineering by high efficiency cell screening

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ABSTRACT

In recent years, several cell-based screening technologies for the isolation of antibodies with prescribed properties emerged. They rely on the multi-copy display of antibodies or antibody fragments on a cell surface in functional form followed by high through put screening and isolation of cell clones that carry an antibody variant with the desired affinity, specificity, and stability. Particularly yeast surface display in combination with high-throughput fluorescence-activated cell sorting has proven successful in the last fifteen years as a very powerful technology that has some advantages over classical generation of monoclonals using the hybridoma technology or bacteriophage-based antibody display and screening. Cell-based screening harbours the benefit of single-cell online and real-time analysis and characterisation of individual library candidates. Moreover, when using eukaryotic expression hosts, intrinsic quality control machineries for proper protein folding and stability exist that allow for co-selection of high-level expression and stability simultaneously to the binding functionality. Recently, promising technologies emerged that directly rely on antibody display on higher eukaryotic cell lines using lentiviral transfection or direct screening on B-cells. The combination of immunisation, B-cell screening and next generation sequencing may open new avenues for the isolation of therapeutic antibodies with prescribed physicochemical and functional characteristics.

1. Introduction

Monoclonal antibodies (mAbs) are among the most successful and frequently used biotherapeutics. As of March 2012, 34 mAbs or antibody formats were approved in either Europe or the US, with a total number of 28 currently marketed molecules [1,2]. About 350 mAb candidates are currently in clinical studies, among them 28 single mAbs and one mAb mixture that are in Phase III corroborating the therapeutic potential of this continuously growing class of therapeutic agents [3]. For antibody discovery, currently two approaches are predominant: animal immunisation and surface display methodologies. Immunisation of wild type or transgenic animals is an effective method for generating antibodies, but immune tolerance may hinder the generation of neutralizing antibodies when antigens are well conserved or toxic to the respective species [4]. Display technologies such as phage display or microbial cell surface are based on the in vitro selection from naïve or immune-libraries [5] and overcome limitations of immune tolerance. Control over selection and screening conditions allow for selecting specificities towards defined antigen conformations or epitopes and in some cases epitopes of the target protein can be addressed that are not detected by antibodies that have been obtained by conventional immunisation [6].

Although the vast majority of approved mAbs are chimeric or humanised antibodies, today more and more entities from phage displayed antibody libraries and from transgenic rodents with functional human antibody repertoires enter clinical development [7,8]. Non-canonical antibodies, e.g. multispecifics [9,10], otherwise engineered or artificial antibodies [11–13] and antibody fragments or domains [14–19] that have been optimized e.g. for enhanced tissue penetration, pharmacokinetics, or effector function constitute about half of the anticancer mAbs in the pipeline [20]. Those antibody derivatives are usually selected from (combinatorial) antibody-libraries via high-throughput identification using various display technologies.

The key feature that enables the identification and isolation of favoured candidates from antibody libraries is the linkage between the antibody variant to the coding genetic information [21]. In general, there are five main display technologies [22] referred to as

Abbreviations: Fab, antigen-binding fragment of an immunoglobulin; FACS, fluorescence-activated cell sorting; FC, flow cytometry; Ig, immunoglobulin; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cells; scFv, single-chain Fv fragment; VL, light chain of an immunoglobulin; VH, heavy chain of an immunoglobulin

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phage display, cell display, ribosomal display, mRNA display and DNA display [23–25], with phage display as the most established one [21]. The technology of phage display has been extensively and elegantly described elsewhere [26–29]. This review exclusively focusses on cellular high-efficiency screening technologies of canonical and non-canonical antibody molecules with a special emphasis on eukaryotic high-efficiency screening. Eukaryotic cell display technologies do not only harbour the benefit of online and real-time analysis and characterisation of library candidates, but also have quality control machineries for proper protein folding which every library member has to pass through before being displayed on the surface of the cell. [7]. In *Escherichia coli*, efficient secretion of functional antibody fragments into the bacterial periplasm and also their display on phage requires co-expression of chaperones and isomerases indicating that the natural bacterial secretion machinery has limited capability in generating functional antibody fragments [30–32]. Using higher or lower eukaryotes for antibody display, library candidates are believed to be displayed most likely well folded and can be expected to have better biophysical properties compared to binders obtained from phage display that relies on bacterial protein production. Further potential advantages include the ability to co-select for high-level expression and stability of glycosylated IgGs simultaneously to the binding functionality. In the past, eukaryotic cell display has been hampered by small library sizes, making direct isolation of high-affinity binders from naïve libraries improbable. This article reviews recent improvements and approaches to enlarge or focus libraries as well as harnessing in vivo affinity maturation strategies for the fast and effective selection of functional therapeutic monoclonal antibodies.

2. Antibody formats used for surface display

This section briefly describes commonly used antibody formats used for surface display without raising a claim of giving a comprehensive overview. A more detailed review on antibody formats can be found elsewhere [33,34]. Fig. 1 shows commonly displayed antibody variants. IgG antibodies are dimers of heterodimers comprising two heavy chains and two light chains. They are bivalent and monospecific. Each antigen binding site is composed of the variable domain of the light chain and the variable domain of the heavy chain. The Fc-part usually mediates effector function e.g. antibody dependent cell cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). Furthermore bispecificity can be introduced into the bivalent IgG structure in different ways by desymmetrisation of the antibody Fc part via engineering residues at the Fc interphase according to a knobs-into-holes principle [35–37]. Bispecificity can also be achieved using various other formats that rely on the combination of antibody domains with different specificities [34,38]. Display of full length IgG is due to molecule complexity difficult to achieve in prokaryotic systems and mainly restricted to eukaryotic cell surface display. Other commonly used antibody formats consist of antibody fragments and are accessible by all display methods including phage and ribosomal display. These include Fab fragments and single-chain variable fragments (scFv) (Fig. 1B and C). Fab fragments are heterodimers of the VH and CH1 domain and the VL and CL domain. ScFvs only consist of the VH and the VL domain connected by a small peptide linker. More recently, single domain antibodies (dAb) have been generated based on single human antibody domains of the VH or VL domain that have been engineered for high specificity, affinity and solubility [39–41]. Fcabs (Fc antigen binding) originate from immunoglobulin Fc-fragments and represent another recently engineered class of alternative antibody formats of small size which in contrast to other alternative scaffolds can elicit immune effector functions [13]. Camelids and sharks express antibodies that are composed only of heavy chains. Hence, antigen binding is mediated by one single domain referred to as VHHs in camelids and vNARs in sharks. Those antibody fragments are naturally highly stable and soluble and it has been shown that those molecules can be utilised to generate high-affinity binders [15,16,19,42,43].

3. Flow cytometry and high-content microscopy

Cell-based screening and selection is essentially conducted by application of high-throughput flow cytometry or high-content microscopy [44]. The most common method used is flow cytometry (FC) or fluorescence-activated cell sorting (FACS) that allows for hundreds of thousands of cells per minute to be analysed according to their size, granularity and fluorescence properties. FC can be applied for multiplexing, with the simplest example being the combined analysis of antibody binding parallel to cell viability assessment. But also more elaborate approaches of monitoring up to 19 different parameters in a single sample by using various cell-surface markers and intracellular cytokine tags have been achieved [45], opening the way for distinct and diversified selection strategies. Both selection and screening of monoclonal antibodies presented on the cell surface can be performed by iteratively enriching cells for both expression level (by display strength) and affinity (via antigen reactivity). However, time-dependent studies on individual cells during screening are not commonly performed and only possible in low throughput after sorting into single colonies. Hence, flow cytometry is neither designed for dynamic analysis of single cells nor observations of spatial localisation of antibodies and target proteins within a cell after endocytotic uptake.

Microscopy, another frequently used method for single-cell analysis is well suited for localisation- and time-dependent

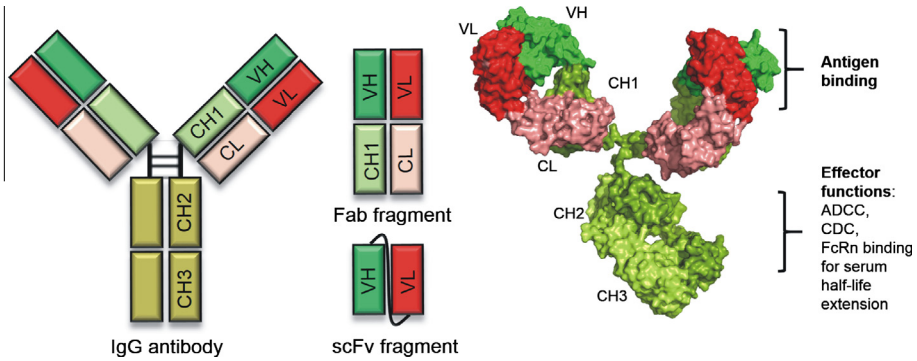

Fig. 1. Schematic presentation of commonly used antibody formats for cellular display systems. Left: antibody molecule of the IgG isotype. Middle: Fab fragment and single-chain variable fragment (scFv), where the variable domain of the heavy chain (VH) is fused to the variable domain of the light chain (VL) by a small peptide linker. Right: space filling model of an antibody (taken von PDB 1IGT). CL: constant domain of the light chain. CH1–CH3: constant domain 1, 2 or 3 of the heavy chain, respectively.