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Targeting epidermal growth factor receptor in tumors: From conventional monoclonal antibodies via heavy chain-only antibodies to nanobodies

Isil Altintas [a], Robbert Jan Kok [a], Raymond M. Schiffelers [a,b,*]

[a] Dept. of Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands
[b] Laboratory Clinical Chemistry and Haematology, UMC Utrecht, Utrecht, The Netherlands

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ABSTRACT

The discovery of naturally occurring heavy chain only antibodies and their further development into small recombinant 'nanobodies' offers attractive applications in drug targeting. Here, we describe the properties of nanobodies that have been developed to target the epidermal growth factor receptor (EGFR) and contrast these to the characteristics of heavy chain only antibodies and conventional antibodies. EGFR is overexpressed in many tumors and is an attractive target for tumor-directed drug targeting.

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1. Passive and active drug targeting to tumors

Tumor-targeted drug delivery takes advantage of the differences between malignant and healthy tissues. The environment of the tumor changes as the tumor grows. Due to increased metabolism and growth, the oxygen supply becomes insufficient and the metabolic breakdown of glucose, forming lactate, decreases the interstitial pH of the tumor tissue. Together, hypoxia and glucose depletion trigger the formation of new blood vessels (angiogenesis) which is crucial for the proliferation, migration and maintenance of the tumor (Dang and Semenza, 1999). Angiogenesis is tightly controlled in healthy tissues. The absence of tightly regulated pathways for the formation of new blood vessels in tumors leads to a heterogeneous vasculature that is tortuous and disorganized. In solid tumors, the gaps between the endothelial junctions are in the range of 300 nm to 1 μm, depending on the tumor type (Rapoport, 2007). This enhanced permeability can be exploited for drug delivery. The phenomenon is known as the enhanced permeability and retention (EPR) effect. Many colloidal drug delivery systems take advantage of this effect by combining a small size (that allows passage through the gaps) with a long circulation time (that increases the statistical probability of extravasation). The colloidal nature of the drug delivery system combines well with the convective transport and limits lymphatic drainage. Active targeting involves the 'directing' of the delivery systems to receptors or target molecules via ligands (Davis, 1997; Marcucci and Lefoulon, 2004). Conventional monoclonal antibodies (mAbs) arguably have been the most popular molecules used as a ligand to target particulate and macromolecular drug carriers.

2. Conventional monoclonal antibodies

Conventional human antibodies are 150 kDa glycoproteins produced by plasma B-cells (Fig. 1a). They are composed of two light (L) and two heavy (H) chains. Both light and heavy chains have a variable and a constant region, at the N and C-terminal domains, respectively. The variable N-terminal domain of both chains determines the antigen specificity and constitutes the complementarity determining region (CDR). The C-terminal part is constant. The terminal C2–C3 constant region of the heavy chain is called the Fc fragment of the antibody. Certain immune cells such as monocytes, macrophages and some lymphocytes have receptors that bind to the Fc fragment (Chames et al., 2009; Huang et al., 2008).

Abbreviations: ADCC, antibody dependent cellular cytotoxicity; Au NP, gold nanoparticles; BSH, sodium borocaptate; CDC, complement dependent cytotoxicity; CDR, complementarity determining region; DDS, drug delivery system; DOX, doxorubicin; EGFR, epidermal growth factor receptor; EPR, enhanced permeability and retention; HCAb, heavy chain antibodies; HSA NP, human serum albumin nanoparticles; ICP-AES, inductively coupled plasma atomic emission spectrometry; IHC, immunohistochemistry; mAb, monoclonal antibodies; mPEG-*b*-, (ω-methoxy-poly(ethylene glycol)-*b*-poly[*N*-(2-hydroxypropyl); p(HPMAm-Lacn), methacrylamide-lactate] (mPEG-*b*-*p*(HPMAm-Lacn)); NP, nanoparticle; P(MDS-co-CES), (poly{(Nmethyldietheneamine sebacate)-co-[(cholesteryl oxocarbonylamido ethyl) methyl bis(ethylene) ammonium bromide] sebacate}; PG, poly(L-glutamic acid); pIgG, porcine IgG; PLA, poly (DL-lactic acid); PLGA, poly (lactide-*co*-glycolide); PTX, paclitaxel; Tar. Lig., targeting ligand; TKI, tyrosine kinase inhibitors; VH, variable heavy chain; VL, variable light chain.

* Corresponding author at: Dept. of Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands.
E-mail address: R.Schiffelers@umcutrecht.nl (R.M. Schiffelers).

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Monoclonal antibodies (mAbs) can be coupled to the surface of colloidal drug delivery systems. They have also been used to form molecular conjugates with drugs and to target receptors or soluble molecules to block pathways involved in tumor progression (Chames et al., 2009; Lee et al., 2009). mAbs as drug targeting ligands can either be internalizing or non-internalizing. Usually the internalizing type is preferred as it facilitates uptake of the drug loaded carrier system and hence increases intracellular drug concentrations. However, non-internalizing mAbs may release the drug into the extracellular space which subsequently can be taken up by cells via conventional routes, such as diffusion or active transport into tumor cells. To circumvent the exposure of Fc-parts, mAb fragments have been used instead of the intact mAb. (Dillman, 1989; Marcucci and Lefoulon, 2004).

Antibodies have been recombinantly rearranged in such a way to increase the avidity upon antigen binding and having a smaller size compared to the intact antibody. For all these fragments it is important to note that the antigen binding domain is composed of two separate chains that need to be in the right spatial orientation for antigen binding.

The Fv fragment, the variable fragments of one light (VL) and one heavy chain (VH), is the smallest antigen binding domain of a mAb. VL and VH are encoded by two different genes and their expression in a prokaryotic system is rather complex. In recombinant approaches, VL and VH are therefore commonly attached via a flexible polypeptide linker. This new recombinant antibody is called the single chain Fv (scFv; see Fig. 2a). However, the monovalent nature of scFv fragments decreases their avidity to ligands. Therefore, two scFv molecules can be linked to each other via a short peptide linker forming the so called diabodies (Fig. 2b) (Hudson, 1998). Diabodies can exert their effect in several ways. One approach is to crosslink tumor cells with immune cells, i.e. cytotoxic T-cells or macrophages. A second approach is to target conjugated drugs or drug carriers binding to tumor specific receptors (Sanz et al., 2005). Minibodies, finally, have an additional CH3 domain in addition to the diabody structure (Fig. 2c). The CH3 domain provides an advantage in increasing the serum half life and the valence of these molecules. The bispecific variable domains target the tumor specific antigens whereas the CH3 domain interacts with the Fc receptors on certain immune cells, thereby triggering antibody dependent cellular cytotoxicity (Shahied et al., 2004).

3. Heavy chain antibodies

Antibodies, with a single chain antigen binding domain, composed of heavy chains only (HCAb), were discovered in 1992 (see Table 1). The discovery was made by a group of students trying to purify the blood serum proteins of dromedaries. In addition to the conventional antibodies present in the serum, they came across an unusual antibody structure, which was devoid of light chains (Muyldermans, 2001; Wolfson, 2006). Later, the presence of these unusual antibodies was further investigated in other animals belonging to the *Camelidae* family. It was found that also llamas and camels had these antibodies without light chains (Hamers-Casterman et al., 1993). The levels of HCAb differ: dromedaries have >50% while levels in llamas are 25–45% (Muyldermans and Lauwereys, 1999; van der Linden et al., 2000). Heavy chain antibodies can also be present in human serum as part of gamma heavy chain disease, but these HCAb

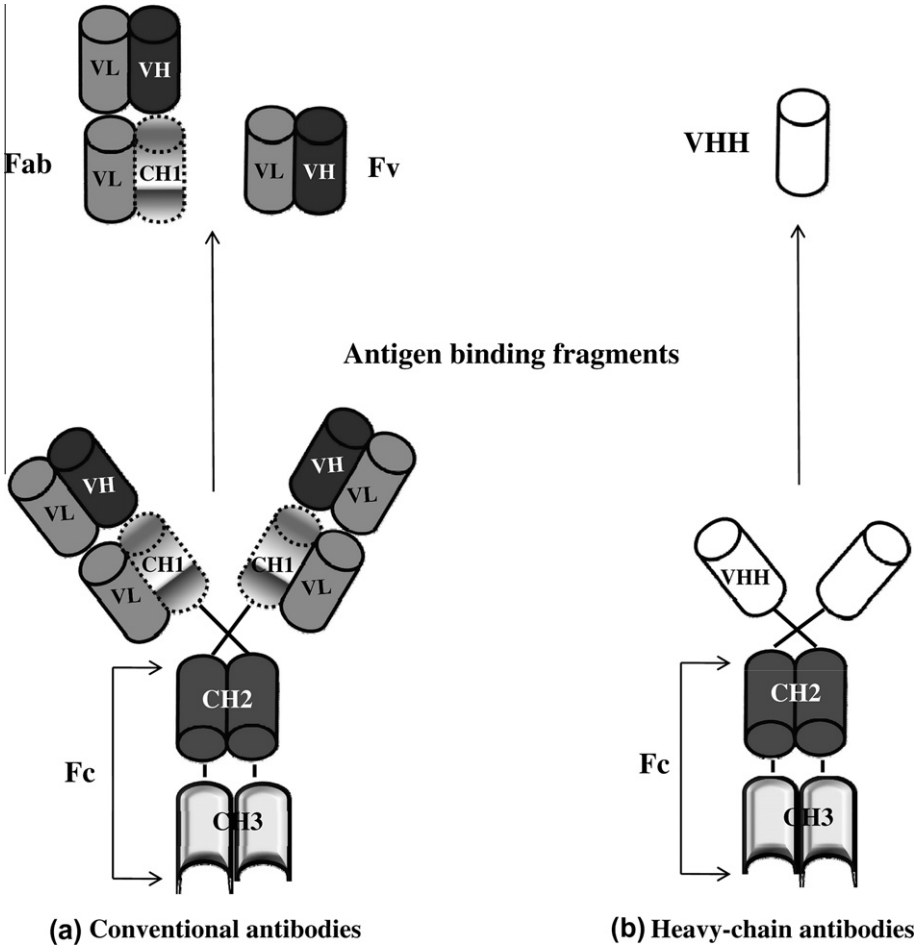

Fig. 1. The structures of (a) conventional antibodies (b) heavy-chain antibodies and their antigen binding domains.

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