Journal of Controlled Release xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review

Targeting tumors with nanobodies for cancer imaging and therapy

Sabrina Oliveira [a,b], Raimond Heukers [a], Jirawas Sornkom [a], Robbert J. Kok [c], Paul M.P. van Bergen en Henegouwen [a,*]

[a] Division of Cell Biology, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
[b] Department of Pathology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands
[c] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 7 June 2013
Accepted 22 August 2013
Available online xxxx

Keywords:
Nanobodies
VHHs
Anti-cancer therapy
Effector domain
Nanoparticle
Targeted drug delivery system
Molecular imaging

ABSTRACT

The use of monoclonal antibodies has revolutionized both cancer therapy and cancer imaging. Antibodies have been used to directly inhibit tumor cell proliferation or to target drugs to tumors. Also in molecular imaging, monoclonal antibodies have found their way to the clinic. Nevertheless, distribution within tumors is hampered by their size, leading to insufficient efficacy of cancer treatment and irregular imaging. An attractive alternative for monoclonal antibodies are nanobodies or VHHs. These are the variable domain of heavy-chain antibodies from animals from the *Camelidae* family that were first discovered in 1993. Stimulated by the ease of nanobody selection, production, and low immunogenicity potential, a number of nanobodies specific to different disease-related targets have been developed. For cancer therapy, nanobodies have been employed as antagonistic drugs, and more recently, as targeting moieties of effector-domaINS and of drug delivery systems. In parallel, nanobodies have also been employed for molecular imaging with modalities such as nuclear and optical imaging. In this review, we discuss recent developments in the application of nanobodies as targeting moieties in cancer therapy and cancer imaging. With such a wide range of successful applications, nanobodies have become much more than simple antagonists.

Contents

1. Introduction . . . 0
2. Nanobodies: Structure and characteristics . . . 0
3. Nanobody applications in cancer immunotherapy . . . 0
3.1. Nanobody antagonists as cancer therapeutic agents . . . 0
3.1.1. Hepatocyte growth factor (HGF, hepapoietin A; scatter factor) . . . 0
3.1.2. Vascular endothelial growth factor receptor-2 (VEGFR2) . . . 0
3.1.3. Epidermal growth factor receptor (EGFR or ErbB1) . . . 0
3.2. Nanobodies as targeting moieties of effector domains . . . 0
3.3. Nanobodies as targeting moieties on drug delivery systems . . . 0
3.3.1. Nanobody-liposomes . . . 0
3.3.2. Nanobody-micelles . . . 0
3.3.3. Nanobody-albumin nanoparticles . . . 0
4. Nanobody for molecular imaging of cancer . . . 0
4.1. Nanobodies in nuclear imaging . . . 0
4.2. Nanobody-targeted ultrasound . . . 0
4.3. Nanobodies in optical imaging . . . 0
5. Perspectives on the applications of nanobodies . . . 0
References . . . 0

1. Introduction

The use of monoclonal antibodies (mAbs) for cancer therapy has been established extensively for over 15 years, with a number of impressive

* Corresponding author. Tel.: +31 30 253 3349.
E-mail address: p.vanbergen@uu.nl (P.M.P. van Bergen en Henegouwen).

0168-3659/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jconrel.2013.08.298

successes both for hematological malignancies and solid tumors treatments [1]. So far, there are 23 mAbs approved by the US Food and Drug Administration (FDA) on the market. Among these, six products are specific for cancer, namely, rituximab (anti-CD20), trastuzumab (directed to HER2), bevacizumab (anti-VEGF), alemtuzumab (anti-CD52), cetuximab, panitumumab, and matuzumab (all targeted to EGFR) [2,3]. These mAbs interfere with the functioning of their target proteins in cancer, either by binding to transmembrane receptors or – in the case of bevacizumab – to the soluble ligand, thereby inhibiting tumor cell proliferation or tumor angiogenesis. As they all possess an intact fragment crystallizable domain, i.e. Fc domain, they can interact with human complement or effector cells of the immune system, which also contributes to their therapeutic effect. mAbs have also found their way to the clinic for molecular imaging. In this case, mAbs are used to target radioactive or fluorescent tracers to the tumor, for either PET/SPECT or optical imaging, respectively [4–6]. Lastly, mAbs are used in a variety of targeted nanomedicines, aiming at tumor cell directed delivery of a cytotoxic payload [7]. It is however fair to state that the application of mAbs in both cancer therapy and imaging needs further improvements. mAbs have a molecular weight of ~150 kDa and dimensions of 14.2 nm × 8.5 nm ×3.8 nm [8], which together with the 'binding site barrier' [9] limit mAb distribution and penetration into the tumor. mAbs typically have several days of half-life in the bloodstream, which for molecular imaging results in high background levels. Moreover, an important concern of mAbs application is their potential to induce immunogenic responses. To avoid unwanted immune responses in patients, mAbs are either completely humanized or produced as a chimeric protein. Altogether, these aspects have urged pharmaceutical companies and scientists to find new antibody formats that provide the same binding specificity of mAbs, but with some of the desired improvements.

As many of the mentioned drawbacks of mAbs are related to their size, large efforts have been made towards the development of smaller antibody formats [10,11]. Naturally derived or synthetic antigen-binding fragment (Fab; ~50 kDa), variable fragment (Fv; ~15 kDa) and single-chain variable fragment (scFv; ~30 kDa) were vastly tested and engineered to overcome the restrictions of the full-length mAbs (Fig. 1) [10,12]. Nevertheless, their average activities are still suboptimal due to lower affinities and limited stability, which is especially the case of scFv [13]. Apart from those mentioned above, the minibody – an engineered antibody fragment made by genetically fusing scFv binding domain to human CH3 – was introduced as another candidate for cancer immunotherapy [14]. Furthermore, synthetic molecules or scaffold proteins, such as affibodies and DARPins (designed ankyrin repeat proteins) have been developed, with important successes [15–17]. However, no report has addressed their potential to induce immunological responses and their added value, compared to the other platforms, still needs to be determined.

By serendipity, a special type of antibody was discovered in animals from the *Camelidae* family by Hamers-Casterman and co-workers in 1993 [18]. These so-called heavy-chain antibodies (HcAbs, ~95 kDa) are fully functional and, despite the absence of light chain and of the first constant domain (CH1), they bind their antigens with similar affinities to those of conventional antibodies [19]. Apart from *Camelidae*, some primitive fish species were also found to produce different types of HcAbs, such as nurse shark and ratfish [20,21]. Interestingly, the variable domain alone of HcAbs (i.e. VHH) was proven to have sufficient antigen binding properties and, as such, can be considered as the smallest naturally derived antigen-binding fragment with the approximate molecular weight of 15 kDa [22,23]. The term 'nanobodies' was employed with respect to their size in nanometer range by the Belgian company Ablynx®, and particularly refers to the VHH from camelid species [23–25]. Another term used for nanobodies is the single domain antibody (sdAb) [26].

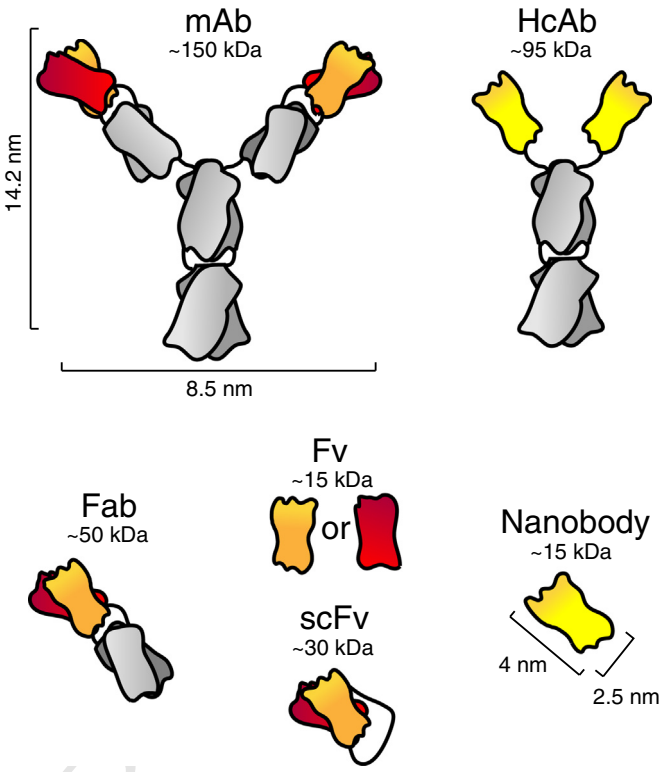

Fig. 1. Antibodies and their fragments. Schematic representation and corresponding molecular weight of (left) a monoclonal antibody, mAb, and its fragments, i.e., Fab', Fv, scFv; and of (right) a heavy chain only antibody, HcAb, together with its antigen-binding fragment, i.e. nanobody or VHH.

2. Nanobodies: Structure and characteristics

In 1994, the first detailed sequence of nanobody encoding genes was published by Muyldermans and co-workers, providing more molecular insights regarding their interaction and binding interface [22]. The nanobody sequences were shown to have a high degree of identity with the human type 3 VH domain (VH3), which most likely accounts for the low immunological potential of nanobodies, as demonstrated in mice [27]. In addition, humanization of nanobodies has been performed before these were translated into the clinic (Ablynx) [25,28,29], further minimizing their immunological potential. In this line, Vincke and colleagues have presented the humanization of dromedary-derived nanobodies resulting in a universal humanized nanobody scaffold [30]. A number of distinctive amino acid substitutions are specifically found in framework 2. In conventional antibodies, this region serves as a part of the hydrophobic VL interface and, consequently, substitutions that have occurred in HcAbs are thought to be the main reason for the high hydrophilicity, stability and higher solubility of VHHs as compared to conventional VH domains, including scFvs. Another interesting difference between VHH and human VH domain is the length of CDRs, which contributes to an increase of the antigen-interacting surface [25,31]. A longer CDR3 in nanobodies allows it to form a fingerlike structure able to extend into cavities on target proteins, which causes nanobodies to bind to unique epitopes [32,33]. In contrast, the binding interfaces of Fabs' and other mAbs' derived fragments are more flat and less flexible, limiting the interactions of mAbs and antibody fragments solely to the surface of antigens [34]. Recently, we have determined the crystal structure of an anti-EGFR nanobody (7D12) in complex with the EGFR ectodomain [33]. This nanobody binds directly to domain III thereby sterically blocking EGF-binding. Interestingly, the 7D12 paratope that is binding to EGFR consists of CDR1 and 3, and the CDR2 makes no contact with EGFR (Fig. 2). Moreover, nanobodies have a high refolding capacity even after being exposed to

Please cite this article as: S. Oliveira, et al., Targeting tumors with nanobodies for cancer imaging and therapy, J. Control. Release (2013), http://dx.doi.org/10.1016/j.jconrel.2013.08.298