Article

A Fibrin-Specific Monoclonal Antibody from a Designed Phage Display Library Inhibits Clot Formation and Localizes to Tumors *In Vivo*

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Abstract

Fibrin formation from fibrinogen is a rare process in the healthy organism but is a pathological feature of thrombotic events, cancer and a wide range of inflammatory conditions. We have designed and constructed an antibody phage display library (containing 13 billion clones) for the selective recognition of the N-terminal peptide of fibrin alpha chain. The key structural feature for selective fibrin binding was a K94E mutation in the VH domain. From this library, an antibody was isolated (termed AP2), which recognizes the five N-terminal amino acids of fibrin with high affinity (K_d = 44 nM), but does not bind to fibrinogen. The AP2 antibody could be expressed in various formats (scFv, small immune protein and IgG) and inhibited fibrin clot formation in a concentration-dependent manner. Moreover, the AP2 antibody stained the fibrin-rich provisional stroma in solid tumors but did not exhibit any detectable staining toward normal tissues. Using a radioiodinated antibody preparation and quantitative biodistribution studies in tumor-bearing mice, AP2 was shown to selectively localize to fibrin-rich F9 murine teratocarcinomas, but not to SKRC-52 human kidney cancer xenografts. Collectively, the experiments indicate that the AP2 antibody recognizes fibrin *in vitro* and *in vivo*. The antibody may facilitate the development of fibrin-specific therapeutic agents.

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Introduction

Monoclonal antibodies represent the largest and fastest growing sector of Pharmaceutical Biotechnology [1–3]. There is an emerging trend, moving from intact immunoglobulins toward “armed” antibody products, in which the antibody moiety serves as a delivery vehicle for suitable payloads for imaging or for therapy applications (e.g., drugs with cleavable linkers, cytokines and radionuclides) [4,5]. Indeed, there is a considerable biomedical interest in the identification of target antigens, which are not expressed in healthy organs, while being abundant under a variety of different pathological conditions [4,6–8].

In principle, fibrin could be considered as a target for pharmacodelivery applications. When blood clots, the soluble plasma glycoprotein fibrinogen is cleaved by thrombin at the level of its alpha and beta chains, yielding a fibrin monomer, which spontaneously polymerizes to form a gel [9]. In the healthy organism, fibrin is virtually undetectable. By contrast, fibrin is abundantly produced under a series of pathological conditions, including thrombus formation [10,11], cancer [12–15] and rheumatoid arthritis [10,16].

Fibrin differs from fibrinogen by the cleavage and removal of two short fibrinopeptides (16 and 14 amino acids in length for the alpha and the beta chain, respectively), which result in new positively charged termini for the two chains (Fig. 1a). A fibrin-specific antibody needs to display no detectable binding affinity toward fibrinogen in order to be useful for biomedical applications, which is present at 1.5–5.5 mg/ml concentrations in the blood plasma. Thus, the generation of selective high-affinity monoclonal antibodies represents a formidable chemical challenge.

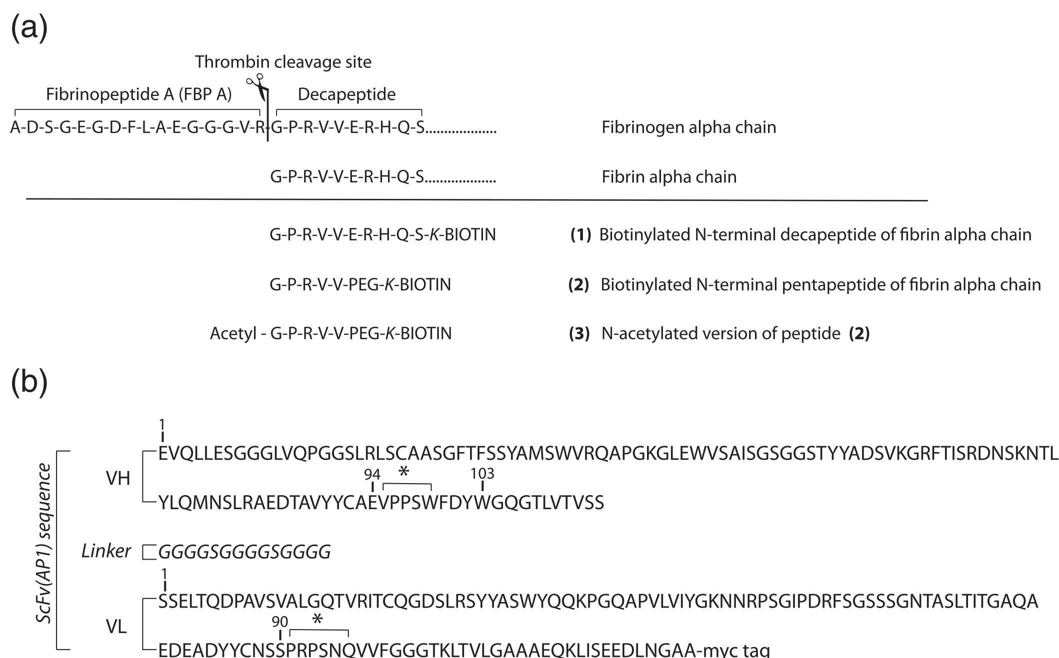

Fig. 1. Fibrin structure, fibrin-derived peptides and fibrin-specific antibodies. (a) The primary sequence of fibrinogen alpha chain is illustrated, indicating the thrombin cleavage site and fibrin-derived peptides, used for antibody selection and testing. (1) Biotinylated decapeptide used for phage display selection (biotin modification: lysine side chain, C-terminal); (2) shorter version of (1) with mini-PEG (*p*oly*e*thylene *g*lycol) spacer [2-(2-(2-aminoethoxy)ethoxy)acetic acid]; (3) acetylated version of peptide (2). (b) Amino acid sequence of the AP1 antibody in scFv format (VH, linker and VL domain). The scFv(AP1) antibody fragment carries a specific mutation at position 94 of VH, resulting from a mutation in the original ETH-2-Gold library. The asterisk indicates residues, which had been combinatorially mutated in that library [17].

Haber and collaborators have pioneered the generation of polyclonal and monoclonal antibodies against fibrin [9,18,19]. One murine antibody (59D8), specific to the beta chain of fibrin, was used for *in vivo* targeting application. A ^{111}In-labeled Fab fragment of 59D8 was studied *in vitro* and in animal models (rabbit and dog), in order to evaluate its potential for the imaging of thrombi and emboli [20]. The antibody was successfully fused to tissue plasminogen activator and to hirudin, with the aim to achieve thrombolytic and anti-thrombotic activities *in vivo*, which were superior to the ones of the non-targeted payloads [21,22].

For biomedical applications, human antibodies should be used, since murine immunoglobulins are immunogenic in patients [3]. Combinatorial libraries and phage technology have revolutionized the way human antibodies are isolated [23,24]. Antibody phage technology allows the panning of very large repertoires of recombinant antibody molecules (typically in scFv or Fab format) onto antigen immobilized on a solid support, followed by the selective recovery and amplification of preferential binders. Selection experiments can be performed in the presence of competitors and are thus ideally suited to drive the enrichment of binders toward antigenic structures of interest [25]. To our knowledge, only one report has been published so far on the selection of antibody phage display libraries against fibrin, yielding one scFv fragment with a weak, but specific, preferential binding to fibrin, compared to fibrinogen (ELISA selectivity: 4:1 at 10 μg/well; 6:1 at 100 μg/well) [26].

In this article, we describe the selection results from large combinatorial phage display libraries against the N-terminal peptide of fibrin, which yielded a small number of specific antibody clones. The sequence of the best binder isolated in these selections (termed AP1) was surprising, as it contained a mutation K94E at the beginning of the CDR3 [complementarity-determining region (CDR)] loop of the VH domain. Having confirmed that the mutation was crucially important for the specific fibrin recognition, we cloned a novel designed antibody phage library (containing 13 billion antibody clones), which incorporated the K94E substitution, together with sequence diversity in the CDR3 loops of heavy and light chains. From this library, a high-affinity anti-fibrin antibody was selected (termed AP2), which inhibited fibrin clot formation *in vitro* and that stained fibrin in tumor sections. The AP2 antibody was also shown to preferentially accumulate to fibrin-rich F9 murine teratocarcinomas *in vivo*, following intravenous administration.

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