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Review

Vascular-homing peptides for targeted drug delivery and molecular imaging: Meeting the clinical challenges

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ABSTRACT

The vasculature of each organ expresses distinct molecular signatures critically influenced by the pathological status. The heterogeneous profile of the vascular beds has been successfully unveiled by the in vivo phage display, a high-throughput tool for mapping normal, diseased, and tumor vasculature. Specific challenges of this growing field are targeted therapies against cancer and cardiovascular diseases, as well as novel bioimaging diagnostic tools. Tumor vasculature-homing peptides have been extensively evaluated in several preclinical and clinical studies both as targeted-therapy and diagnosis. To date, results from several Phase I and II trials have been reported and many other trials are currently ongoing or recruiting patients. In this review, advances in the identification of novel peptide ligands and their corresponding receptors on tumor endothelium through the in vivo phage display technology are discussed. Emphasis is given to recent findings in the clinical setting of vascular-homing peptides selected by in vivo phage display for the treatment of advanced malignancies and their altered vascular beds.

Contents

Abbreviations: ANXA 1, annexin A1; APA, aminopeptidase A; APN, aminopeptidase N; APP, aminopeptidase P; BBB, blood–brain barrier; CT, computed tomography; CREG1, cellular repressor of E1A-stimulated genes 1; DDL4, delta like ligand 4; DLP, dynamin-1 like protein; Eph-B2, ephrin type-B2; Eph-B4, ephrin type-B4; ERBB2, epidermal growth factor tyrosine kinase receptor 2; ERK1/2, extracellular-signal-regulated kinase 1/2; FGF8b, fibroblast growth factor 8b; aFGF, acidic fibroblast growth factor; bFGFR, fibroblast growth factor receptor b; FN-ED-B, fibronectin extra-domain B; GRP78, glucose regulated protein 78; HGG, high-grade glioma; HSP90, heat-shock protein 90 kDa; IL11R, interleukin 11 receptor; MAP2, microtubule-associated protein 2; MGMT, methylated methylguanosine methyltransferase; NG2, neuron-glial antigen 2; NGR, asparagine-glycine-arginine; NSCLC, non-small cell lung cancer; OPA-1, Optic Atrophy-1; PCR, polymerase chain reaction; PDGFR, platelet derived growth factor receptor; PET, positron emission tomography; PRGD2, pegylated arginine-glycine-aspartic acid dimer; RGD, arginine-glycine-aspartic acid; SCID, severe combined immunodeficiency; SCLC, small cell lung carcinoma; SPECT, single photon emission computed tomography; TEM-5, tumor endothelia marker 5; TEM-8, tumor endothelia marker 8; TF, tissue factor; TNFα, tumor necrosis factor alpha; VCAM, vascular cell adhesion molecules; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor

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1. Introduction

The vascular feature, markedly modified under pathological conditions, is an address system that allows the specific targeting towards blood vessels. The heterogeneous expression of proteins in the vasculature has been profiled by the in vivo phage display technology, a powerful method used to identify peptides homing specifically to normal and diseased vasculature. Vascular homing peptides are optimal ligands for the targeted delivery of imaging agents or drugs for the treatment of cancer, cardiovascular diseases (restenosis, atherosclerosis, hypertension, ischemia), and neurological disorders (stroke, Alzheimer's disease) [1–3]. Importantly, they provide an efficient mean of discriminating between normal cells and tumor-associated endothelial cells, thus, controlling tumor growth independently of the cell type. Tumor vasculature, structurally and functionally different compared to the vasculature of normal tissues, is highly disorganized with vessels strikingly tortuous and leaky. Indeed, neoformed vessels are discontinuous, leaky and present a deregulated expression of a number of molecules such as integrins, endothelial cells growth factor receptors, cell surface proteoglycans, proteases, and extracellular matrix components [1] (Fig. 1). In particular, proteins functionally important for tumor angiogenesis, which represent potential targets for anti-angiogenic therapy, are vascular endothelial growth factor receptor (VEGFR), integrins, delta-like ligand 4, ephrin-B4, ephrin-B2, tumor endothelial markers 5 and 8, annexin A1, and fibronectin extra-domain B [4] (Fig. 1).

In vivo phage display has been widely used to analyze the structural and molecular diversity of normal and tumor vasculature [1,2,5]. The pioneering in vivo phage display study was aimed at discovering brain vasculature targeting peptides [6]. To date, sequences capable of targeting vasculature of normal tissue or organs, such as brain, kidney, lung, muscle, pancreas, thymus, and mammary gland have been discovered [7].

The construction and use of phage libraries include millions of polypeptides expressed within the coat proteins of filamentous bacteriophages, such as protein 3 (pIII), protein 6 (pVI) and protein 8 (pVIII) [7,8]. The phage display of peptide libraries is based on the concept that the epitopes of interest can be targeted with foreign proteins expressed on the phage. Phage particles binding to a certain epitope are isolated and transduced back into bacteria which are then grown to expand the selected phage population [7,8]. A consistent number of peptides with high specificity and affinity have been isolated from phage display libraries using affinity selection (*panning*) and used in different fields [5,9]. To date, several comprehensive reviews describing the phage display technology steps, such as phage libraries, *biopanning* strategies, and animal models are available [1,7,10,11]. Here, we review advances on new candidate peptides identified by in vivo screening phage display libraries and, looking towards clinical challenges, provide an update of current clinical trials evaluating novel targeted drugs and imaging agents.

2. Principles of the in vivo phage display technology

The phage display technology is based on the exposure of combinatorial peptide libraries on the surface of recombinant phages [8]. Two are the main types of phage systems; the phage vector and phagemid vector. Filamentous phage f1, fd and M13 (Ff phages) are the main tools in the phage display as they are very stable under extreme pH and temperature conditions and in the presence of DNase or proteolytic enzymes [8]. The engineered expression of random peptide libraries on the coat proteins of filamentous bacteriophage consists in the expression of a unique peptide by each phage obtained by cloning a segment of peptide-coding DNA into surface protein genes (pIII and pVIII). Interestingly, the lambda phage engineered to display multiple copies of peptides or large protein domains offers the possibility of dual display of large proteins (antibody fragment and a reported/effector moiety) on the capsid using both the head- and the tail-based display platforms. Such bifunctional phage nanoparticles can be particularly useful for diagnostic and therapeutic delivery [12].

The basic components of the phagemid, filamentous-phage-derived vectors containing the replication origin of a plasmid, include the replication origin of a plasmid, the restriction enzyme recognition sites, the gene of a phage coat protein, the intergenic region, the selective marker, the promoter, the DNA segment encoding a signal peptide, and a molecular tag which facilitates screening of phagemid-based library [13]. Major advantages of phagemid vectors are represented by the small genome that can accommodate a larger foreign DNA fragment, high transformation efficiency, stability under multiple propagations, variety

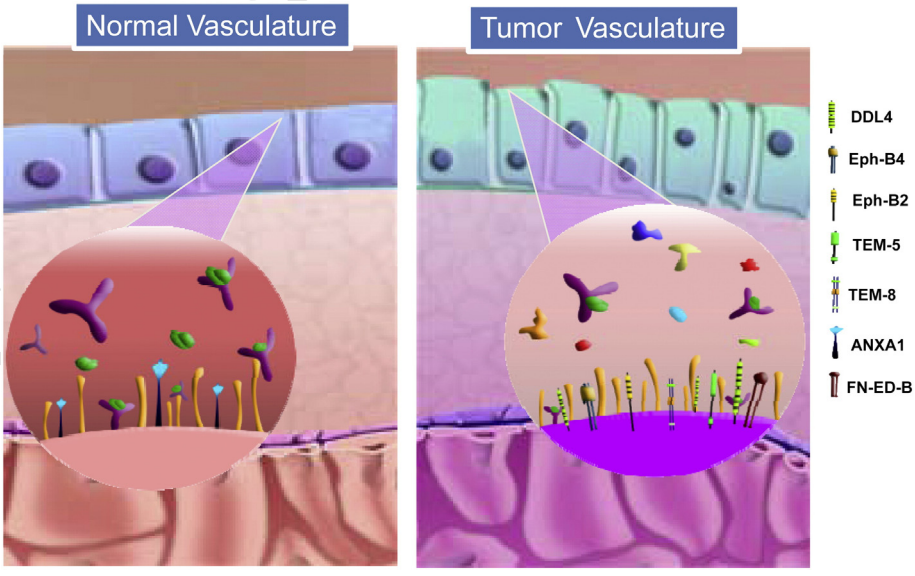

Fig. 1. *Targeting endothelial cells as anticancer strategy.* The molecular diversity of the luminal endothelial cell surface provides the basis for developing targeted molecules using in vivo phage display through which it is possible to decipher the molecular signature of (A) normal vasculature and (B) tumor vasculature. Tumor vessels, structurally and functionally different from normal vasculature, are highly disorganized, strikingly tortuous, and with a leaky architecture. They express molecules that are not present in normal blood vessels, such as delta like ligand 4 (DDL4), ephrin type-B4 (Eph-B4), ephrin type-B2 (Eph-B2), tumor endothelia marker 5 (TEM-5), tumor endothelia marker 8 (TEM-8), annexin A1 (ANXA 1), and fibronectin extra-domain B (FN-ED-B).

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