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Biomedical aspects of targeted delivery of drugs to pulmonary endothelium

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Drug targeting to selected subcellular compartments of the pulmonary endothelium may optimise treatment of many diseases. This paper describes endothelial determinants that are potentially useful for such targeting, including endothelial ectopeptidases, cell adhesion molecules and novel candidates identified by high-throughput methods, as well as the means to achieve optimal subcellular targeting of drugs in the endothelium that have been explored in cell culture and animal studies. Criteria for determining the applicability for targeting include accessibility, specificity, safety and subcellular precision. The effects of endothelial delivery of therapeutic agents, including the effects mediated by the intervention in the function of the target determinants, must be characterised in the context of given pathological conditions.

Keywords: ACE, drug delivery, endothelium, ICAM-1, nanocarriers, PECAM-1, vascular immunotargeting

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1. Introduction

The primary medical goal of advanced drug delivery systems is to improve therapeutic interventions by making them more effective and safe. In the context of drug action, effective means mechanistically specific, precisely localised, adequately powerful and optimally timed, whereas safe means inflicting minimal adverse side effects, both local and systemic. Economic concerns, such as the conservation of drugs by reducing doses, are somewhat less of an incentive. Usually, the more advanced drugs are more expensive. Nevertheless, advancing relevant technologies, obtaining novel information on features of targets (e.g., cells) of interest under normal and pathological conditions, as well as using targeting systems for modelling and studying pathological processes, represent additional benefits.

Diverse means for drug delivery (i.e., liposomes, polymer carriers, blood components) and targeting (peptides, antibodies) have been proposed and are currently undergoing intense preclinical and, to a lesser extent, clinical testing. Most of these means are modular in nature and can be employed for drug delivery to diverse targets in the body. However, the design of each particular delivery system must fit the nature of its target and the pathological processes. From this perspective, drug targeting to the pulmonary vasculature represents a specific paradigm, in many aspects different from similar tasks targeting other compartments, such as tumours, heart or brain.

The goal of this paper is to give a brief overview of biomedical perspectives of targeted drug delivery to the pulmonary vasculature. It focuses on drug delivery-related features of this compartment, describes components that are potentially useful for targeting in the context of pertinent pathological processes and discusses examples of current strategies providing localised effects in the pulmonary vasculature. In order to comply with space limitations and to avoid overt superficiality, the review does not discuss the design of particular formulations but rather presents general principles important, in the author's opinion, for the design and testing of diverse

strategies. Presumably, these strategies can be employed for targeting various classes of therapeutic agents but this review focuses on biotherapeutics, such as enzymes proposed for the treatment of pulmonary disease conditions. It only marginally describes gene therapies and the delivery of genetic materials, a huge area on its own that should be reviewed elsewhere. Research activities focused on the identification of new determinants for drug targeting to the pulmonary vasculature are discussed in Section 5.5. The focus is predominantly on features of drug delivery systems directed to endothelial determinants that have demonstrated potential use for therapeutic interventions aimed at the management of pathological processes in the pulmonary vasculature, including inflammation, thrombosis and oxidative stress.

2. Pulmonary vascular endothelium: a therapeutic target

The pulmonary vasculature is a unique anatomical and functional compartment exerting diverse vital functions [1]. First, the capillaries surrounding the alveoli are intimately involved in gas exchange and blood oxygenation, which is, arguably, the main, yet certainly not the only, lung function. Second, the precapillary arterioles and capillary network serve as an anatomical filter for thrombi, aggregates of activated or damaged blood cells and other types of emboli (e.g., lipid, gas) in venous blood, which would otherwise lodge in the cerebral vasculature and cause brain ischaemia. Third, ectoenzymes expressed on endothelial cells (ECs) lining the luminal surface of pulmonary vessels perform numerous functions, including the activation and inactivation of circulating peptides and other bioactive molecules. Fourth, pulmonary ECs control vascular fluid permeability by maintaining a continuity of a closely connected cellular monolayer and thereby preventing plasma extravasation and oedema. The pulmonary endothelium also plays a key role in homing leukocytes (i.e., about one third of the resting neutrophil population resides in the pulmonary vasculature), and their activation and recruitment into the lung tissue under pathological conditions.

However, due to its unique anatomical function, which can be characterised as a filter for both inhaled air and dirty venous blood carrying products of excretion and peripheral tissue damage, the pulmonary vasculature is vulnerable to the damaging effects of many pathological factors; for example, inhaled damaging environmental factors including oxidants, pollutants, microparticulate matters, dust, tobacco and burn smoke, and pathogens initiate pulmonary inflammation, often causing damage to the vascular compartment. This, in turn, may cause systemic disorders due to both hypoxia and hyperactivation of leukocytes, complement and thrombotic systems in the pulmonary vessels. Alternatively, systemic activation of these defense systems may lead to oxidative stress and severe collateral damage in the pulmonary microvasculature, due to its function as a blood filter and extremely large surface area [2]. Acute lung injury, a severe syndrome with high mortality, which develops as pulmonary failure in response to sepsis, trauma or haemorrhage, is an example of the latter scenario [3,4]. Furthermore, both ECs and smooth muscle cells (SMCs) in the pulmonary arterioles are intimately involved in the development of pulmonary hypertension, the mechanism of which has not yet been fully elucidated. In addition, the pulmonary vasculature is susceptible to tumour metastases, due to its blood filter function and the expression of adhesion molecules anchoring circulating tumour cells [5,6].

Therefore, the pulmonary vasculature is involved in many pathological conditions, both local and systemic and, as such, represents an important target for diverse diagnostic, prophylactic and therapeutic interventions. However, most of the agents proposed for such a use have no natural or built-in mechanism(s) allowing their specific location (i.e., targeting) into this compartment, resulting in adverse effects and suboptimal effectiveness, if it is even effective. This article gives a brief overview of drug delivery strategies designed to improve the effectiveness and specificity of such interventions, in the context of lung pathophysiology.

3. Pulmonary vasculature: target characteristics

Intratracheal administration (e.g., aerosols) is a unique route that permits the achievement of a high local concentration of a drug in the lung [7]. Despite significant technical challenges, including drug inactivation, this clinically proven strategy is optimal when pulmonary airspace or interstitium are the sites of drug action [8]. Furthermore, the pulmonary route is suitable for the systemic administration of drugs, including some hormones capable permeating the air–blood barrier.

However, the tracheal route is suboptimal for agents that supposedly act in the vascular compartments of the lungs. First, deposition of inhaled drugs in the lung tissue is heterogeneous, patchy and stochastic. Second, most drugs permeate poorly into the distal airways and alveoli, where transfer from the air to blood compartments occurs, a process that is not sufficiently effective for most drugs. These limitations are especially acute in cases of biotherapeutic delivery, such as proteins and genetic materials (i.e., relatively large and unstable agents) [9]. As a result, only a small fraction of intratracheally delivered drugs can reach pulmonary vascular cells, in particular, the endothelium. Finally, this fraction of the drug is rapidly eliminated by circulating blood. Therefore, the latter factor, permitting systemic effects of drugs delivered via the lungs, eventually compromises their local effects in the pulmonary vasculature.

In contrast to the intratracheal route, intravascular administration is naturally designed for the delivery of circulating compounds to the pulmonary endothelium, which is a preferred target for drugs circulating in the bloodstream. First, pulmonary vasculature is the first major capillary network encountered by intravenously injected drugs. Second, it contains about one-third of the total surface of ECs in the

body. Third, in contrast to any other organ in the human body, lung vessels receive the entire cardiac output of venous blood, whereas all other organs share an equal volume of the arterial blood. Fourth, a relatively slow perfusion rate through high-capacity, low-resistance pulmonary vessels kinetically favours binding of circulating ligands to the endothelium. In theory, therefore, agents that possess specific affinity to ECs should accumulate in the lungs after intravascular administration, even if target determinants are common throughout all types of ECs in the body (i.e., pan-endothelial determinants).

Blood perfusion patterns in the lungs should be taken into consideration in terms of vascular drug delivery. First, the lower lobes of both left and right lungs are perfused more effectively then the apical lobes. In fact, a similar inequity of ventilation effectiveness exists between basal and apical areas of the lungs, providing ventilation-perfusion matching. Second, even under normal conditions, a substantial fraction of pulmonary capillaries throughout the lungs is only transiently perfused. These 'silent vessels' form a reserve of perfusion capacity that can be recruited in cases of venous blood redistribution into the pulmonary circulation; for example, under physical stress [10] or pathological conditions such as heart diseases [11]. Third, perfusion and vascular permeability are transiently elevated at sites of acute inflammation, although they may be reduced at sites of chronic inflammation, at the formation of fibrous masses or tumour growth [12-14]. Fourth, perfusion is usually reduced in hypo- or non-ventilated areas due to a paradoxical vasoregulation in the lungs [15]. The latter phenomenon is due to the contraction of small muscular arterioles in the lungs in response to hypoxia [16]. In contrast, in the systemic circulation (e.g., in the brain or heart) hypoxia induces vasorelaxation. Interestingly, pulmonary veins play an important role in the regulation of vascular resistance in this organ [17]. Finally, partial or complete occlusion of pulmonary vessels (e.g., by fibrin thrombi or activated white blood cells) compromises delivery of circulating agents downstream and, depending on the extent of blood stasis, even upstream of the occlusion site.

An incomplete list of drugs and diagnostic agents, whose therapeutic activity can be improved by effective and specific delivery into the pulmonary vasculature, includes the following: contrast agents and isotopes for the visualisation of blood flow and pathological processes; anti-inflammatory, antithrombotic and antioxidant agents to alleviate lung damage caused by these intertwined syndromes; toxic agents for the eradication of tumours or pathologically proliferating cells in sites of vascular stenosis in pulmonary hypertension; protease inhibitors; enzyme replacement therapies to alleviate lysosomal disorders in storage diseases; growth factors and their inhibitors; nitrogen oxide (NO)-donors and; at least in theory, genetic materials for gene therapies.

Despite the diversity of chemical classes of these drugs, many of which are biotherapeutics (e.g., proteins and nucleic acids), most of them do not accumulate in the lungs after intravascular injection. Drug delivery systems are being designed in order to provide such an accumulation and thus enhance effectiveness and reduce systemic adverse effects. In some cases, these systems also help to optimise subcellular localisation of drugs (e.g., providing anchoring on the luminal surface for antithrombotic agents, lysosomal delivery of enzyme replacement therapies or nuclear delivery of DNA). The following sections will focus on the key characteristics of drug delivery strategies and systems serving these goals.

4. Means for drug delivery to the pulmonary vasculature

General problems impeding the use of many drugs include unfavourable pharmacokinetics (e.g., insufficient circulation time due to a rapid hepatic uptake, renal clearance or other elimination pathways) and the inactivation on route to targets due to inhibitors and metabolising systems in the bloodstream [18]. Some of these problems can be circumvented by using stealth technologies that mask drugs or their carriers by polyethyleneglycol coupling (pegylation), which decelerates their elimination, reduces immune recognition and prolongs circulation [19,20]. Drug encapsulation into nanocarriers including liposomes [21-23], polymersomes [24] or other polymer carriers [25,26] reduces their systemic adverse effects [27].

However, only small fractions of long-circulating formulations accumulate in the areas of therapeutic interest, including the lungs [28,29]. One approach to improve the delivery of a drug to vascular areas of interest is to load it into biocompatible microspheres with a 20 – 50 μm diameter that mechanically lodge in the precapillary arterioles downstream to the injection site, thus creating an elevated local level of the drug or imaging agent. Naturally, pulmonary vasculature is a target for intravenously injected microspheres [30]; for example, clinically used albumin microspheres labelled with ^{99}technecium, ^{111}indium or other isotopes can be visualised in a γ-camera and can help to show the perfusion of the pulmonary vasculature, providing an additional tool for the diagnosis of pulmonary embolism.

A similar principle, lodging in the precapillary and capillary vascular bed in the lungs, is also being explored in the context of gene therapy, using cationic liposomes complexed with negatively charged plasmid DNA, which rapidly form multi-micron aggregates in the blood and lodge in the pulmonary precapillary bed [31]. In addition to mechanical retention of aggregated DNA–liposomes, such polyplexes retaining the residual net positive charge bind to negatively charged components of the endothelial glycocalyx (e.g., chondroitin–sulfate). This technique has been employed for transgene expression in the lungs and in angiogenic ECs in tumours. However, in many cases the transgene expressed in the lung tissue after intravenous injection of DNA/cationic liposomes is localised in SMCs but not in ECs. The nature of this phenomenon and its potential significance in terms of gene therapies remain to be elucidated more fully.

However, the mechanical or electrostatic retention of small aggregates does not allow the selective delivery of cargo into certain cell types (e.g., ECs) nor control over the subcellular localisation of the delivered cargo. Furthermore, blood flow rapidly eliminates drugs released from a lodged carrier, thereby reducing the time of their contact with target cells, and thus compromising their delivery to the target vascular cells.

In contrast, numerous studies in animals and humans have documented that the coupling of diagnostic or therapeutic agents, as well as nanocarriers loaded with these agents, with affinity moieties provides preferential delivery of the cargo to the pulmonary vasculature [32-35]. Some authors call such affinity moieties 'vectors', although this terminology may be confusing in the context of gene therapies, where vectors are defined as genetic constructs. Diverse affinity moieties including sugars, hormones and receptor ligands (e.g., transferrin) are being explored in the context of drug targeting to ECs.

Drugs can be chemically conjugated with affinity moieties (e.g., using covalent bi-functional agents) that crosslink them either directly or to polymer backbones increasing the loading capacity and valency of the binding to target cells [25]. Non-covalent conjugation methods (e.g., using crosslinking antibodies or proteins such as streptavidin, for example) are also being explored, although to a more limited extent, in animal studies [34]. Reproducible synthesis of conjugates with standard structures and FDA-acceptable levels of homogeneity represent a significant challenge in terms of industrial scaling-up, quality control and clinical use of chemical conjugates. The genetic fusion of protein drugs with protein affinity moieties using recombinant methods provide stable, homogeneous and relatively easy to scale-up and good manufacturing practice-produce therapeutic agents. Furthermore, molecular redesign of the protein components of fusion constructs permits the optimisation of their features, such as the deletion of unnecessary parts of molecules or the insertion of point mutations allowing products with novel, favourable pharmacokinetics and/or functional features. Finally, affinity moieties can be coupled to the surface of drug vehicles, such as liposomes, polymer nanocarriers or protein carriers. In general, carriers with a diameter within the 50 – 500 nm range (i.e., the size allowing free circulation through capillaries in the vascular system [36] and, in some cases permitting intracellular delivery into ECs [37]) have been employed for targeted drug and gene delivery to the pulmonary vasculature.

Arguably, IgG antibodies directed to specific endothelial surface determinants represent the most popular class of affinity moieties. Such targeting methods have modular structure and amenable standard techniques for induction, purification, conjugation and modification. Advanced techniques are available for the production of monoclonal and recombinant antibodies and their fragments, such as single-chain antibody fragment (scFv), the minimal fully competent antigen-binding site of an antibody; their deimmunisation and humanisation by the replacement of their parts overtly alien to a human body; artificial enhancement of their affinity (i.e., recombinant maturation); and oligomerisation that in some cases facilitates internalisation [38]. The vascular immunotargeting of drugs to the pulmonary vasculature, an expanding area of current biomedical research using antibodies and their fragments directed to specific endothelial surface determinants, will be considered in detail in the following section.

5. Vascular immunotargeting to the pulmonary endothelium

In order to be useful for targeted drug delivery to the pulmonary vasculature, a target determinant must meet at least the following four major criteria:

- Accessibility. A target should be present on the luminal surface of the pulmonary endothelium to permit the delivery of sufficient amount of a drug. Intracellular components are not useful for drug delivery, unless they become exposed on the lumen under pathological conditions (e.g., selectins). Furthermore, steric accessibility of the surface binding site(s) should be sufficient to harbor affinity carriers circulating in the bloodstream, ranging in size from small fusion proteins to carriers 100 – 300 nm in diameter. The surface expression of a determinant should be sufficient for targeting in disease conditions, which can reduce the surface density of some endothelial molecules due to pathological shedding, suppressed synthesis or the adhesion of activated leukocytes. In cases of transiently or intermittently expressed targets, the identified time window should suffice for targeting.
- Specificity. The level of target antigens in the blood or in non-ECs accessible to the blood should not achieve levels at which they compromise targeting; for example, ECs have transferrin receptors, which are also abundantly exposed in hepatic cells accessible to blood. As a result, transferrin-targeted drugs accumulate in the liver, and, to some extent, in the brain (as the cerebral endothelium is enriched in transferrin receptors), with a little targeting in the lungs. Counterparts of the target determinant circulating in the blood, such as the soluble form of transmembrane glycoproteins or P-selectin on platelets, will compromise delivery to the endothelium. However, absolute specificity is not necessary; targeting to the endothelium will suffice if a competing determinant is localised in a counterpart cell type inaccessible to blood. On the other hand, panendothelial determinants can be used for drug delivery to the pulmonary vasculature due to its privileged target features.
- Safety. Targeting should not cause harmful side effects to ECs. The binding of targeted drugs may activate the shedding and/or internalisation of target determinants, or otherwise inhibit or activate them, which may lead to detrimental effects (e.g., thrombomodulin; see Section 5.1). Engagement and crosslinking of endothelial determinants

may induce signalling and endothelial activation; processes whose potential side effects must be rigorously tested. Ideally, binding of an antibody–drug complex to a target antigen should cause additional therapeutically beneficial side effects (e.g., cell adhesion molecules; see Section 5.2).
- Precision. Docking to a surface determinant should provide a proper subcellular localisation of a drug; for example, depending on the therapeutic goal, an antibody–drug complex should either be retained on the cell surface (e.g., antithrombotic drugs) or undergo trafficking to a proper subcellular compartment (e.g., the nucleus in the case of DNA or lysosomes in the case of enzyme-replacement therapies).

No affinity carrier can suit all therapeutic needs. Specific therapeutic goals require different secondary effects mediated by binding to the endothelium, drug targeting to different subpopulations of ECs (e.g., resting versus inflammation-engaged endothelium) and to diverse cellular compartments. Targeted delivery of antioxidants, antithrombotics or NO-donors to normal or resting ECs can be useful for either prophylaxis or therapies [32,39-41]. On the other hand, specific recognition and drug delivery to abnormally activated or pathologically altered endothelium may permit more specific means for the treatment of such maladies as localised tumour growth and inflammation. Therefore, diverse affinity carriers sometimes directed to relatively similar endothelial targets, for example, cell adhesion molecules, or even binding to different domains of the same target molecule can be employed to more fully capitalise on unique opportunities offered by vascular immunotargeting. Examples of pulmonary vascular immunotargeting to specific endothelial determinants are discussed below.

5.1 Constitutively expressed transmembrane glycoproteins enriched in the pulmonary endothelium: angiotensin-converting enzyme and thrombomodulin

Historically, the first animal studies in the field of targeted drug delivery to the pulmonary endothelium, initiated in the late 1980s, employed monoclonal antibodies against two constitutively expressed endothelial surface glycoproteins: angiotensin-converting enzyme (ACE) pioneered by Danilov *et al.* [42-45] and thrombomodulin studied by Kennel *et al.* [46-48]. The fact that these determinants are enriched in the pulmonary microvasculature provides an additional factor favouring the specificity of immunotargeting.

ACE is a transmembrane glycoprotein expressed on the endothelial luminal surface, converting angiotensin I (Ang I) into Ang II, a vasoactive peptide that exerts vasoconstricting, prooxidant, prothrombotic and pro-inflammatory activities [49-51]. The pulmonary vasculature is enriched with ACE: nearly 100% of ECs in the alveolar capillaries are ACE-positive versus < 15% ACE-positive ECs in the extra-pulmonary capillaries [44]. Radiolabelled anti-ACE accumulate in the pulmonary vasculature after intravascular and intraperitoneal injections in rats, cats, primates and humans [43,52]. Diverse tracing compounds and drugs conjugated with anti-ACE accumulate selectively in the lungs after intravenous injection in rats [43,44,52].

Recently, anti-ACE has been used successfully for retargeting of viruses to pulmonary ECs in rats [53,54]. Heteroconjugates that consist of anti-ACE and antibody directed to a viral fibre protein recognising cellular receptors (CARs) pursues a dual function: it blocks natural viral tropism to CARs and thus attenuates viral infection in non-target organs, the liver first of all, and redirects it towards the ECs in the lungs [54]. Importantly, combining anti-ACE-mediated targeted delivery, blocking binding to CAR and the insertion of an endothelium-specific promoter in the genetic construct carried by the viral envelope permits the augmentation of the pulmonary specificity of transgene expression by several orders of magnitude [53]. The insertion of such endothelium-specific promoters as used by genes encoding vascular endothelial growth factor receptors (Flk-1) or endothelin, as well as blocking natural viral tropism by antibodies or peptides, currently represent a general approach for enhancing endothelial specificity of gene therapies, both viral and non-viral [55-58].

ACE also inactivates substance P and bradykinin, a peptide stimulating NO production, although Ang II may stimulate NO production by ECs [59]. Some anti-ACEs block its active site and/or facilitate ACE shedding from the endothelium by specific secretases regulated by metalloproteases [45,60,61]; however, other anti-ACEs enable ACE to retain its function. Therefore, using anti-ACEs directed to different epitopes enables targeting strategies to be developed that either retain or inhibit ACE activity, enhancing flexibility and therapeutic applicability of the strategy. ACE inhibition may be beneficial in conditions associated with vascular oxidant stress, thrombosis, ischaemia and inflammation. Figure 1 illustrates immunotargeting to ACE.

Pro-inflammatory agents including oxidants and cytokines suppress ACE expression in the endothelium [62-64]. Such agents may inhibit therapeutic targeting to ACE under certain pathological conditions. On the other hand, ACE inhibition or shedding due to targeting may lead to the reduction of Ang II formation and elevated levels of bradykinin. In some clinical settings including acute hypotension, vascular collapse and oedema, these secondary effects may lead to adverse consequences. However, in some settings such as in hypertensive patients, ACE inhibition may provide secondary beneficial effects [32,39,41,52].

However, anti-ACE is a good candidate for targeting drugs to the pulmonary endothelium for diagnostic, prophylactic and, perhaps, therapeutic goals; for example, the targeting of anti-ACE-conjugated isotopes can be used for the visualisation of pulmonary vasculature [42]. Furthermore, ECs internalise anti-ACE and anti-ACE conjugates, which, therefore, delivers drugs intracellularly [39]. Antioxidant enzymes conjugated with anti-ACE (e.g., catalase) accumulate in rat lungs *in vivo* [65] and protect perfused rat lungs against H_2O_2 [66]. Targeting ACE does not cause acute harmful reactions in animals and humans [45,52].

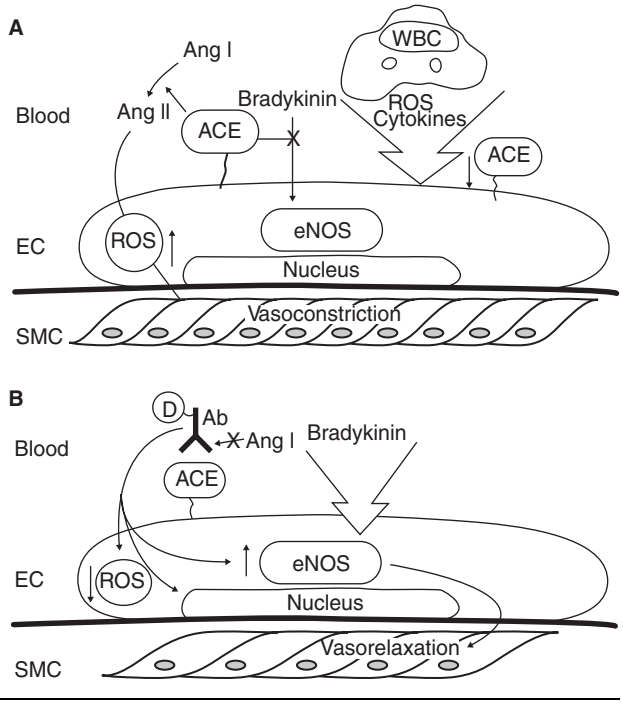

Figure 1. Immunotargeting to ACE. A. ACE is normally expressed on the endothelial surface (left) but inflammatory factors (e.g., ROS and cytokines released from activated leukocytes) downregulate the density of ACE molecules on the endothelial surface (right). **B.** Anti-ACE delivers drugs to the endothelium and undergoes internalisation. This paradigm can be used for the delivery of antioxidant enzymes (to intercept intracellular ROS) or genetic materials. In addition, anti-ACE inhibits ACE. This attenuates Ang I conversion, protects bradykinin (both of these effects lead to vasorelaxation) and suppresses pro-oxidative effects of Ang II. Reproduced with permission from MUZYKANTOV VR: Targeting pulmonary endothelium. In: *Biomedical aspects of drug targeting.* VR Muzykantov *et al.* (Eds), Kluwer Academic Publishers, Boston, MA, USA (2002):129-148 [39].

Ab: Monoclonal antibody (anti-ACE) conjugated with drugs; ACE: Angiotensin-converting enzyme; Ang: Angiotensin; D: Drug; EC: Endothelial cell; eNOS: Endothelial nitric oxide synthase; ROS: Reactive oxygen species; SMC: Smooth muscle cell; WBC: White blood cell.

Thrombomodulin is an endothelial target determinant similar to ACE in many aspects except function. Quite appropriate to its name, thrombomodulin acting in concert with proteins C and S converts thrombin into an antithrombotic enzyme that inactivates coagulation factors [67]. Hence, any intervention that may compromise thrombomodulin function, including targeting drugs that may block, inhibit or shed thrombomodulin, has the potential to shift the balance towards thrombosis [68,69]. Indeed, some of such interventions induced or aggravated thrombosis in animal models [48]. However, due to the fact that intravascularly injected thrombomodulin antibodies accumulate in rat and mouse lungs, this affinity moiety has been extensively used in model targeting of liposomes, genetic materials and diverse injurious agents to the pulmonary endothelium in these laboratory animals [70]. One potential application of thrombomodulin targeting is the modelling of human vascular pathologies (e.g., thrombosis, inflammation or oxidative stress) localised in the pulmonary vasculature in laboratory animals [32].

5.2 Constitutively expressed cell adhesion molecules: platelet-endothelial adhesion molecule-1 and intercellular adhesion molecule-1

These determinants, which belong to the immunoglobulin superfamily of transmembrane glycoproteins, are relatively evenly distributed throughout ECs in the vasculature. Therefore, their ligands can be used for vascular immunotargeting to diverse vascular areas including cardiac and cerebral, especially if they are infused via a vascular catheter inserted in a conduit artery [71]. However, due to the factors previously described (privileges of the pulmonary endothelium as a vascular target), antibodies directed against these constitutive pan-EC adhesion molecules tend to accumulate in the lungs after intravenous injection and represent good candidates for targeting either the normal and/or pathologically altered endothelium [35].

For example, platelet-endothelial adhesion molecule-1 (PECAM; CD31) is predominantly localised in intercellular borders in the endothelial monolayer [72]. Platelets and white blood cells also express PECAM but at levels that are several orders of magnitude lower than ECs, and, therefore, have no substantial effect on the binding of circulating anti-PECAM to ECs. PECAM is involved in complex, but not completely elucidated, mechanisms of cellular recognition, adhesion and trans-endothelial migration of leukocytes, which are key to the pathogenesis of many disease conditions [73]. Hence, the effects of PECAM engaging, blocking and metabolism imposed by anti-PECAM must be carefully studied in the context of potential side effects, including cellular signalling [74]. However, blocking PECAM by anti-PECAM attenuates inflammation and leukocyte-mediated injury [75]. Thus, anti-PECAM targeting may offer secondary benefits in conditions associated with inflammation.

PECAM is stably and abundantly expressed in ECs, with millions of anti-PECAM binding sites that are not suppressed by cytokines and oxidants. This permits a robust PECAM-targeted drug delivery to either normal or pathologically altered vasculature, for either prophylaxis or therapy. Active reporter enzymes conjugated to anti-PECAM have been shown to accumulate and display their functional activity in the pulmonary endothelium as soon as 10 min after intravenous injection in mice and pigs [71,76,77]. Targeting of anti-PECAM–catalase conjugates protects ECs against H_2O_2 toxicity in cell cultures [78,79], and protects the pulmonary vasculature against acute oxidant stress in mice and rats [70,80].

ECs bind anti-PECAM without internalisation but do internalise multimeric anti-PECAM complexes that are within the 100 – 350 nm diameter range [78,81], regardless of the chemistry and mechanism of formation of such multivalent conjugates [34]. This feature permits the use of anti-PECAM as

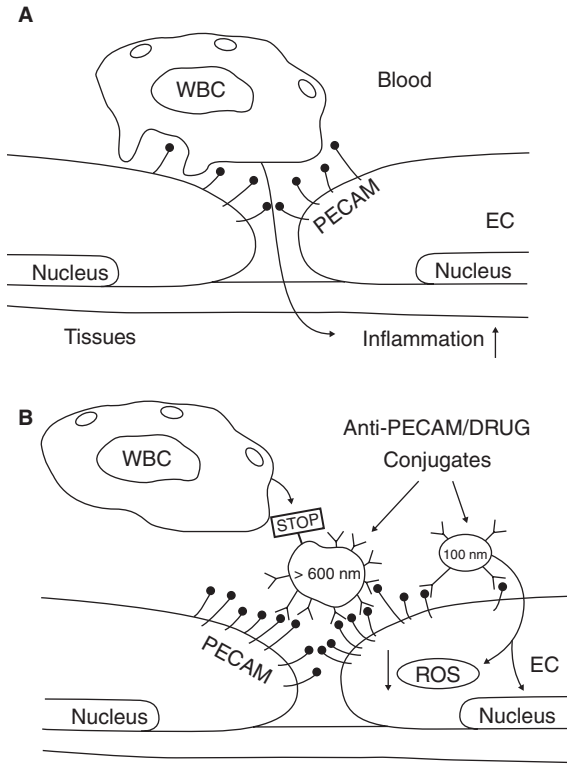

Figure 2. Immunotargeting to PECAM-1. A. PECAM-1 is constitutively expressed by the endothelium, predominantly in the intercellular contacts. It supports WBC transmigration and facilitates inflammation in the tissues. **B.** Large (> 600 nm diameter) anti-PECAM conjugates poorly internalise in the ECs, whereas smaller counterparts (100 – 300 nm diameter) enter the cells and can be used for intracellular delivery of genetic materials and antioxidant enzymes to detoxify ROS. In addition, anti-PECAM conjugates may suppress inflammation via blocking WBC transmigration. Reproduced with permission from MUZYKANTOV VR: Targeting pulmonary endothelium. In: *Biomedical aspects of drug targeting*. VR Muzykantov et al. (Eds) Kluwer Academic Publishers, Boston, MA, USA (2002):129-148 [39].
EC: Endothelial cell; PECAM: platelet-endothelial adhesion molecule; ROS: Reactive oxygen species; WBC: White blood cell.

an affinity moiety to targeting drugs either to the endothelial surface (e.g., using monomolecular conjugates and scFv constructs), or intracellularly. In addition, multivalent, high-affinity binding of anti-PECAM nanocarriers to the endothelium greatly enhances pulmonary targeting [44,78]. Thus, anti-PECAM targeting is being explored for the delivery of reporter enzymes and genetic materials for the transfection of pulmonary endothelium in cell cultures [81] and in laboratory animals [82]. Figure 2 illustrates some characteristics of vascular immunotargeting to PECAM.

Intercellular adhesion molecule-1 (ICAM-1; CD54) is another immunoglobulin superfamily surface glycoprotein with a short cytoplasmic domain, transmembrane domain and large extracellular domain. It is normally expressed by ECs at a surface density that, by various estimates, ranges from modest to relatively high ($2 \times 10^4 - 2 \times 10^5$ surface copies per cell) [83]. Some other cell types also express ICAM, yet the major fraction of blood-accessible ICAM is exposed on the luminal surface of endothelium. Therefore, after intravascular injection, ECs represent the major target for anti-ICAM. Indeed, a robust and specific binding of ICAM antibodies and anti-ICAM conjugates to the vascular endothelium after intravenous administration has been documented in animals, with major fractions of the injected traced conjugates accumulate in the lungs [84,85].

Pathological stimuli, such as oxidants, cytokines and abnormal shear stress stimulate surface expression of ICAM by ECs via the activation of nuclear transcription factors and *de novo* synthesis of ICAM [86]. As a result, inflammation and other pathological conditions (modelled in laboratory animals by the administration of endotoxin, cytokines, hyperoxic lung injury and autoimmune reactions) enhance ICAM surface density in the pulmonary endothelium [87] and, therefore, do not suppress but rather markedly facilitate ICAM targeting [84,85,88,89]. Therefore, ICAM-1 seems to be a very attractive target for the delivery of diagnostic and therapeutic agents to the pulmonary endothelium [85,90]. Conjugation of anti-ICAM to liposomes [91] or polymer carriers [35] may enhance the effectiveness of drug delivery.

ICAM is a counter receptor for integrins on leukocytes, supporting firm adhesion of activated white blood cells to ECs and thus promoting inflammation [92]. In addition, ICAM serves as a natural ligand for certain viruses [93]. ICAM antibodies suppress leukocytes adhesion, thus producing anti-inflammatory effects in animal models and clinical pathological settings associated with vascular injury, such as acute inflammation, ischaemia/reperfusion and oxidative stress [94-96]. The anti-inflammatory effect of multivalent anti-ICAM conjugates may be even more potent due to the higher affinity/valency of the conjugate binding and the downregulation of surface ICAM due to internalisation via cell adhesion molecule (CAM)-mediated endocytosis (which has been described above for PECAM). Similar to PECAM, ICAM may also serve as a signalling molecule; however, the significance of this signalling, in the context of both normal vascular physiology and pathology remains to be more fully elucidated [97].

Interestingly, the internalisation of anti-ICAM follows the same pattern as described for PECAM: ECs internalise multivalent anti-ICAM conjugates that are within 100 – 400 nm diameter but not monomeric anti-ICAM or very large conjugates ≥ 0.5 µm [98]. Recent studies by Muro *et al.* [35,37] revealed that to internalise anti-ICAM and anti-PECAM conjugates via constitutively non-internalisable determinants ECs employ a novel, previously unknown internalisation pathway, which differs from clathrin- and caveoli-mediated endocytosis and CAM-mediated endocytosis. Briefly, key features of this pathway include PECAM or ICAM clustering by multivalent

anti-CAM complexes that triggers signalling events including the activation of protein kinase C, Src family kinases, Rho-dependent kinase and dynamin. These events lead to the fast formation of actin stress fibres, involving a transmembrane sodium–proton exchanger (NHE1) and resulting in the formation of endocytic vacuoles and the internalisation of cell-bound conjugates [35,37].

Therefore, subcellular localisation of anti-PECAM and anti-ICAM conjugates can be controlled by their size; for example, monomeric anti-ICAM carriers permit targeted delivery of antithrombotic agents to the endothelial surface [85], whereas polyvalent anti-ICAM conjugates deliver antioxidant and lysosomal enzymes to the endothelial interior [35,37]. Interestingly, ICAM-1 dissociates from anti-ICAM carriers early after internalisation and recycles to the plasma membrane and can, therefore, serve for multiple cycles of intracellular delivery [99].

In addition, the pace and destination of intracellular traffic of cargos internalised via PECAM-1 and ICAM-1 can be regulated by auxiliary pharmacological agents; for example, alteration of the proton–sodium balance in endosomal-lysosomal compartments, such as using an alkalinising agent chloroquine or specific ionophors, decelerates degradation of proteolysis-susceptible cargos and may permit their recycling to the plasma membrane [99,100]. On the other hand, disruption of endothelial microtubules blocks lysosomal traffic and dramatically prolongs duration of the therapeutic activity of internalised drugs [100].

In summary, antibodies to EC adhesion molecules PECAM-1 and ICAM-1 apparently meet five criteria of targeting and provide a versatile and potentially practically useful means for targeted drug delivery to and into the pulmonary ECs. Figure 3 illustrates several modes of potential applications of this drug delivery system.

5.3 Inducible endothelial cell adhesion molecules: selectins and vascular cell adhesion molecule-1

There is a class of potential endothelial targets that are normally absent on the vascular lumen but become exposed under pathological conditions. In theory, these determinants are ideal for therapeutic (but not prophylactic) drug delivery, although this avenue is more difficult to pursue in clinical settings than targeting constitutive determinants.

For example, inflammatory mediators, cytokines, oxidants and abnormal shear stress induce rapid mobilisation of P-selectin, which is normally stored intracellularly, to the EC surface [87]. In fact, within 10 – 30 min after exposure to some of these pathological mediators, P-selectin appears on the endothelial lumen in cell cultures and laboratory animals. Furthermore, the same agents induce *de novo* synthesis and surface expression of E-selectin by activated endothelium within several hours after challenge [92]. An Ig-superfamily cell adhesion molecule vascular cell adhesion molecule-1 (VCAM-1) is also exposed on the surface of activated ECs [72]. Selectins and VCAM-1 facilitate adhesive interaction of activated white blood cells with ECs [101].

Therefore, antiselectins and anti-VCAM represent attractive affinity moieties for drug delivery to activated pulmonary endothelium, with a potential secondary benefit of attenuation of leukocyte adhesion [87,96]. Experiments in cell cultures and limited animal studies support this hypothesis and show that selectins may permit the targeting of drugs to cytokine-activated endothelium [102,103]. Interestingly, ECs constitutively internalise E- and P-selectins via clathrin-coated pits [104-106]. Therefore, anti-E-selectin targeted liposomes or conjugates internalise via clathrin-coated pits within ECs, enabling the intracellular delivery of conjugated liposomes [107], anti-inflammatory drugs [107,108] and genetic materials [109]. The readers interested in the specific aspects of endothelial internalisation, intracellular sorting and trans-cellular transport of ligands of endothelial surface determinants are referred to a recent review on this topic [37].

However, selectins are transiently exposed on the surface of stressed ECs. As such, the kinetics and persistence of selectins exposure following endothelial activation are difficult to follow even in animal studies, where the exact initiation of cytokine activation could be easily controlled; thus, the transient character of surface exposure hinders the targeting of selectins. Furthermore, even at the activation peak, selectins are exposed at relatively low surface densities; hence, robustness of the targeting may be suboptimal for therapeutic interventions requiring the delivery of large doses. However, targeting selectins seems to be a very attractive avenue for the diagnostic visualisation of activated endothelium in inflammation foci by the delivery of conjugated isotopes [110] or ultrasound contrasts [111,112].

5.4 Antibodies directed to specific endothelial domains

ECs contain diverse domains in the plasma membrane, both relatively static (e.g., intercellular junctions) and dynamic, including caveoli; for example, glycoprotein 85 (gp85), identified by Ghitescu, is predominantly localised in the thin part of the EC body that separates alveolar and vascular compartments and lacks main organelles (avesicular zone) [113,114]. gp85 monoclonal antibodies accumulate in rat pulmonary vasculature without internalisation and deliver conjugated cargos into the pulmonary vasculature [115]. In theory, a human counterpart of this antigen could be an interesting candidate for drug delivery to the surface of alveolar capillaries.

On the other hand, animal studies showed that the pulmonary endothelium in rats contains surface determinants localised to cholesterol-enriched plasma membrane microdomains, including caveoli. Antibodies of determinants localised to caveoli such as gp60 and gp90 accumulate in the pulmonary vasculature after intravenous injection in rats, enter the EC and traverse endothelial barriers [116].

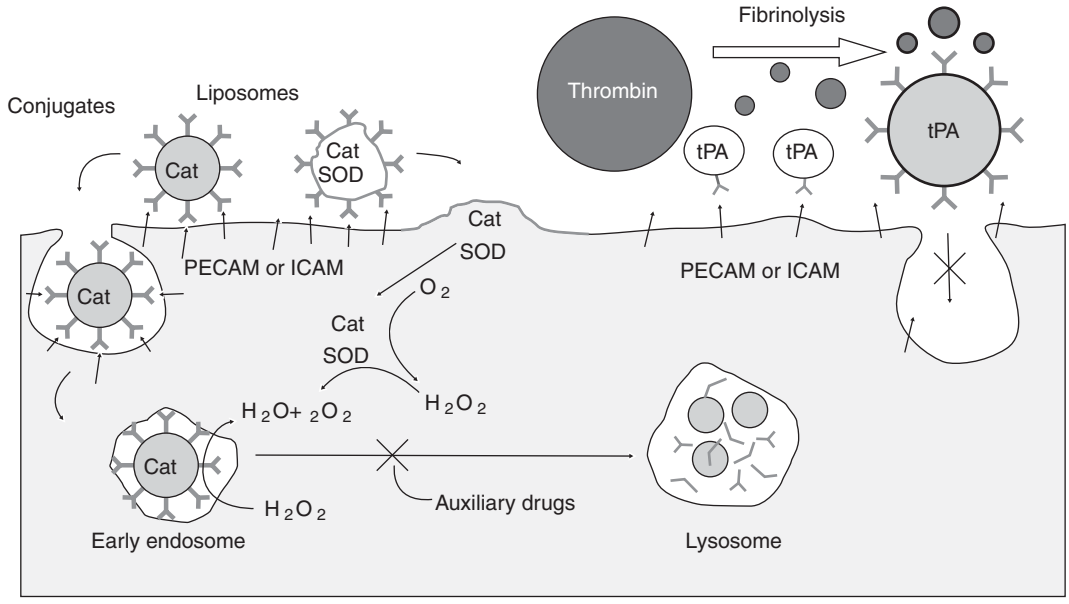

Figure 3. Vascular immunotargeting of antioxidant and fibrinolytic enzymes to constitutive EC adhesion molecules.
Left: containment of vascular oxidative stress. Multivalent conjugates of 100 – 300 nm diameter, consisting of antioxidant enzymes (AOE, Cat and SOD) and carrier PECAM-1 or ICAM-1 antibodies, bind to ECs, enter cells via CAM-mediated endocytosis and detoxify ROS in the cell interior. Auxiliary drugs decelerating lysosomal trafficking and/or degradation can be employed to prolong the duration of the protective effects. A fraction of the conjugates transiently bound to the endothelial surface intercepts external ROS and inhibits leukocytes adhesion via blocking of ICAM-1 or PECAM-1 (not shown). As an alternative strategy, anti-CAM stealth immunoliposomes bound to the endothelium can fuse with the plasma membrane, thus delivering their AOE payload into the cytosol; this tentative strategy is suitable for the delivery of SOD/Cat tandem for orchestrated detoxification of both superoxide anion and hydrogen peroxide ROS. Right: boosting of fibrinolytic activity on the endothelial luminal surface. ECs poorly internalise both small monomolecular and large micron-sized anti-CAM conjugates. Plasminogen activators (e.g., tPA) delivered in the form of such conjugates are retained on the surface of ECs and facilitate fibrinolysis of clots lodged in the vasculature. Reproduced with permission from MURO S, MUZYKANTOV VR: Targeting of antioxidant and antithrombotic drugs to endothelial cell adhesion molecules. *Curr. Pharm. Des.* (2005) **11**(18):2383-2401 [35].
AOE: Antioxidant enzymes; CAM: Cell adhesion molecule; Cat: Catalase; EC: Endothelial cell; ICAM: Intercellular adhesion molecule; PECAM: Platelet-endothelial adhesion molecule; ROS: Reactive oxygen species; SOD: Superoxide dismutase; tPA: Tissue plasminogen activator.

Caveolar-mediated endocytosis and transcytosis, mediated by the interactions of the coat protein caveolin with cell signalling and cytoskeletal molecules, play an important role in endothelial transport functions [117-122]. Interaction of a protein ligand (including antibodies) leading to receptor clustering in caveoli and the activation of specific signalling pathways plays a critical role in the initiation of transcytosis [123,124]. For example, caveolar clustering of endothelial albumin binding protein gp60 has been shown to increase transendothelial permeability via the mediation of phosphorylation events, including signalling through the Src family of tyrosine kinases [125,126]. Interestingly, chemical modifications of caveolar ligands, such as albumin nitration (that may take place in oxidant stress and inflammation) stimulate transcytosis even further [127].

Caveolar transcytosis pathways are envisioned as a means for transcellular delivery of therapeutics, which could be achieved by targeting caveolae-located receptors; for example, after intravenous injection tracers conjugated with antibodies directed against specific antigen gp90 localised in the pulmonary endothelial caveolae undergo transport through the pulmonary endothelium [116]. Although the function of gp90 and the potential effects of its inhibition by targeting are not completely understood, it is tempting to speculate that caveolar targeting will permit more effective extravascular drug delivery. Pending identification of the functions and human counterparts of these caveoli-localised determinants, they currently represent interesting candidates for drug delivery to (and, perhaps, beyond) the pulmonary endothelium.

5.5 Quest for novel determinants: endothelial genomics, proteomics, phage-display and plasma membrane proteomics

Determinants described in the previous sections have been defined mostly (although not exclusively) in hypothesis-driven research endeavours, in which affinity moieties have been produced to target identified endothelial molecules with relatively well-known location, functions and regulation. Their testing in cell cultures and animal studies provided (and

continue to provide) important additional information related to the role of these determinants in vascular biology and pathology, as well as defined the validity and limits of the ligands as affinity carriers. Although this approach does not warrant that a candidate determinant will be of practical use, at least it sets a reasonable framework for its testing in a given biomedical context, taking into account known distribution and functions of the target.

Thrombomodulin was an exception to this rule. In this case, targeting studies have been initiated by the production of a monoclonal antibody recognising an unidentified antigen highly enriched in the pulmonary capillary endothelium [47]. This work produced exciting data showing high specificity and effectiveness of pulmonary targeting using this antibody, and eventually led to the identification of the target molecule as thrombomodulin. However, so far hopes for a practical use of this targeting strategy in a human practice faded, due to the danger of potential grave adverse effects of thrombomodulin inhibition.

However, the very idea of discovery-driven quest for novel target determinants that would provide potentially more specific or effective targeting, or that permit entry into unique subcellular sorting pathways and to attain unique secondary effects, is extremely appealing. The development of high-throughput techniques including functional genomics, phage display libraries and proteomics has provided a biotechnological basis for strategies that have been employed to define novel determinants, including those in the pulmonary vasculature. This section will briefly introduce some key approaches and try to analyse objectively their advantages and limitations.

Early efforts in the identification of specific EC surface determinants and markers of functional state or vascular source of ECs have been hampered by limited inability of standard methods to maintain specific EC phenotype in culture; for example, the comparison of cell cultivation under static versus controlled flow conditions reveals that ECs grown under static conditions rapidly lost many functional and structural characteristics including surface expression of many specific marker determinants [128-134]. However, flow is not the only significant factor of physiological and pathological environment that governs specific endothelial phenotype [135,136]. Therefore, the effectiveness of the quest for new endothelium-specific determinants is based on the adequacy of strategies for analysis of endothelial determinants *in situ* or in cells freshly harvested from a selected vascular area. Some of these strategies developed in the last decade will be briefly introduced below.

Most of these strategies are focused on the identification of specific markers of pathologically altered vascular areas, such as sites of inflammation, atherosclerosis, thrombosis, disturbed blood flow, oxidative stress or tumour growth. Targeting subendothelial determinants exposed to the bloodstream at the sites of vascular injury and endothelial denudation can also be contemplated in some of these pathological conditions [137]. However, in the context of targeting the pulmonary vasculature, the endothelium *per se*, including resting, ECs is the most important target.

For example, comparing the mRNA of ECs obtained from different areas of vasculature, either normal or pathologically altered, in animal models or in human specimen characterises a relative abundance of messages for a practically unlimited number of proteins [138,139]. Furthermore, the use of laser-capturing techniques and high-fidelity polymerase chain reaction permits the analysis of mRNA obtained from a few hundred ECs harvested in the area of interest [136]. However, this analysis, called functional genomics, does not necessarily give direct correlations with actual levels of proteins themselves expressed in particular endothelial phenotype. Furthermore, this method provides no information on subcellular localisation of proteins of interest and their spatial accessibility to circulating ligands.

The analysis of proteins and their peptide digests in lysates obtained from these specimens, using methods including mass spectrometry and 2D-electrophoresis, provides an insight into relative levels of proteins [140,141]. Most of the proteomic studies focused on endothelium-used cultivated cells as the source of protein for analysis [142,143], thus providing data on generic, degenerated phenotype of cells deprived their normal milieu. Furthermore, the use of this information in terms of design of drug delivery systems is limited by the lack of insight into subcellular distribution, surface accessibility and density of these proteins, their post-translational modifications (e.g., glycosylation) that may alter their conformation, epitope structure and harbouring capacity for targeting drugs.

Proteomics of endothelial plasma membranes, for example, obtained by silica perfusion through the vasculature of interest (for description of an original method see [144]), remarkably provides more focused and precise information on molecular topography of surface determinants throughout endothelia in organs of interest, including lungs [134,145]. In addition to the identification such 'zip codes', characteristic to selected organs or vascular areas, separation methods allowing proteomic analysis of specific domains of endothelial plasma membranes, such as caveoli [146], permit micron- and nanoscale range cartography of surface of an EC [145,147]. Using this platform in rats, Schnitzer and colleagues found that the injection of radiolabelled antibody to aminopeptidase P, which is enriched in the pulmonary vasculature, permits the visualisation of lung blood vessels [145]. On the other hand, the injection of toxic isotope conjugated with an antibody against annexin A1, which is expressed in tumours, permitted the eradication of tumours [145]. Translation of these intriguing findings into drug delivery strategies should include a careful and rigorous analysis of secondary effects and subcellular destination of targeting these determinants; for example, aminopeptidase P has been shown to play several important physiological roles, including the degradation of bradykinin [148]. Therefore, unintended interventions in functions of this enzyme may cause potentially harmful effects, including vascular oedema.

The application of phage display library technique repesents an alternative high-throughput approach for the identification of site-specific binding sites in selected

vascular areas, normal and pathologically altered [149,150]. This method uses repetitive cycles of intravenous injections of phages encoding stochastic peptides, which may bind to sites accessible to the circulation, followed by the identification of specific phages homing into selected tissues and the eventual isolation of the homing determinants [151]. This method becomes more popular in the analysis of specific endothelial surface determinants, especially those characteristic of pathologically altered endothelium in tumours and atherosclerotic lesions, both in cell cultures and *in vivo* [55,152-157]. Some of the affinity ligands defined by this methodology, including those binding to previously identified endothelial determinants including adhesion molecules and ectopeptidases, are currently being explored for vascular targeting of imaging probes and drugs in animals [158]. The fact that only targets accessible to the bloodstream can be identified by this approach represents both limitations (e.g., this method of vascular topography is not inclusive) and advantages (e.g., a built-in feature of selection of accessible targets) of this strategy.

High-throughput analyses including functional genomics, phage display *in vivo* and endothelial plasma membrane proteomics provide a wealth of information on vascular topography and represent powerful tools in the ongoing quest for novel target determinants. Furthermore, these methods are designed to favour the selection of determinants that meet the criteria of specificity and accessibility. Monoclonal antibodies and other ligands produced using such candidate determinants as immunogen or selection probe afford an ultimate test of their spatial accessibility to circulation [145,159]. However, the medical use of unknown binding sites identified by these methods depends on whether they meet criteria of safety and precision.

6. Expert opinion

Due to its specific anatomic location, close proximity to external air and venous blood filtering, the pulmonary vasculature is involved in diverse vital functions and suffers from a plethora of external and internal insults. Both acute and chronic injury and disorders of the pulmonary vasculature have grave consequences, greatly increasing morbidity and mortality. Therefore, it represents an extremely important site for interventions using various prophylactic and therapeutic agents, whose action could be markedly improved by their specific delivery to the target.

The pulmonary vasculature is a very specific target in several aspects. First, in contrast to many other organs, where drugs are delivered beyond the vascular wall, ECs lining the luminal surface of the pulmonary blood vessels represent the most important therapeutic site in this organ. Second, the pulmonary vasculature is privileged in that it binds a major fraction of the drugs, even if they are targeted to common endothelial determinants. Third, traditionally, drug delivery strategies are designed in the context of oncological settings (e.g., for the delivery of toxic agents to eradicate tumour cells and diagnostic agents to localise and visualise tumours). In contrast, the pulmonary vasculature represents a preferable site for therapeutic or/and prophylactic action of various classes of undamaging agents including antithrombotic, anti-inflammatory and antioxidant drugs, as well as enzyme replacement therapies (e.g., for alleviation of genetic defects). Design of delivery systems for targeting non-toxic compounds means a specific mindset including in-depth considerations for potential side effects inflicted by engaging, blocking or other interventions in the functions of target determinants.

At least four criteria must be met in order for an endothelial determinant to be useful in the context of targeted drug delivery to the pulmonary vasculature: specificity, accessibility, safety and subcellular precision. Many endothelial determinants that are potentially useful for drug delivery have been identified recently by proteomics of endothelial plasma membrane, phage display library selections *in vivo* and tracing of labelled antibodies. High-throughput, discovery-driven approaches, proteomics and phage display provide a basis for the mapping vascular lumen and for the identification of novel determinants specific for or enriched in defined areas of pulmonary vasculature or endothelial domains. However, due to limited insight into functions and the metabolism of these determinants, some of them will not be used for drug delivery due, for example, to safety concerns. However, it is most likely that all these determinants can be explored in animal models for the design of cell- or domain-specific probes or models of human pathologies, for example. However, in order to justify the development of a drug delivery system directed to an endothelial determinant and proposed for a clinical use, such a determinant and affinity carrier(s) must be carefully selected in the context of a particular pathological process that will be treated. Final selection must be based on the hypothesis-driven exploration accounting for the target functions, side effects of inhibition by targeting and the subcellular localisation of drugs.

Some determinants, such as ACE, aminopeptidase P, ICAM and PECAM, can be used for targeting either normal (i.e., prophylaxis) or pathologically altered (i.e., therapy) endothelium, whereas selectins permit the specific recognition of pathological endothelium. Functions of these endothelial determinants are well understood, which sets limits of their use due to potential inhibition or activation, in a given pathological context. This aspect (i.e., unintentional side effects caused by interventions into functional activity[ies] of the target molecules), acutely appreciated in the post-Vioxx era, will remain a key issue in the development of targeted delivery systems.

Targeting caveoli provides an exciting avenue for the intracellular and transcellular targeting in the pulmonary vasculature. Careful selection of targets and the modulation of such features of antibody–drug conjugates as valency and size provide powerful tools for the control of intracellular uptake and trafficking of cargos. Auxiliary pharmacological agents affecting shedding, internalisation and intracellular traffic of targeted drugs can also help to control precise localisation and

duration of the effects of targeted drugs. These therapeutic parameters can be further fine-tuned by capitalising on specific features of drug carriers, including relatively labile liposomes, polymer nanocarriers with built-in degradation and release rates and membrane permeating moieties.

Targeting of the active reporter and therapeutic cargos, including enzymes, genes and viruses, has recently been achieved in intact animals and animal models of human pathologies, thus providing a basis for the preclinical evaluation of the strategy and its translation into the clinical domain. These targeting systems promise unique advantages for the delivery of specific therapeutic agents in diverse clinical settings and, therefore, must be carefully tested in terms of the robustness, effectiveness, specificity and effects of the targeting, including the effects mediated by intervention in the functions of target determinants. Based on analysis of the literature, personal research interests or clinical experience, one can select specific human pathologies, such as acute lung syndrome, hyperoxic ventilation injury, lung transplantation, pulmonary oedema, thrombosis, hypertension, ischaemia/reperfusion and inflammation, as the most relevant and promising areas for the application of targeted drug delivery to the pulmonary vasculature.

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