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Review

Methods for the identification of vascular markers in health and disease: From the bench to the clinic

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ABSTRACT

Several diseases are characterized by changes in the molecular composition of vascular structures, thus offering the opportunity to use specific ligands (e.g., monoclonal antibodies) for imaging and therapy application. This novel pharmaceutical strategy, often referred to as "vascular targeting", promises to facilitate the discovery and development of selective biopharmaceuticals for the management of angiogenesis-related diseases. This article reviews novel biomedical applications based on vascular targeting strategies, as well as methodologies which have been used for the discovery of vascular markers of pathology.

Contents

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1. Vascular targeting

Ever since the pioneering work of Rudolf Virchow, diseases are frequently studied at the cellular level, with the aim to identify pathological abnormalities at the molecular level and at the site of disease, and to correct them by pharmacological intervention. However, it is becoming increasingly recognized that many diseases have a vascular defect component in their etiology or in their manifestations. For example, cancer is characterized by an uncontrolled proliferation of transformed cells, but the growth of solid tumors heavily relies on the formation of new blood vessels which provide oxygen and nutrients to the developing neoplastic mass [1]. Similarly, Alzheimer disease is characterized by abnormalities of neuronal cells (e.g., amyloid plaques, neurofibrillary tangles), but vascular changes appear to presage the neuropathological manifestations [2].

In the adult, the vasculature is essentially quiescent. In health, endothelial cells lining blood vessels divide only rarely and for specialized purposes (e.g., menstrual cycle, hair follicle cycle, fetus development). By contrast, the florid formation of new blood vessels is a pathological manifestation in wound healing and in many diseases associated with the growth of new blood vessels ("angiogenesis"), such as rheumatoid arthritis, psoriasis, diabetic retinopathy, age-related macular degeneration and atherosclerosis [3].

The realization that the neo-vasculature in cancer and other diseases can be different, at the molecular level, compared to the quiescent vasculature in normal organs, has stimulated the development of biomedical strategies, aimed at the inhibition of angiogenesis [4] or at vascular pharmacodelivery strategies ("vascular targeting"; [5–7]). One of the attractions of delivering bioactive molecules to new blood vessels is certainly the accessibility of these structures in vivo. For example, small organic molecules (e.g., cytotoxic agents) do not efficiently localize at the tumor site compared to normal organs, due to high interstitial pressure, irregular vasculature and multidrug resistance proteins [8]. By contrast, vascular targeting antibodies (i.e., monoclonal antibodies specific to markers of angiogenesis) can exhibit a striking ability to preferentially localize at the tumor site [9] and at other sites of the disease [10–14].

Monoclonal antibodies represent the most relevant class of biopharmaceuticals in terms of sales and growth potential [15]. While the majority of antibodies used in the clinical practice are directed against cellular antigens, recent ex vivo immunofluorescence studies have revealed that certain antibodies do preferentially localize to vascular structures in vivo (e.g., Herceptin in breast cancer; [16]), due to impaired extravasation and to their capture by perivascular antigen-containing cells [17]. Furthermore, the first inhibitors of angiogenesis introduced in the market are monoclonal antibodies specific to VEGF-A, an angiogenic factor (i.e., Avastin™ and Lucentis™; [4]).

When planning antibody-based vascular targeting strategies, one would intuitively privilege the use of monoclonal antibodies specific to antigens located in the luminal aspect of pathological blood vessels (Fig. 1), assuming that these structures should be more readily accessible from the bloodstream, as they do not require antibody extravasation. However, one should consider that luminal antigens on endothelial cells may display a limited abundance, thus forcing the use of low stoichiometric doses of targeting antibody, with severe consequences on the in vivo targeting efficiency and kinetics [18]. For this reason, antigens located in abluminal structures of pathological blood vessels (e.g., abundant and stable components of the modified extracellular matrix) may represent equally attractive targets for pharmacodelivery applications [19].

In this review, we survey some of the most relevant experimental methodologies, which have been used for the

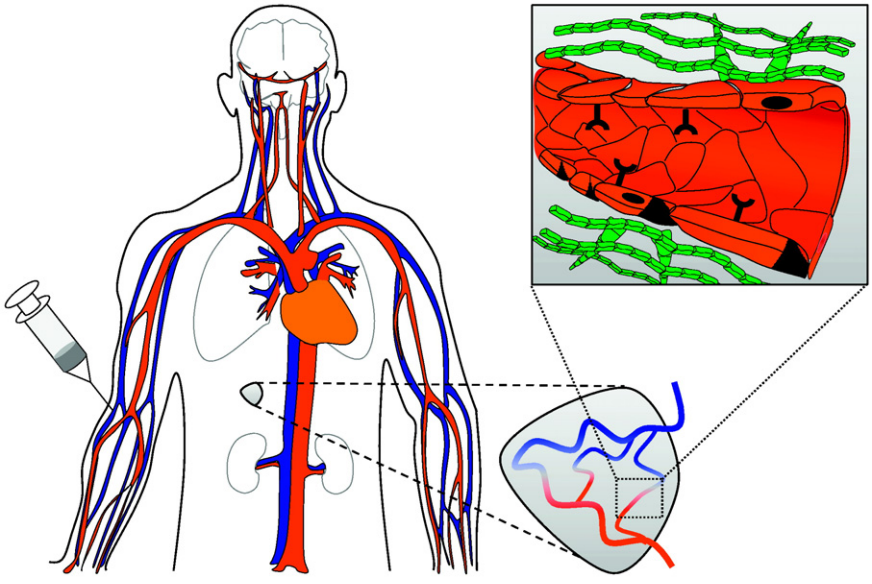

Fig. 1 – Illustrative depiction of the concept of tumor vascular targeting. Upon its intravenous injection, the targeted drug homes to a tumor-induced antigen either expressed on the luminal endothelial membrane (black) or in the perivascular extracellular space (green).