Vascular Targeting Agents and Strategies


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Vascular targeting agents are made up of two parts, a targeting moiety that is usually an antibody or growth factor that binds to specific vessel markers in diseased tissue and an effector moiety that induces the therapeutic effect, typically damage to vessels or occlusion of vessels. To limit toxicity, surface markers are needed that allow recognition of blood vessels in diseased tissues, and not vessels in normal tissues.

In tumors, neoplastic cells, stromal cells, or inflammatory cells impose a hostile environment on the tumor endothelium by secreting cytokines, secreting acidic metabolites that cause low pH, consuming oxygen resulting in hypoxia, and causing oxidative stress. These stresses cause upregulation of genes that are not normally expressed in endothelium. Tumor endothelium is dividing and remodeling, so it expresses proliferation and remodeling markers. Also, the basement membrane around angiogenic vessels is different (e.g., the ED-B domain of fibronectin is deposited around tumor vessels but is absent from the basement membrane of mature vessels). The endothelium of tumor vessels is discontinuous and allows antibodies that are in the blood to gain access to basement membrane components that are normally concealed. The tumor endothelium can also acquire markers from the environment, such as neutrophil-derived markers or bind growth factors (e.g., VEGF) to form complexes that are excellent markers of angiogenic vessels.

Markers that have been used successfully for vascular targeting of tumors include: (1) angiogenesis and remodeling markers, e.g., VEGF receptors, complexes of VEGF with receptors, \( \alpha, \beta \_3 \) integrin, fibronectin ED-B domain, endoglin, anthrax toxin receptor, MMP2 and 9, CD13, and prostate specific membrane antigen (PSMA); (2) cell adhesion molecules, e.g., VCAM1, E-selectin, VE cadherin; (3) markers of oxidative stress, e.g., the lipid, phosphatidylserine (PS). PS is normally confined to the internal surface of the plasma membrane of vascular endothelium but gets transposed to the external surface in response to oxidative stresses in tumors. Also, blood proteins that bind to the exposed PS, such as the annexins, can provide good tumor vessel markers.

Effectors that have been used successfully in rodent model systems include: (1) toxins, e.g., diphtheria toxin, ricin, and gelonin; (2) coagulation-inducing proteins, e.g., tissue factor; (3) cytokines IL2, IL12, and TNFa; (4) apoptosis-inducing factors, such as the Raf1 gene product, and the mitochondrial membrane disrupting peptide used by Pasqualini and coworkers; (5) radioisotopes, both \( \alpha \) and \( \beta \) emitters; (6) liposomal encapsulated effectors such as doxorubicin; and (7) photosensitizers such as SnChe6.

Effectors are not always needed, because naked antibodies can recruit the body’s own defense systems to destroy tumor vessels. Tarvacin is a naked anti-PS antibody which is currently in Phase I clinical trials in patients with various solid tumors. Tarvacin is currently in
the form of a chimeric antibody. It has a murine Fv region that binds to PS in a β2-glycoprotein I-dependent fashion. The Fv region is linked to human IgG1 constant regions. It is a nontoxic antibody at therapeutic doses even though antibodies of this type are sometimes associated with antiphospholipid syndromes. PS is usually confined to the internal surface of endothelium and is unavailable for binding to antibodies. But, in tumors, the endothelial cells are exposed to reactive oxygen species generated in response to hypoxia, cytokines, neutrophils, thrombin, or low pH. Lipid peroxidation probably occurs, generating calcium fluxes into the endothelium that activate the transporters that export PS to the luminal surface of the vessels. PS is one of the cleanest markers for tumor vessels yet discovered. It is absent from normal vessels including those in the ovary, a site of physiological angiogenesis, and the pancreas, a site of high vascular permeability. Treatment of mice with Tarvacin markedly reduces tumor growth in multiple tumor models, especially when combined with irradiation or cancer chemotherapy. It causes vascular damage resulting in disappearance of vessels in the center of the tumor with a surviving outer rim of tumor. This pattern of vascular damage is typical of vascular targeting agents and is thought to occur because vessels in the outer rim are subjected to lower interstitial pressures. Tarvacin recruits macrophages that bind to and damage the tumor endothelium, causing occlusion. This action is primarily an Fc-dependent process, macrophages and NK cells being the predominant effector cells. In addition, Tarvacin appears to cause a blockade of PS-mediated anti-inflammatory signals by switching the cytokines made by macrophages from TGFβ, a quiescence signal, towards TNFα and IL1, which are inflammatory mediators. Normally the macrophages produce TNFα and very little TGFβ, but when they are exposed to intact or apoptotic cells with PS on their external surface, they switch to producing TGFβ and little TNFα. This mechanism may explain why macrophages engulf PS-expressing apoptotic cells but do not engage in inflammatory responses to them. The TGFβ may also stimulate VEGF production by tumor cells as part of the proangiogenic response of macrophages. So, Tarvacin homed to tumor vessels, induces host cells to attack the tumor vessels, and may then re-educate macrophages to mount cytotoxic responses rather than proangiogenic responses towards PS-expressing tumor vessels and tumor cells.

A second application of vascular targeting is ligand targeted liposomal chemotherapy being developed in the Ponzoni laboratory in Genoa, Italy in collaboration with the Allen laboratory in Alberta, Canada. Liposomes are PEGylated to provide stealth and then NGR peptides are attached that recognize CD13 on activated endothelial cells and pericytes. The liposomes are loaded with doxorubicin. Upon intravenous injection, the liposomes enter the tumor vasculature, bind to CD13, get taken up, and doxorubicin is released into endothelial cells and pericytes causing regression of the tumor vasculature. In addition, because the tumor vessels are leaky, the liposomes leak into the tumor interstitium and form a depot of drug. So, NGR-liposomes localize to tumor vessels and pericytes, extravasate into stromal tissue, and are taken up by endothelial cells and pericytes causing regression of tumor vessels.

A third example of vascular targeting is an application that is being explored for treatment of choroidal neovascularization (CNV). This is a collaboration between the Campochiaro laboratory at Johns Hopkins and the Rosenblum laboratory at M.D. Anderson in Houston, TX. The drug is a fusion protein generated by ligation of VEGF121 cDNA to cDNA for gelonin, a plant toxin that inhibits protein synthesis. The construct is expressed in E. coli and forms a disulfide bonded VEGF/rGel dimmer. VEGF/rGel binds to VEGFR2 receptors resulting in internalization into endocytic vesicles and transport to the Golgi-ER complex. Gelonin is released into the soluble phase of cytoplasm where it turns off protein synthesis by damaging
ribosomes. Either intravenous or intravitreous injection of VEGF/rGel causes regression of CNV, ischemia-induced retinal neovascularization, or neovascularization in rhodopsin promoter/VEGF transgenic mice. The basis of the targeting is the increased expression of VEGFR2 on endothelial cells participating in retinal or choroidal neovascularization. This results in homing of VEGF/rGel and selective damage to new vessels within the eye.