

## **Unmasking IV-accessible vascular targets specific to solid tumors and single organs by tissue subfractionation, subtractive proteomics and molecular imaging**

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Molecular imaging and medicine can benefit from the discovery of new tissue-specific targets that are inherently accessible to agents injected intravenously. Using our recently developed tissue subfractionation techniques for purifying luminal endothelial cell plasma membranes and caveolae directly from normal organs as well as tumors, we have generated multiple distinct high-resolution proteomic maps of the endothelial cell surface directly in contact with the circulation. We have used mass spectrometry, de novo sequencing, and database searching coupled with antibody generation for immunoblotting, tissue immunostaining, intravital microscopy, and SPECT imaging in vivo to compile an extensive proteomic database including multiple validated IV-accessible vascular targets specific to single organs or solid tumors. Novel organ- and even tumor-induced targets have been identified in mouse, rat, monkey and human tissues and then used to image the vasculature of whole organs and tumors selectively after intravenous injection of monoclonal antibodies in rodents. SPECT imaging rapidly visualizes the tissue-specificity of immunotargeting with high sensitivity and objectivity. Our new antibodies show clearly that tissue-specific delivery to individual solid tumors or single normal organs can indeed be achieved in vivo at levels approaching the theoretical expectations of the vascular targeting strategy. Up to 90% of the IV-injected dose of antibody can accumulate in a single tissue within 30 min. For antibodies specifically targeting caveolae, both tissue staining and live dynamic imaging by intravital microscopy reveal rapid targeting of a single vascular bed with extensive transport across the endothelial cell barrier leading to much improved tissue penetration. Electron microscopy shows additional details including selective antibody entry and binding within caveolae followed by rapid transcytosis and uptake by underlying cells inside the tissue. Our novel profiling strategy directly reveals distinct molecular signatures for the endothelial cell surface in each major organ (lung, kidney, liver, heart & brain) and in solid tumors including breast, lung, kidney, liver, brain and prostate. This new development and optimization of several key technologies encompassing tissue subfractionation, subtractive proteomics and molecular imaging permits rapid discovery and validation of tissue-specific targets that are inherently accessible to agents injected intravenously. Profiling IV-accessible vascular & caveolae targets is an important logical step not only for achieving site-directed pharmacodelivery & selective molecular imaging in vivo but also for overcoming the normally restrictive endothelial cell barrier to transport drugs & even genes to their intended target cells inside the tissue.