Ligands Designed for Targeting Nanoparticles Differentiate Normal and Atherosclerotic Tissue

Pillai J, Ruegsegger MA Biomedical Engineering Department, Division of Cardiovascular Medicine, and Davis Heart & Lung Research Institute, The Ohio State University, Columbus, OH 43210

Statement of Purpose. Cardiovascular disease, particularly atherosclerosis, remains one of the largest health issues in the United States. Because the progression of coronary plaque occurs over years and decades, the possibility exists to detect the growing plaque using non-invasive imaging modalites, and provide suitable clinical and drug administration to control or even reverse the condition. Nanotechnological approaches are being developed in our lab to engineer multifunctional nanoparticles that incorporate targeting, MRI-contrast, and drug delivery capabilities. In this particular study, the targeting capability of ligands designed for these nanoparticles has been demonstrated. The cell-surface ligands directed to endothelium have been tested and analysed for binding to atherosclerotic tissues and model endothelial cell lines.

Methods. In these experiments, three targeting ligands (antibodies) directed against human markers were tested for their use in targeting endothelium covering atherosclerotic plaque in an ex vivo rabbit model as well as on human aorta endothelial cells (HAECs) in vitro. To perform immunohistochemical (IHC) experiments on ex vivo tissue samples, 7 micron thick sections were prepared from two rabbit models. New Zealand (NZ) rabbit aorta samples represented healthy endothelium, while the Watanabe (WAT) rabbit aorta has been identified as a good model of spontaneous plaque formation in the coronary artery and other arterial vessels. All antibodies were tested at the maximal recommended titer of 10 mg/ml and incubated for 90 minutes. A secondary Goat antibody directed against mouse carried a biotin tag that was targeted by a streptavidin-conjugated Horse Radish Peroxidase (HRP) tertiary. These were incubated for 60 and 45 minutes, respectively. Finally, the staining agent, diaminobenzidine (DAB), was activated in the presence of HRP to give brown coloration in the case of a positive result. Goat serum was used as a blocking buffer as recommended by manufacturers (it blocks against non-specific binding of the secondary). Negative controls were included by adding Mouse IgG1K (to test non-specific binding), a control without the primary antibodies (to test non-specific binding of the secondary) and a control with only blocking buffers. An identical sequence of reagents was used for the model cell studies in vitro. Images were taken with a high-resolution optical microscope with CCD camera for image capture.

Results / Discussion. The results from the IHC ex vivo experiments showed that one of the targeting ligands demonstrated differential binding between the healthy and plaque-laden tissues. The staining from this ligand was highly specific to endothelium on both tissue types, with negligible non-specific staining of the other cell types within the tissue. The images also indicated that the targeting ligand could differentiate between the tissue samples. In the healthy New Zealand rabbit tissue, the luminal stain was observed as a dark, single layer. Conversely, the stain was almost absent on the Watanabe tissue section of endothelial layer covering plaque (Figure 1). These results suggest information about the endothelium cell surface, namely that markers present at the surface of healthy endothelium may be absent (down-regulated) in disease-state endothelium. For this particular ligand, then, the effect is that the plaque is identified through negative selection. Also important is that the targeting ligand only binds to endothelium and not other cells, such as fibroblasts or smooth muscle cells.

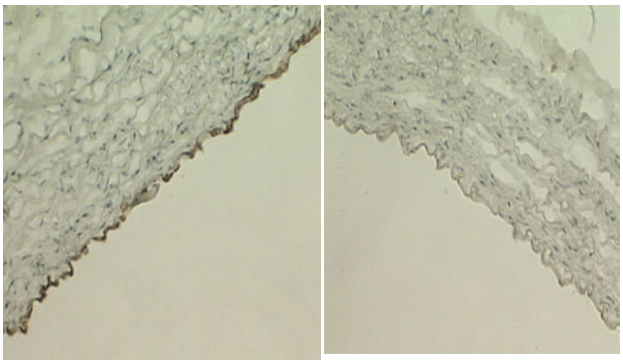

Figure 1. Immunohistochemistry tissue cross-sections showing differential binding of an endothelial cell surface marker. (Left) Targeted-staining to endothelial layer only of healthy NZ rabbit aorta. (Right) Absence of stain indicated targeting ligand did not bind to endothelium of plaque-laden tissue.

Conclusion. The ability to target specific tissues, normal and disease-state, is a critical component for developing nano-scale particles that can detect, analyse, and deliver drugs to the desired location. This study has demonstrated that antibody-based ligands can be used to differentially bind endothelium in the disease-state, i.e. atherosclerotic plaque. Future studies extending from this research will explore the binding potential of ligands to other disease-state endothelial markers, such as inflammatory, thrombogenic, and cell-cell/cell-extracellular matrix adhesion markers. This targeting approach is particularly relevant to nanoparticle design, since it provides both a vascular pathway and disease-specific target for their localization and subsequent detection in vivo.

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