

## **Phage display selection of tissue-specific homing peptides and their theragnostic applications**

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### **ABSTRACT**

Several homing peptides that are specific to tumor cells, atherosclerotic plaques, stroke lesion, apoptotic cells and phosphatidylserine have been isolated by phage display in our laboratory. For example, the Bld-1 peptide is specific to bladder tumor cells and the AP-1 to atherosclerotic plaques. These peptides could be used in targeted delivery either alone or in combination with nanoparticles. We have demonstrated that the Bld-1 peptide can be homing to bladder tumor in vivo and showed a tumor specific PET imaging in rat and mouse models. Moreover, we assembled the Bld-1 peptide with hydrophobically-modified glycol chitosan (HGC) nanoparticles for carrying an anticancer drug and a nearinfrared optical imaging dye for targeted delivery and imaging simultaneously. The AP-1 peptide alone or assembled with HGC nanoparticles could home to atherosclerotic plaques in LDL receptor-deficient mice. These results demonstrate the potential of the homing peptide technology for affinity-based drug targeting and molecular imaging.

### **1. INTRODUCTION**

#### **1.1. Molecular diversity of pathological tissues**

Growing evidence shows that pathological tissues and even their vessels are distinct from their normal counterparts at molecular levels and put their own signature on the tissue and vasculature. For example, tumor blood vessels and atherosclerotic plaques carry distinct molecular markers like  $\alpha v\beta 3$  integrin and increased vascular cell adhesion molecule-1 on endothelial cell surface, respectively (Ruoslahti, 2002; Libby, 2002).

#### **1.2. Phage display selection of tissue-specific homing peptides and their applications**

In vivo and in vitro screening of phage libraries have provided peptides that specifically recognize disease-specific signatures (Lee et al., 2007; Pasqualini and Ruoslahti, 1996). Phage libraries can have as many as  $10^{10}$  different peptides (Smith, 1985). An example of a homing peptide is the three-amino-acid sequence RGD motif that homes to tumor vascular endothelial cells through binding to the  $\alpha v\beta 3$  integrin. Homing peptides can be used to targeted delivery of

therapeutic and imaging agents or to decorate the surface of nanoparticles containing these agents and thus can enhance the efficacy of the treatment while reducing the side effects.

## 2. MATERIALS AND METHODS

### 2.1. Screening of phage library specific for bladder tumor and PET imaging

A T7 phage library displaying CX7C random peptides was used for screening. Phages that preferentially bind to HT-1376 bladder tumor cells were selected and sequenced. A synthetic peptide was radiolabeled with [ $^{124}\text{I}$ ] and administered into tumor-bearing nude mice for PET imaging.

### 2.2. Phage display for atherosclerotic plaque-specific peptides and optical imaging

The phage library was screened for peptides that bind to cells from primary atherosclerotic plaques. A synthetic peptide was conjugated with HGC nanoparticles containing Cy7.5 dye and administered into LDL receptor-deficient mice fed on a high cholesterol diet.

## 3. RESULTS AND DISCUSSION

### 3.1. PET imaging of bladder tumor with [ $^{124}\text{I}$ ]-labeled Bld-1 peptide

The most promising bladder tumor-specific peptide was selected and named as Bld-1. [ $^{124}\text{I}$ ]-labeled Bld-1 peptide was intravenously administered into HT-1376 tumor-bearing nude mice. After circulation for 5 h, PET imaging was taken (Figure 1).

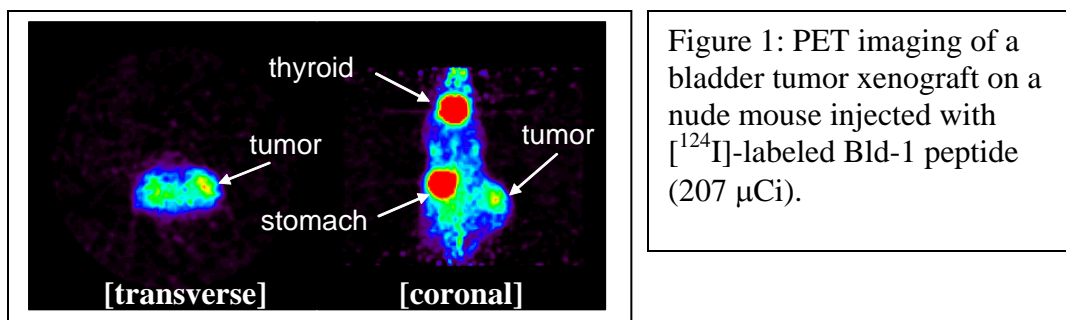


Figure 1: PET imaging of a bladder tumor xenograft on a nude mouse injected with [ $^{124}\text{I}$ ]-labeled Bld-1 peptide (207  $\mu\text{Ci}$ ).

### 3.2. Optical imaging of atherosclerosis with AP-1 peptide assembled with HGC-Cy7.5

AP-1, a promising atherosclerotic plaque-specific peptide, was assembled with HGC-Cy7.5 nanoparticles and intravenously administered into LDL receptor-deficient mice. After circulation for 6 h, imaging was taken using Optix explorer (Figure 2).

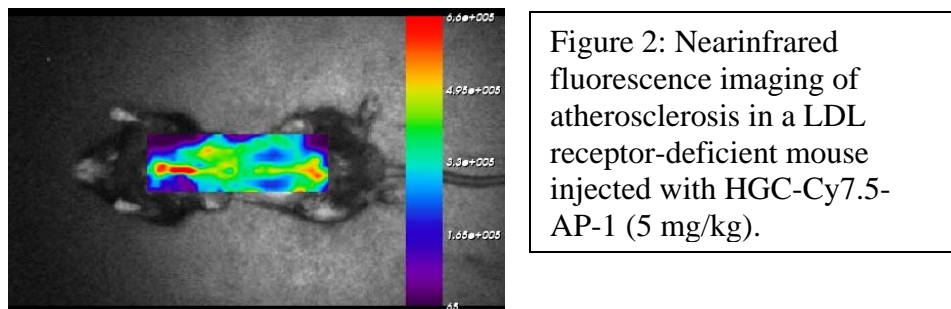


Figure 2: Nearinfrared fluorescence imaging of atherosclerosis in a LDL receptor-deficient mouse injected with HGC-Cy7.5-AP-1 (5 mg/kg).

#### 4. CONCLUSIONS

The tissue-specific Bld-1 and AP-1 peptides identified by phage display may be useful as a targeting moiety for selective drug delivery and molecular imaging of bladder tumor and atherosclerosis, respectively.

#### 5. ACKNOWLEDGMENTS

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