

# In vitro and in vivo reactivity of heparin-binding peptides with human and murine tumors.

Jonathan S. Wall<sup>1,2</sup>, Amy K. LeBlanc<sup>2</sup>, Tina Richey<sup>1</sup>, Alan Stuckey<sup>2</sup>, Murthy Akula<sup>2</sup>, George Kabalka<sup>2</sup>, Emily Martin<sup>1</sup>, Sallie Macy<sup>1</sup>, and Stephen J. Kennel<sup>1,2</sup>.

Departments of <sup>1</sup>Medicine or <sup>2</sup>Radiology, University of Tennessee Graduate School of Medicine, Knoxville, TN.

## Introduction

The ability to specifically target tumors and, in particular, metastases is critically important for the accurate diagnosis, disease staging and treatment of patients with cancer. The most commonly used radiotracers for non-invasive tumor imaging are <sup>18</sup>F-DG and <sup>18</sup>F-FLT (PET) and <sup>99m</sup>Tc-labeled octreotide (SPECT). Both <sup>18</sup>F-DG and <sup>18</sup>F-FLT accumulate at sites of hypermetabolic activity and/or cell division, such as tumors in the case of <sup>18</sup>F-DG, sites of inflammation, the myocardium and the brain are additional areas of accumulation. Octreotide binds the somatostatin receptor which is expressed in high concentrations on many neuroendocrine and pancreatic tumors. In contrast to <sup>18</sup>F-DG and <sup>18</sup>F-FLT, tumor targeting by peptides affords an opportunity for both imaging and therapy for use in patients with cancer.

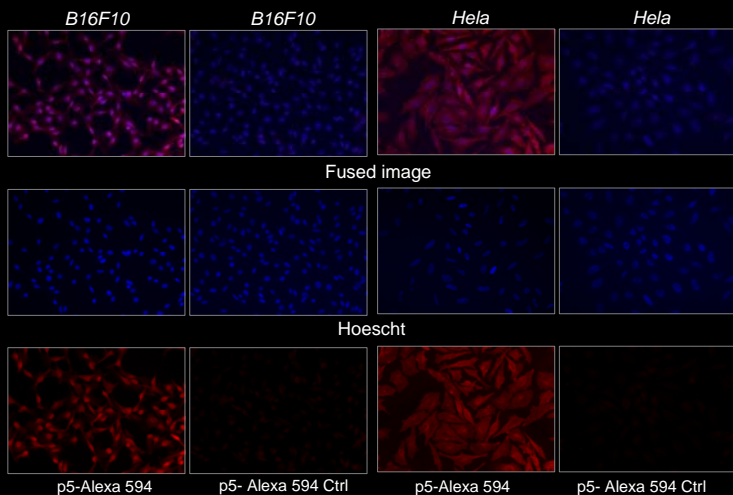
Here we describe our preliminary observation that heparin-binding peptides can target hypersulfated, tumor cell surface-expressed proteoglycans. Peptides labeled with biotin for histological staining or radioiodinated peptides for in vivo SPECT/CT imaging specifically detected tumors in mice. Based on our initial observation that the heparin-binding peptide, p5, bound basal cell carcinoma tumor in a tissue overlay assay, we surveyed the reactivity of this, and related peptides, with a series of tumor core biopsies and for reactivity with murine melanoma pulmonary metastases in C57B/6 mice by using SPECT/CT imaging and micro-autoradiography.

GGGYS KAQKA QAKQA KQAQK AQAQK AKQAK Q

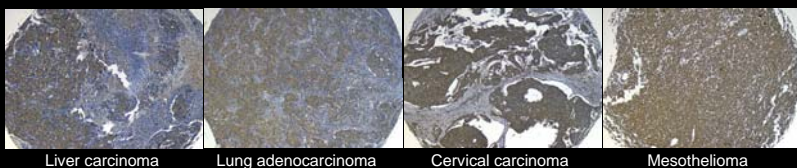
|                             | Peptide p5                   |
|-----------------------------|------------------------------|
| MW <sup>1</sup>             | 3,303.7                      |
| Theoretical pI <sup>1</sup> | 10.31                        |
| Net charge                  | +8                           |
| Source                      | chemically synthesized       |
| Target in amyloid           | heparan sulfate proteoglycan |
| Catabolism                  | kidney                       |

**Figure 1.** Primary Structure of peptide p5 and table of physical properties.

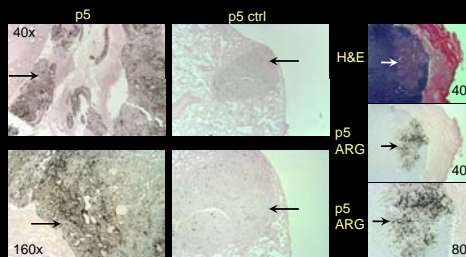
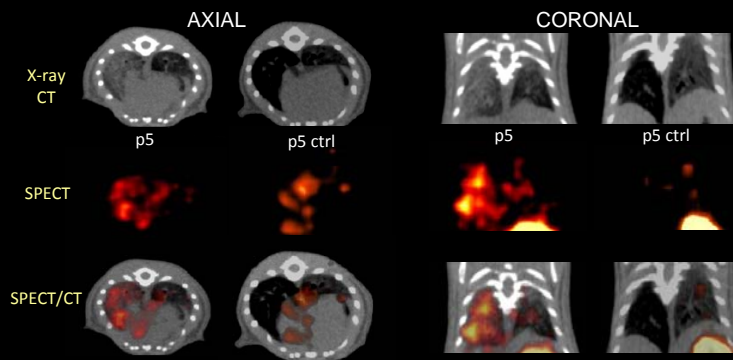
<sup>1</sup>Calculated using, [www.expasy.org/cgi-bin/protparam](http://www.expasy.org/cgi-bin/protparam)



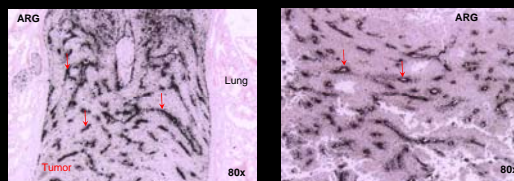
**Figure 2.** Peptide p5 binds murine melanoma, B16F10 and human cervical cancer, HeLa cells in culture. Alexa-594 (red)-labeled p5 or control peptide was incubated with cells in culture and the interaction visualized by using fluorescence microscopy.



**Figure 3.** Peptide p5 binds a ligand expressed by carcinoma cells as well as other tumors such as mesothelioma. Biotinylated peptide p5 was used to stain a human tissue array. The presence of reactivity with tumor cells was evidenced by brown diaminobenzidine staining.



**Figure 4.** Peptide <sup>125</sup>I-p5 binds pulmonary colonies of B16F10 in C57Bl6 mice. SPECT/CT imaging revealed the co-localization of peptide with tumors that were visible in axial and coronal CT images. Notably, a control, size-matched peptide (ctrl), did not bind the tumors. The lungs and regional lymph nodes were excised post mortem and micro-autoradiographs (ARG) prepared. The presence of <sup>125</sup>I-p5 was seen as black staining in both pulmonary tumors and those that metastasized to the regional lymph nodes.



**Figure 5.** A p5 peptide variant was shown to preferentially associate with blood vessels within the B16F10 pulmonary tumors in C57Bl6 mice by using autoradiography.

## Results and Discussion

Biotinylated peptides were shown to bind preferentially to human carcinomas histochemically in a panel of 35 biopsy samples. Reactivity was particularly strong with liver, cervical and lung carcinomas. No reactivity was observed with sarcomas or lymphomas, but modest binding was observed with human melanoma. This reactivity was confirmed by fluorescence microscopy using biotinylated peptide and HeLa human carcinoma and B16F10 murine melanoma cells grown in culture. Finally, by using SPECT/CT imaging, we detected pulmonary colonies of B16F10 cells in C57Bl/6 mice and demonstrated co-localization of <sup>125</sup>I-labeled p5 peptides but not control peptides with the lesions by microautoradiography. Binding of the <sup>125</sup>I-p5 peptide was not uniform throughout the tumor mass, and one peptide specifically associated with tumor vasculature.

Heparanase expression by tumor cells results in the truncation of heparan sulfate glycosaminoglycan chains and their subsequent increased sulfation. We have now shown that heparin-binding peptides, such as p5, bind preferentially to carcinoma and melanoma tumors of both human and murine origin. Although preliminary, these data support the further investigation of these and similar reagents for the radiodetection and radiotherapy of appropriate tumors.