

Integrin-specific near infrared optical imaging agent for tumor-induced angiogenesis detection in mice

Joy L. Kovar¹, Rose A. Skopp¹, Melanie A. Simpson², and D. Michael Olive¹

¹LI-COR Biosciences, Lincoln, NE 68504; ²University of Nebraska-Lincoln, NE 68588-0664

ABSTRACT

Tumor-induced angiogenesis is a common feature of malignant neoplasms including melanoma, glioma, prostate, and breast cancer. Integrins, heterodimeric glycoprotein receptors, mediate cell-to-cell and cell-to-matrix interactions and are over-expressed in the tumor microenvironment. The Arg-Gly-Asp (RGD) tripeptide is a specific ligand for the integrin receptor $\alpha_v\beta_3$, to which it binds with high affinity. We sought to develop a near infrared dye labeled RGD probe for monitoring diseases related to $\alpha_v\beta_3$ receptor over-expression. A cyclic-RGD peptide was conjugated to the near infrared dye, IRDye[®] 800CW (ex/em 778/794 nm). Specificity and sensitivity of the dye-labeled agent for multiple tumor cell types (i.e., U87, A431, PC3M-LN4, and 22Rv1) were evaluated using the In-cell Western plate-based assay. Increasing concentration of IRDye 800CW RGD (0-500 nM) produced a dose-dependent response while incubation with unlabeled RGD (0-10 μ M), in addition to IRDye 800CW RGD (0.5 μ M), blocked the labeled agent, confirming specificity to the target receptor. Subsequent *in vivo* studies were performed to identify the optimal dose and imaging time point post-injection in nude mice bearing subcutaneous tumors derived from glioblastoma (U87) and epithelial carcinoma (A431). Mice

were imaged with the Pearl[™] Imager and in all cases, IRDye 800CW RGD localized to tumor tissue with good signal-to-noise characteristics. Successful *in vivo* competition for binding of IRDye 800CW RGD was achieved by administering a dose of unlabeled RGD prior to labeled RGD injection, upon which fluorescence was reduced by 72%, confirming specificity of the targeting agent. These data demonstrate IRDye 800CW RGD will be an effective optical imaging tool for the study of tumor biology in mice.

INTRODUCTION

Integrins are a family of heterodimeric glycoprotein receptors consisting of an alpha and beta subunit which mediate cell-to-cell and cell-to-matrix interactions. The $\alpha_v\beta_3$ integrin is over-expressed on many tumor cell types and on endothelial cells of tumor-associated angiogenic vessels. Inappropriate expression of this receptor plays a critical role in the regulation of tumor growth, progression, and metastasis.[1] Biomarkers specifically targeting cell surface receptors have widespread efficacy in noninvasive optical detection of cancer and other diseases.[2,3] Several

groups have reported the use of fluorescently labeled cyclic-pentapeptides [2,4,5] for the detection of integrin over-expression in tumor specific tissue in mice.

Near infrared (NIR) optical imaging (650-900 nm) is a technology well suited to the preclinical evaluation of disease and molecular processes. Its appeal is partially attributable to the optical sensitivity achieved in this spectral range, in which the inherent autofluorescence of tissues and water is relatively low.[6-8] IRDye 800CW excitation and emission maxima are ideally situated in the NIR spectrum to yield superior sensitivity and low background when imaging in mice.[9] Prior dual-labeling of a cyclic-RGD peptide with ¹¹¹In and IRDye 800CW showed effective, specific targeting of $\alpha_v\beta_3$ in melanoma cells.[10]

Our objective with this study was to produce an IRDye 800CW RGD optical imaging agent for the detection of tumors in which $\alpha_v\beta_3$ is over-expressed. Subsequent characterization of IRDye 800CW RGD confirmed the agent is specific to tumor tissue both *in vitro* and *in vivo*, providing another imaging tool for preclinical evaluation of tumor biology in mice.

CONCLUSIONS

Our studies demonstrate IRDye 800CW RGD maintained specificity for human tumor cell targeting *in vitro* and in mouse xenografts. We have optimized and characterized a non-invasive optical imaging agent, IRDye 800CW RGD, for tracking tumors over-expressing integrin receptors in mice.

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RESULTS

Labeling:

❖ Cyclo-(Arg-Gly-Asp) containing peptide was labeled with IRDye 800CW NHS ester (LI-COR Biosciences) and purified by chromatography.

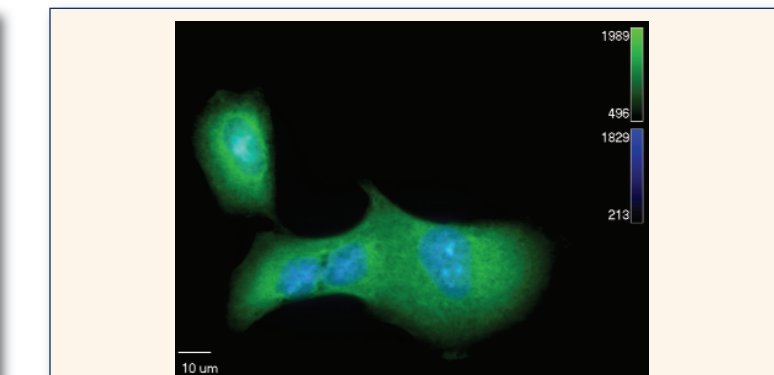
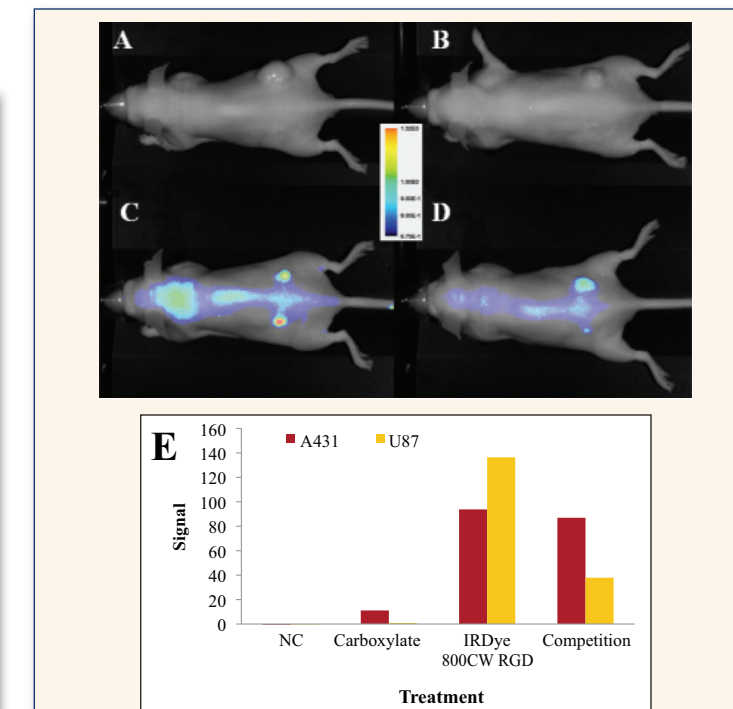
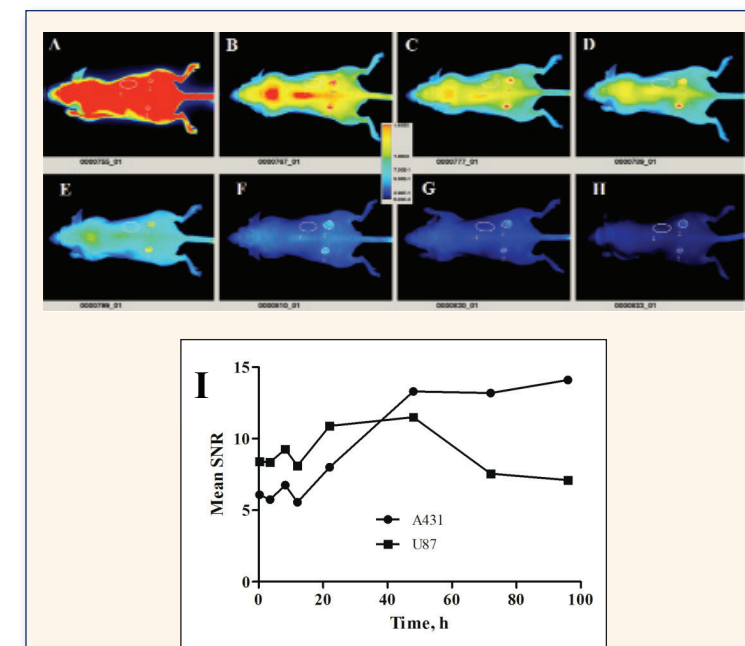
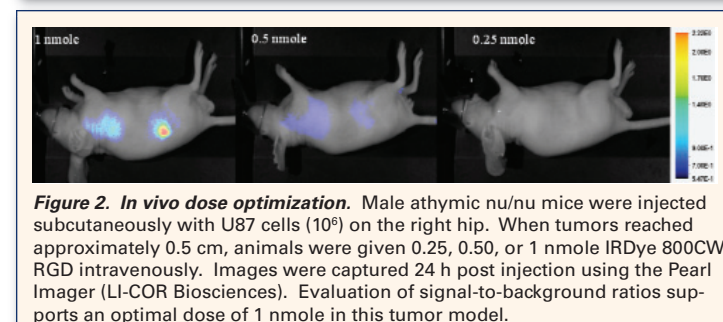
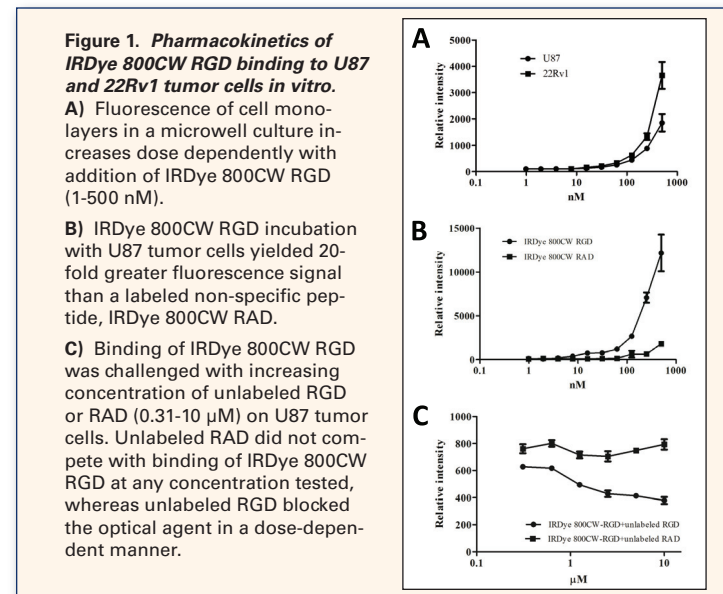


Figure 5. Deconvolution fluorescent microscopy confirms internalization of IRDye 800CW RGD. Lung carcinoma cells were incubated with IRDye 800CW RGD (6.25 μ M, shown in green) for 3 h followed by a 5 min incubation with Sytox green nuclear stain (1 μ M, shown in blue). Image was captured using an Olympus IX 71 / IX 81 inverted system microscope (40X oil).