Cancer cells are often characterized by a high metabolic rate exemplified by a high glucose consumption rate. This biological activity has been exploited for noninvasive imaging by positron emission tomography using glucose analogues such as 2-fluoro-2-deoxy-D-glucose (FDG) as a tracer for localized signal. In this work, our goal was to adapt a similar methodology for optical imaging of tumors in mice.

We selected a fluorophore with maximal excitation and emission wavelengths in the near-infrared (NIR) spectral range (700-900 nm), where low absorption coefficients of tissues allow greater optical sensitivity, deeper tissue penetration, and low autofluorescence. The NIR fluorophore, IRDye® 800CW (excitation/emission of 778 nm/794 nm), was covalently coupled to 2-deoxy-D-glucose (2DG). The resultant conjugate was evaluated first for specificity and sensitivity in vitro. Specificity of binding to an In-Cell Western assay, in which concentration dependence of label uptake was established by fluorescence changes in a high throughput microplate format. Uptake of the labeled agent was specifically blocked in a dose-dependent manner by addition of unlabeled 2-deoxy-D-glucose. Subsequent in vivo studies were conducted to optimize dosing, clearance, and optimal time-point post-injection for signal capture in nude mice. A research prototype imaging assay was used to detect IRDye 800CW signal, which was used to characterize the IRDye® 800CW 2DG optical agent in subcutaneous tumors derived from either an epithelial carcinoma (A431 cells), colorectal carcinoma (SW620 cells), or prostate carcinomas (PC3M-LN4 and 22Rv1). In all cases, the tumors were clearly imaged with good signal-to-noise characteristics. This pilot demonstration suggests that IRDye® 800CW 2DG will be a general optical imaging tool for studying tumor biology in mice.

**RESULTS**

**Labeling**

IRDye® 800CW 2-deoxy-D-glucose hydrochloride (2DG, Sigma-Aldrich, St. Louis) was labeled with IRDye 800CW NHS ester (LI-COR Biosciences) and purified by chromatography.

We labeled 2DG with a near infrared fluorophore, IRDye 800CW, and tested its ability to accumulate in tumors in mice. Specific staining of several different tumor cell lines was evaluated in vitro by dose dependence of fluorescent signal and competition with unlabeled 2DG. A prototype imager optimized for IRDye® 800CW detection was then used to quantify the signal strength derived from this targeting agent in tumors. The sensitivity of the agent was such that even with the high signal-to-noise ratio of the targeting is unknown, IRDye® 800CW 2DG may be a useful agent for tumor imaging in vivo.

**Administration**

Administration of 10–20 nmol of the agent allowed the tumors to be clearly visualized at 24 hours post-injection.

**RESULTS**

**Labeling**

2-amino-2-deoxy-D-glucose hydrochloride (2DG; Sigma-Aldrich, St. Louis) was labeled with IRDye 800CW NHS ester (LI-COR Biosciences) and purified by chromatography.

**IN VITRO BINDING AND COMPETITION ASSAYS**

Confirmation of binding and specificity of NIR-conjugated 2DG was accomplished with an In-Cell Western (ICW) whole cell fluorescence microplate assay.

**RESULTS**

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IRDye® 800CW 2DG was effective for tumors derived from A431 (epidermoid), 22Rv1 (prostate), and SW620 (colon) cell lines. Uptake of the agent differed with tumor type.

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