

Monitoring Progression of Prostate Tumors in Mice By Receptor-Targeted Near Infrared Optical Imaging

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ABSTRACT

Progression of prostate cancer to metastatic disease is a leading cause of cancer death in men. Mechanisms of prostate cancer growth, invasion, and metastasis are studied in mice by surgical implantation of tumor cells in the prostate. Use of sensitive noninvasive imaging techniques to track progression in this model would greatly facilitate screening of therapeutics. In this report, we have specifically targeted prostate tumor cells with EGF conjugated to IRDye™ 800CW and used near-infrared optical fluorescent imaging to plot a time course of tumor growth following orthotopic injection.

Two model human cell lines were used in the study. PC3M-LN4 cells are well characterized for prostate tumorigenesis and eventual spontaneous metastasis to paraaortic lymph nodes. 22Rv1 cells were previously uncharacterized. Twelve NOD/SCID mice were evaluated using EGF-IRDye™ 800CW and a prototype near infrared 2D imaging system optimized for IRDye™ 800CW detection. Five mice per cell line received weekly tail vein injections of EGF-IRDye™ 800CW (1 nmol). One mouse per line was preserved as a dye-negative control. Weekly imaging of tumor growth was performed 96 hours post-dye injection. Despite multiple intervening tissue layers and depths of up to 1 cm, tumors were readily detected in several prostates as early as 2 weeks, and in all the mice by 3 weeks. Fluorescence continued to intensify specifically in the prostate over the remainder of the study. Tumor images were analyzed for signal-to-noise ratio and correlated at termination to tumor volume and wet weight, as well as signal intensities per square millimeter in tumor sections. Absence of lymph node metastases in 22Rv1-injected animals was noted. Specific fluorescence of tumor cells in positive nodes of PC3M-LN4-injected mice was also confirmed by imaging upon termination. This is the first longitudinal study of prostate cancer progression in mice using near-infrared fluorescence.

INTRODUCTION

Human prostate cancer initially arises as a neoplasia within the prostate epithelium. Progression of the tumor to invasive, metastatic disease may occur over a period of years, and is delayed by androgen ablation therapies in early stages. Tumor cells that resume growth independently of androgens will frequently metastasize to lymph nodes and bone. Complications from bone metastasis are the most common cause of prostate cancer mortality.

To study mechanisms of metastatic progression, we utilize a pre-clinical model in which human prostate tumor cells are surgically implanted into the prostates of immunocompromised mice. Studies of such mechanisms could be advanced by use of non-invasive imaging to detect growth and regression of tumors in response to treatments or manipulations. In previous experiments, we determined that systemic injection of a fluorophore-conjugated human growth factor, EGF-IRDye™ 800CW, led to EGF receptor-mediated accumulation of the dye specifically within subcutaneously growing tumors (Kovar *et al.*, 2005). The tumors were then easily visualized in anesthetized mice by near infrared optical imaging. In this study, we sought to extend these findings to the longitudinal tracking of tumors in murine prostate and identification of spontaneous lymph node metastases.

MATERIALS AND METHODS

Six-week-old male NOD/SCID mice were injected orthotopically with 10⁵ tumor cells. Two human prostate tumor cell lines were evaluated: PC3M-LN4, a highly tumorigenic and metastatic cell line, and 22Rv1 cells, previously uncharacterized in this model system. The biomarker, EGF-IRDye™ 800CW, was used for detection of tumors. On week 1, post-tumor cell implantation, 1 nmol of dye-conjugate was injected via the tail vein. A time course of dye clearance using signal to noise ratio (SNR) as a readout established the maximum specificity at 96 hours. Images were collected weekly for five weeks at 96 hours after dye injection. A competition challenge was included, in which C225 anti-EGF receptor blocking antibody was used to confirm specificity of the probe in the prostate tumors and lymph nodes.

Near-infrared fluorescence imaging of isoflurane-anesthetized live animals was performed with a prototype LI-COR® Biosciences small-animal imager (Lincoln, NE). Images were acquired and analyzed with Wasabi software from Hamamatsu Photonics (Hamamatsu City, Japan).

At the termination of the study, tumors were imaged, excised, weighed, caliper measurements taken, and tissue sections prepared.

RESULTS AND DISCUSSION

A pictorial view of prostate tumor growth in NOD/SCID mice from both cell lines used in this study is shown in Figure 1.

When imaging internal tumor growth in a longitudinal study, a major concern is the ability to follow and correctly estimate tumor size. Figure 2 (PC3M-LN4 tumor) and Figure 3 (22Rv1 tumor) show representative images for one animal gathered over the experiment's time period. In previous work with this cancer model system and the prototype LI-COR Biosciences small-animal imager, a threshold SNR of 3 was determined to be the limit of detection for positive tumor tissue (Kovar *et al.*, 2005). Therefore, only pixels that met or exceeded the SNR standard in the region of interest were included in the estimation of tumor size over time. Area (mm²), tumor signal intensity per mm², and peak SNR were determined. These parameters showed similar trends over time for both prostate tumor cell lines. We were able to monitor tumor growth effectively with EGF-IRDye™ 800CW in the orthotopic tumor model with tumor depths of up to 1cm.

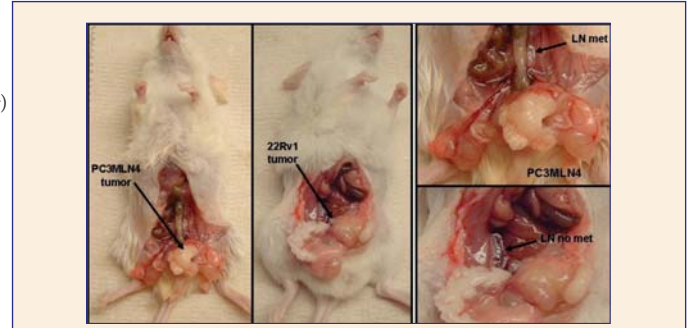


Figure 1. Modeling human prostate cancer progression. Surgical orthotopic implantation and spontaneous metastasis to lymph nodes are shown for PC3M-LN4 and 22Rv1 cell lines.

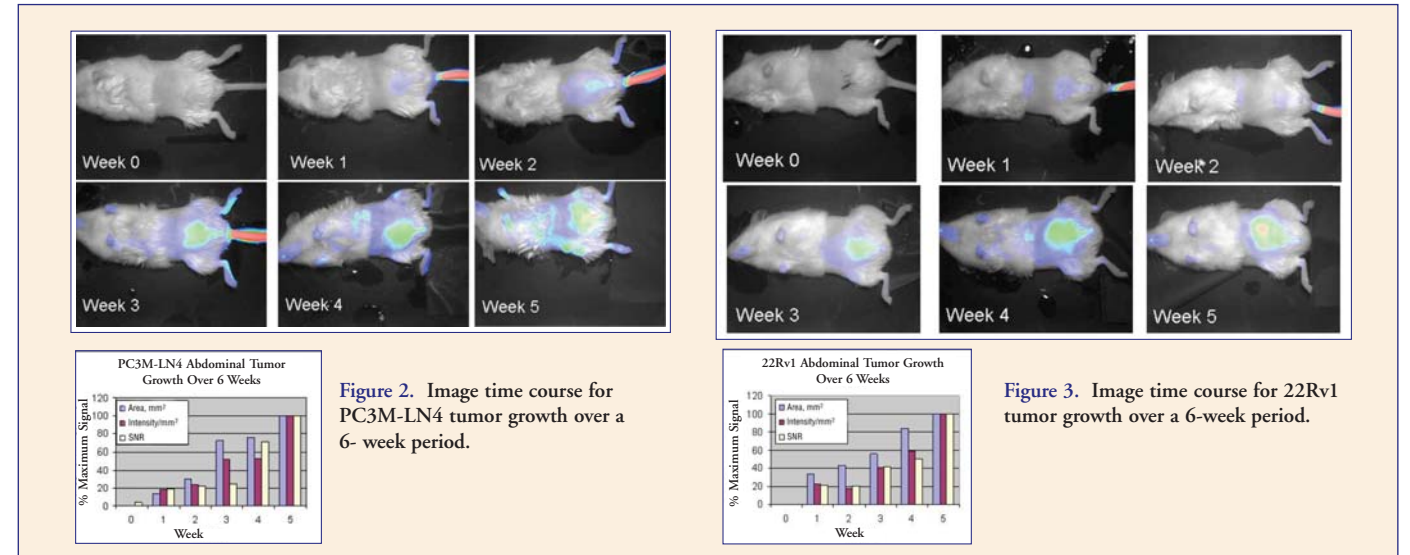


Figure 2. Image time course for PC3M-LN4 tumor growth over a 6-week period.

Figure 3. Image time course for 22Rv1 tumor growth over a 6-week period.

When tumor volume (mm³), weight (mg), and SNR were compared, the 22Rv1 treatment group maintained a higher average in all analysis categories relative to PC3M-LN4 tumors (Figure 4).

Further confirmation of the specificity of EGF-IRDye™ 800CW for the prostate tumors was achieved by recovering tumors and lymph nodes at the termination of the study. Frozen tissue sections from the challenge animals were scanned on the Odyssey® Infrared Imaging System (LI-COR Biosciences) for signal intensity and area determinations. Analysis showed that when C225, a monoclonal antibody that blocks the EGF receptor, was given 24 hours prior to the EGF-IRDye™ 800CW, approximately 39% blocking was achieved (Figure 5). Deposition of the EGF-IRDye™ 800CW throughout the tumor was evident in both sections receiving labeled biomarker.

A primary objective of this study was to determine lymph node involvement with both cell lines and to evaluate EGF-IRDye™ 800CW as an indicator of metastasis. PC3M-LN4 is known to metastasize to the paraaortic lymph nodes, while 22Rv1 was uncharacterized. At the termination of the study, prostate tumors and lymph node regions were imaged in the open abdominal cavities. No enlargement or sign of metastases were noted for animals with 22Rv1 prostate tumors, even though those tumors were, on average, 3-fold larger by weight and volume compared to the PC3M-LN4 prostate tumors. Lymph nodes for animals with PC3M-LN4 prostate tumors appeared enlarged and opaque, visually exhibiting the characteristics of metastases. When imaging lymph nodes in an opened abdomen with a large prostate tumor, signal from the lymph nodes, though detected, is dampened by the high signal in the region (Figure 6). However, if the tumor is excised or covered, the signal in the lymph nodes is clearly evident.

Lymph nodes from the challenge animals were excised and scanned on Odyssey to confirm the presence of EGF-IRDye™ 800CW in paraaortic lymph nodes from the PC3M-LN4 animals (Figure 7). Although large prostate tumors developed in mice receiving 22Rv1 cells, these animals showed no signs of metastasis to the lymph nodes by week 6. The excised PC3M-LN4 nodes were analyzed in Odyssey and signal per area (mm²) determined. Comparisons of the lymph nodes from EGF-IRDye™ 800CW and C225+ EGF-IRDye™ 800CW treatments showed approximately 34% blockage in the lymph nodes compared to the 39% noted in the prostate tumors. These results suggest that the effect of C225 is extended to the site of metastasis, further confirming the specificity of the biomarker with this tumor model system.

CONCLUSIONS

We have shown that unlike PC3M-LN4 tumors, 22Rv1 tumors exhibited no spontaneous metastasis to paraaortic lymph nodes, even with large prostate tumors present. Use of the EGF-IRDye™ 800CW biomarker and non-invasive optical fluorescent imaging allowed tumor size to be tracked in deep abdominal tumors, as well as in lymph nodes, confirming metastases and providing the researcher with immediate confirmation without the need for histology. *In vivo* signal antagonism by C225 antibody confirms the specificity of the EGF-IRDye™ 800CW biomarker.

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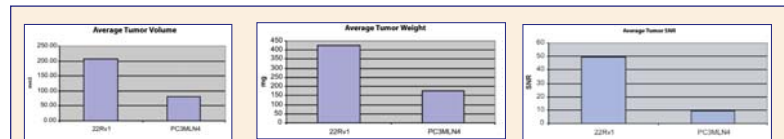


Figure 4. Averages for 22Rv1 and PC3M-LN4 prostate tumors after excision. SNR were calculated from images taken on week 6.

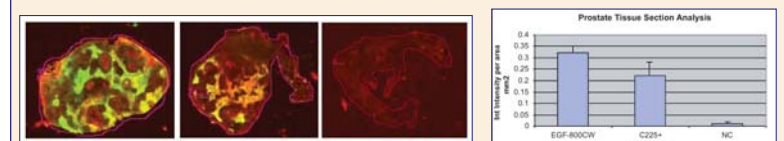


Figure 5. Frozen tissue section analysis of challenge mice (PC3M-LN4 derived tumors; n=4) showing a 39% signal reduction when C225 treatment preceded EGF-IRDye™ 800CW. Green signal represents 800 nm = EGF-IRDye™ 800CW; red signal represents 700 nm = autofluorescence of tissue; yellow signal represents overlap of the two channels.

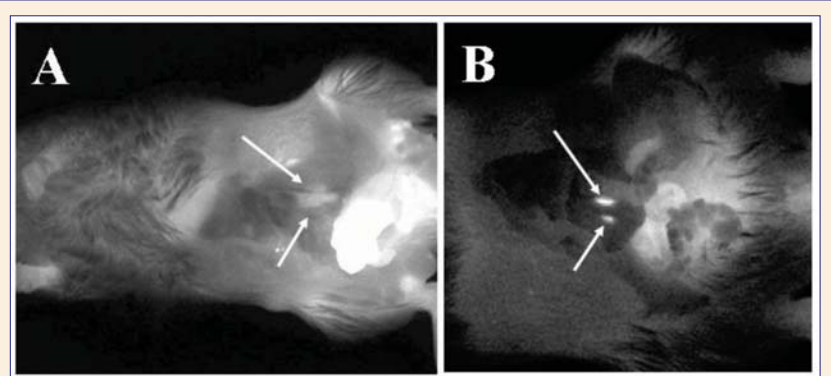


Figure 6. Panel A shows large lymph nodes and tumor. Arrows point out the nodes. Panel B shows that when the tumor is removed the lymph nodes become clearly visible due to the presence of labeled tumor cells.

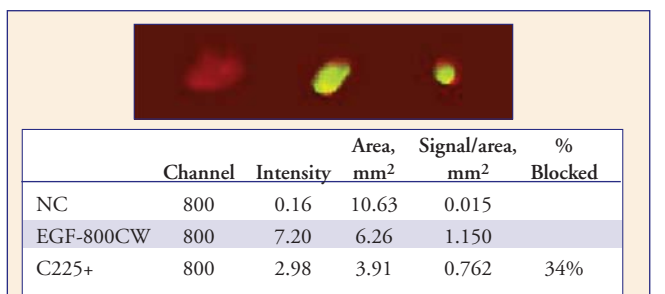


Figure 7. Lymph nodes from positive animals (PC3M-LN4 derived tumors) along with a negative control node (left). Green represents signal from 800 nm channel and red represents signal from the 700 nm channel (autofluorescence and background).