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Multiscale Optical Molecular Imaging of Cancerous Cells and Environments *In Vivo*

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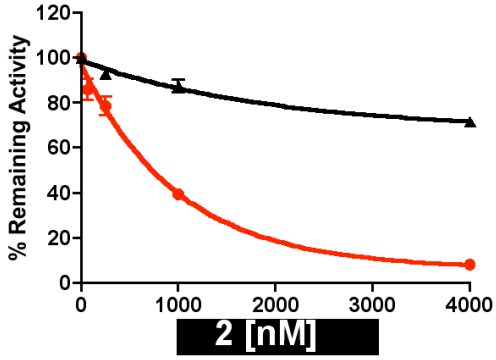
Modern instrumentation and optical probes allow us to examine quantitatively dynamic processes within ever more complicated biological systems, including live animals. Diagnostic imaging of cancers to date is limited by the non-selective accumulation of imaging agents in normal tissues. Thus, to broaden the applicability of these optical approaches novel quantitative approaches and new probes must be developed. We have developed *in vivo* detection tools for several different cancer related proteins and their activity. Two examples of this work are presented here.

To examine the interplay between tumor cells and the microenvironment during early breast cancer metastasis, we developed a technique for ex-vivo imaging of murine tissue explants using two-photon microscopy [1]. Breast cancer metastasis was compared in the liver and the lung, by imaging both organs 24 hours after the injection of the same polyoma middle T-initiated murine mammary tumor cell line. In contrast to the lung, where the majority of the tumor cells remained intravascular at 24 hours, the majority of tumor cells in the liver had extravasated by 24 hours. In the liver, the majority of imaged cells transitioned from an intravascular location to an extravascular site between 12 hours (30.2% extravascular) and 24 hours (52.7%). Within the liver microenvironment, the average colony size of the micrometastatic lesions increased 4-fold between days 5 and 12. Histologic analysis of these lesions determined that by day 12 the micrometastases were heterogenous, consisting of both tumor cells and von Willebrand Factor-positive endothelial cells. Further analysis with iv-administered lectin indicated that vessels within the micrometastatic tumor foci were patent by day 12. These data present the use of two-photon microscopy to highlight the differences in early metastatic patterns between two important secondary sites in breast cancer progression that may have future implications for therapy.

We have also developed a suite of specific imaging agents for cyclooxygenase-2 (COX-2) expression, and have utilized these agents as a tool for COX-2-targeted imaging of tumors [2]. The imaging agents are synthesized derivatives of NSAIDs containing functional groups targeted to COX-2 and containing specific imaging functionalities for red or near infrared (NIR) fluorescence. For this purpose, several NSAIDs (indomethacin, reverse indomethacin, celecoxib) were linked to a wide range of fluorophores through variable length tethers. Fluorophore moieties selected for conjugation included fluorescent coumarin, NBD, Alexa Fluor dyes, TMCR, TECR, 5-ROX, nile blue, cy5 or cy7-dyes, NIR-dyes, and IR-dyes (IRdye800)**.** These fluorescent NSAID conjugates were evaluated for inhibitory potency against purified COX-1 and COX-2 enzymes, and selective COX-2 inhibitors were then tested for their ability to inhibit the enzyme in intact head and neck squamous carcinoma cells. One compound, LM-4777 that uses a rhodamine derived fluorescent tag, was identified as the most promising lead compound in the series. LM-4777 is a selective COX-2 inhibitor with an IC_{50} of 92 nM in intact cells. We next investigated the efficacy of LM-4777 as an imaging agent *in vivo*. *In vivo* fluorescence imaging of nude mice bearing COX-2-expressing tumor xenografts on the left rear flank showed significant uptake of the probe in the xenograft, documented as bright red fluorescence at 3.5 h post-administration of 2 mg/kg of LM-4777 in DMSO. A negative control with nude mice bearing HCT116 xenografts showed minimal fluorescence. We

evaluated the targeting of tumors in the C57BL/6J-*Min*/+ mouse adenoma model and in the C57BL/6 Lewis lung carcinoma (LLC) model, both of which are COX-2 expressing tumors. We administered LM-4777 by retro-orbital injection into *min* mice, followed by removal and fixation of the small intestines and colon after 2 h. Images showed bright LM-4777 fluorescence in the small intestinal polyps compared to the surrounding tissue at a ratio of > 20:1, polyp: normal tissue. Control and LLC-mice were dosed with LM-4777 (2 mg/kg, i.p.). Fig. 2 shows the COX inhibition curveand an in vivo image of the compound targeting a tumor on the mouse flank. Thus, LM-4777 successfully targeted the COX-2-expressing tumors in nude mouse xenograft, as well as in vitro and cell culture models.



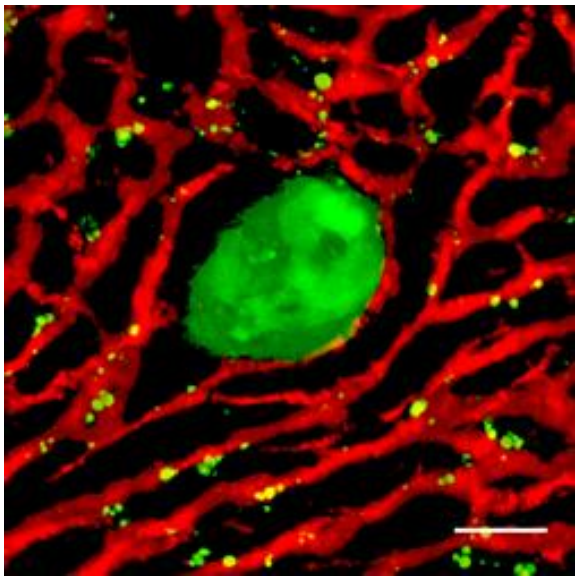


Figure 1: Two-photon micrograph of R221A-GFP tumor foci (green) in the liver at 5 days after tumor cell injection. Lectin-Qdot585 used to label the vasculature (red).

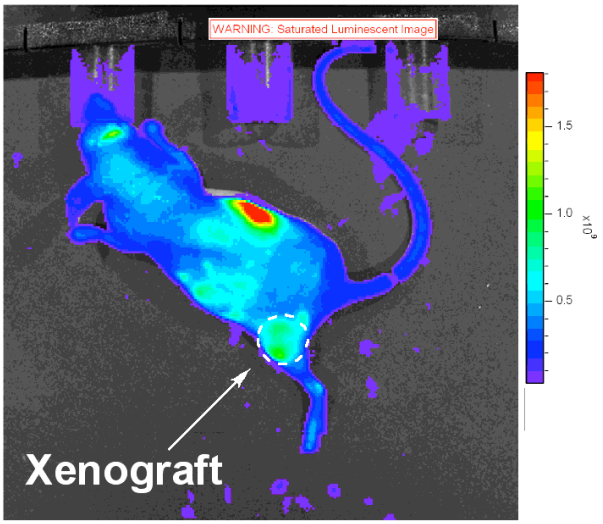


Figure 2: S Figure 5. *In vivo* labeling of COX-2-expression. a, COX enzyme inhibition curve. **b,** Nude mouse bearing a 1483 HNSCC xenograft on the left flank dosed (ip) with 2 mg/kg LM-4777. Images taken in a Xenogen IVIS (DsRed filter, 1 s) system at 3.5 hrs post-injection.

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