Multivalent Fluorescent Probes for In Vivo Tumor Targeting

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Abstract

Conventional fluorescence probes are monovalent molecules that interact with cellular or extra-cellular target molecules through affinity binding. To improve the binding affinity and sensitivity of detection of the fluorescence probe, we describe a novel strategy for the synthesis designed to contain multiple targeting moieties and a near infrared fluorescent dye Iflour750 for optimal in vivo detection. In this study, we applied several multivalent probes in vivo tumor targeting and monitored the targeting process using fluorescence imaging. Deoxyglucose based fluorescent probes have been applied to tumor targeting as the high metabolic rate of the tumor cells causes preferential labeling of the tumor mass. With a multivalent 2-desoxy-D-glucose Iflour750 (2-DG-IF750) probe, we demonstrated sensitive in vivo targeting as compared to a control mono-valent 2DG probe in several tumor models, including PC3M-luc2 prostate tumor cells, MDA-MB-231-luc2 mammary for tumor cells as well as LLC-luc and H460-luc lung cancer cells. Distinct tumor signal was visualized within a few hours after intravenous delivery and reached the peak at approximately between 2-34 hours. With a multivalent cytochrome (CDO) binding fluorescence probe Nifluoxin (NIT), we observed an interesting targeting of CDO negative cancer cells HT116-luc2, but not the CDOx positive HT29-luc2, hypothetically due to selective binding to CDOX. We also tested a multivalent RGD probe targeting integrin α5β3, the expression of which is associated with tumor angiogenesis and metastasis. With our Fluorocoxib (DG) probes, we demonstrated in vivo targeting of U87-MG, HT1080-luc2, HT116-luc2 and HT29-luc2 tumor cells. Finally, we demonstrated that optical imaging was conveniently applied in evaluating the PK/PD profile of the probes. Our multivalent probes showed a fast clearance with a half-life of less than 1 hour, which is a desirable feature for achieving maximal tumour/background contrast. In summary, we have developed a new class of multi-valent fluorescence probes with improved specificity and sensitivity of tumor targeting as compared to mono-valent analogues. Utilization of this class of multivalent probes may help to perform preclinical models, as imaging guided tumor surgery is at the verge of clinical applications.

Results

2-DG-IF750 Clearance

Figure 3. Female Nu/Nu mice were i.v. injected with 10 nmol of 2-DG-IF750 probe and imaged with IVIS Spectrum at indicated time points. Data presented as tumor to background ratio ± SEM.

2-DG-IF750 Probe vs. IF750 Dye for Tumor Targeting

Figure 4. Female C57Bl mice were i.v. injected with 10 nmol of 2-DG-IF750 probe and imaged with IVIS Spectrum at indicated time points. Data presented as tumor to background ratio ± SEM.

Bioimunescence and jCT Imaging of Metastatic MDA-MB-231-luc2 Tumors

Figure 5. A. PC3M-luc2 cells (3 million) were sc injected to the right flank of Nu/Nu mice. At day26, mice were i.v. injected with (RGD)4-IF750 probe (2 nmol/mouse) and imaged with IVIS Spectrum at indicated time points using 745nm excitation and 800 nm emission filter set.

B. Detection of Apoptosis and Necrosis of PC3M-luc2 Tumors

Figure 6. A. Specific tumor targeting by the 2-DG-IF750 probe as compared to the IF750 dye in naive mice. Fluorescence images showed specific signal from 2-DG-IF750 injected mice but not the IF750 injected control (A). Co-localization of the 2-DG-IF750 probe with the Cox2 binding domain in black. In vitro binding of the FluoroCoxib A probe to Cox2 positive HT29-luc2 cells was shown after imaging with NuanceFX. Staining of the cytoplasm by the probe was displayed in red. Nuclei staining with DAPI was shown. Bioluminescence images showed comparable tumor signal from both 2DG-IF750 and IF750 injected mice. (B) Quantification of fluorescence signal from tumor (left flank) and background (right flank). Data presented as tumor to background ratio ± SEM.

Bioluminescence and jCT Imaging of Metastatic MDA-MB-231-luc2 Tumors

Figure 7. A. Specific tumor targeting by the 2-DG-IF750 probe as compared to the IF750 dye in naive mice. Fluorescence images showed specific signal from 2-DG-IF750 injected mice but not the IF750 injected control (A). Co-localization of the 2-DG-IF750 probe with the Cox2 binding domain in black. In vitro binding of the FluoroCoxib A probe to Cox2 positive HT29-luc2 cells was shown after imaging with NuanceFX. Staining of the cytoplasm by the probe was displayed in red. Nuclei staining with DAPI was shown. Bioluminescence images showed comparable tumor signal from both 2DG-IF750 and IF750 injected mice. (B) Quantification of fluorescence signal from tumor (left flank) and background (right flank). Data presented as tumor to background ratio ± SEM.

B. Detection of Apoptosis and Necrosis of PC3M-luc2 Tumors

Figure 8. A. Specific tumor targeting by the 2-DG-IF750 probe as compared to the IF750 dye in naive mice. Fluorescence images showed specific signal from 2-DG-IF750 injected mice but not the IF750 injected control (A). Co-localization of the 2-DG-IF750 probe with the Cox2 binding domain in black. In vitro binding of the FluoroCoxib A probe to Cox2 positive HT29-luc2 cells was shown after imaging with NuanceFX. Staining of the cytoplasm by the probe was displayed in red. Nuclei staining with DAPI was shown. Bioluminescence images showed comparable tumor signal from both 2DG-IF750 and IF750 injected mice. (B) Quantification of fluorescence signal from tumor (left flank) and background (right flank). Data presented as tumor to background ratio ± SEM.

Conclusions

- Multivalent 2-DG-IF750 probe showed specificity on the tumors. This probe exhibited a short half-life and is ideal for achieving a high signal to background ratio. Although not shown, the 2-DG-IF750 probe is superior to a monovalent 2-DG fluorescence probe in both sensitivity and specificity.
- Tumor targeting with (RGD)4-IF750: Imaging guided tumor surgery was performed with NuanceFX system. Targeting of the 2-DG-IF750 probe towards the PC3M-luc2 tumor caused preferential labeling of the tumor mass. With a multivalent 2-deoxy-D-glucose Iflour750 (2-DG-IF750) probe, we demonstrated sensitive in vivo targeting as compared to a control mono-valent 2DG probe in several tumor models. In this study, we applied several multivalent probes in vivo tumor targeting and monitored the targeting process using fluorescence imaging. Deoxyglucose based fluorescent probes have been applied to tumor targeting as the high metabolic rate of the tumor cells causes preferential labeling of the tumor mass. With a multivalent 2-desoxy-D-glucose Iflour750 (2-DG-IF750) probe, we demonstrated sensitive in vivo targeting as compared to a control mono-valent 2DG probe in several tumor models, including PC3M-luc2 prostate tumor cells, MDA-MB-231-luc2 mammary for tumor cells as well as LLC-luc and H460-luc lung cancer cells. Distinct tumor signal was visualized within a few hours after intravenous delivery and reached the peak at approximately between 2-34 hours. With a multivalent cytochrome (CDO) binding fluorescence probe Nifluoxin (NIT), we observed an interesting targeting of CDO negative cancer cells HT116-luc2, but not the CDOx positive HT29-luc2, hypothetically due to selective binding to CDOX. We also tested a multivalent RGD probe targeting integrin α5β3, the expression of which is associated with tumor angiogenesis and metastasis. With our Fluorocoxib (DG) probes, we demonstrated in vivo targeting of U87-MG, HT1080-luc2, HT116-luc2 and HT29-luc2 tumor cells. Finally, we demonstrated that optical imaging was conveniently applied in evaluating the PK/PD profile of the probes. Our multivalent probes showed a fast clearance with a half-life of less than 1 hour, which is a desirable feature for achieving maximal tumour/background contrast. In summary, we have developed a new class of multi-valent fluorescence probes with improved specificity and sensitivity of tumor targeting as compared to mono-valent analogues. Utilization of this class of multivalent probes may help to perform preclinical models, as imaging guided tumor surgery is at the verge of clinical applications.

Methods

Structures of the Multivalent 2DG-IF750 and RGD-IF750 Probes

Figure 1. Structure of the Multivalent 2DG-IF750 and RGD-IF750 Probes.

Figure 2. Study Design and Schedule

Tumor implantation I.V. injection of cancer cells. At 2-3 weeks, mice were sc injected with 2-DG-IF750 probe (10 nmol/mouse) and imaged with IVIS Spectrum at indicated time points using 745nm excitation and 800 nm emission filter set.

Figure 3. Study Design and Schedule

References