Optical imaging of bombesin and transferrin receptor expression is comparable to ¹⁸F-FDG in assessing early drug efficacy

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Abstract

Physical measurement of tumor volume reduction is the most commonly used method for assessing tumor progression and treatment efficacy in mouse tumor xenograft models. The time for a therapeutic to achieve tumor regression can be long, and detection of gross changes can require repeated drug dosing and tumor measurements for several weeks. However, ¹⁸F-FDG PET imaging of altered glucose metabolism has been shown to be a more sensitive tool for early cancer detection/diagnosis as well as treatment assessment; cancer cells are known to have abnormally increased cellular metabolism that can be inhibited by drug treatment. To illustrate this, we used HCT-116 human colorectal tumor xenografts in nu/nu mice with sorafenib treatment, a clinically approved tyrosine protein kinase inhibitor (5 days/week of therapeutic treatment at 40 mg/kg for 2 weeks). Treatment is known to inhibit PDGFR and VEGFR, as well as Raf kinases involved in regulating energy metabolism in cancer cells. GB PET/CT imaging of sarofenib-treated mice using ¹⁸F-FDG revealed a significant drop in tumor metabolism with as little as 2.3 days of treatment, a time in which there is typically little or no effect on tumor size. This approach was relatively low throughput, and required special procedures to accommodate use of radioactivity, but offered the important option of daily imaging of tumor status. We also explored alternative optical imaging approaches that could offer higher throughput and as well as potential for multiplex imaging. There is no effective fluorescent equivalent of ¹⁸F-FDG, so we focused on bombesin- and transferrin-receptor as potential biomarkers for drug-induced inhibition in tumor metabolism. Bombesin receptors are upregulated in a variety of tumors and are important in energy metabolism and tumor growth. The rapid recycling kinetics also make this receptor highly sensitive to cellular metabolic changes. Transferrin receptors are also upregulated in most tumors and provide critical iron transport function vital for their increased enzymatic, proliferative, and metabolic requirements. We used targeted near infrared (NIR) fluorescent imaging probes, Bombesin/FlaMe® 680 (BRs-680) and Transferrin-Vivo® 750 (TA5-750), to monitor changes in receptor expression in HCT116-luc tumor xenografts during the course of sorafenib treatment. Interestingly, both BRs-680 and TV-750 fluorescence imaging (FLI) on the IVIS® SpectrumCT yielded data quite similar to our results using ¹⁸F-FDG PET, reduction in probe signal can be measured as early as 48-72 hours, in the absence of significant reduction of tumor size/viability. As expected, both PET and FLI were also highly effective at imaging sorafinib effects 7.8 days later, with datasets in good agreement with physical measurement of changes in tumor size. These results suggest that BRs-680 and TV-750 can serve as fluorescent surrogates for ¹⁸F-FDG PET both in measuring early metabolic changes and ultimate therapeutic outcomes following cancer treatment.

Optimization of sorafenib treatment in HCT116-luc tumor using bioluminescence imaging (BLI) and tumor volume assessment

A. Efficacy of high dose sorafenib (120 mg/kg)

B. Dose optimization of sorafenib in HCT116-luc tumor model to provide early window of apparent non-responsiveness by BLI and tumor volume

1. Treatment as early detection

2. Conceptual model for tumor biology changes in progression and treatment

3. PET imaging of ¹⁸F-FDG detects metabolic changes in tumors 2-3 days after treatment, prior to changes in tumor volume

4. Fluorescence imaging using bombesin- and transferrin-receptor specific probes is effective in assessment of high dose sorafenib treatment outcome

5. Fluorescence imaging of BRs-680 and TV-750 detects early therapeutic response with low-dose sorafenib in HCT116 tumor-bearing mice

6. Comparison of PET and optical approaches to imaging early metabolic changes in tumors in response to treatment

Summary

In these studies we demonstrated the synergistic use of bioluminescence, fluorescence and PET imaging to depict a comprehensive picture of tumors’ responses to drug treatment. Combining the intrinsic bioluminescence generated within the tumor and the extrinsic fluorescence generated using imaging probes, we were able to acquire both viability and metabolic data, respectively, to detect early biological changes in response to targeted cancer therapy prior to changes in viability status. Our results show that significant disturbances in metabolic activity occurred very early, within 2-3 days of treatment, and our FLI approach was corroborated by the gold standard in metabolic imaging, ¹⁸F-FDG PET. Both PET and FLI offer their own unique advantages, but we believe the optical imaging method may be a reasonable alternative for pre-clinical development of anti-cancer drugs that are designed to target tumor energy metabolism.