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Cover Story

Quantitative non-invasive imaging of target engagement in small animals

Targeted drug delivery is essential for enabling personalized medicine. Nevertheless, even after decades of intense research efforts and clinical evaluation, especially in the case of oncology, targeted drug delivery is still in its infancy stage. Despite an increasing refined understanding of cancer biology and the development of potent anticancer drugs, efficient drug delivery specifically to cancer cells and subsequent intracellular transfer has been elusive. One of the difficulties in studying intracellular drug delivery is the lack of tools to evaluate it in live subjects noninvasively. Current approaches are either invasive or unable to discern between cell-bound ligands and unbound ligands present near the cancer cells.

In this issue, the research team lead by Professors Intes and Barroso presents a novel Macroscopic Fluorescence Lifetime Imaging Forster Resonance Energy Transfer (MFLI-FRET) platform to quantify ligand-receptor binding in breast cancer xenograft tumors in live intact mice [1]. It is based on measuring FRET by estimating the reduction of the fluorescence lifetime of the donor fluorophore when it is within 2-10 nm to one or more acceptor fluorophores. Most importantly, MFLI-FRET can discriminate soluble fluorescently labeled ligands from those bound by dimerized receptors. Hence, MFLI-FRET reports on molecular events at the macroscopic level in live, intact animals.

The Intes-Barroso team capitalized on the homodimeric nature of the transferrin receptor (TfR) to quantify transferrin (Tf) internalization into cancer cells by measuring FRET between receptor-bound Tf-labeled donor and acceptor near-infrared (NIR) fluorophore pairs. The transferrin receptor (TfR) is typically overexpressed in cancer cells in solid tumors due to their increased metabolism and proliferation. The receptor-ligand target engagement in tumor xenografts was validated by comparing FRET levels (related to the fraction of the bound transferrin) from *in vivo* imaging with the results of histological analysis of intracellular ligand accumulation and receptor density in excised tumors. The authors demonstrated a strong correlation between FRET levels and transferrin internalization into tumor cells despite the significant heterogeneity of tumors regarding their size and cellular density. In contrast, no correlation between MFLI-FRET and TfR levels was observed, underscoring the insufficient link between receptor density and intracellular drug delivery. This has an important implication that receptor expression and tumor drug delivery cannot be estimated via *ex vivo* immunohistochemistry. MFLI-FRET measurements correlate with ligand binding to tumor cells, but strikingly, not with ubiquitously used *ex vivo* receptor expression assessment. Thus, a high level of receptor expression, which is a major criterion for determining targeted therapy strategy, does not guarantee the efficacy of treatment because of drug internalization issues.

The significant advantage of MFLI-FRET imaging is its versatile nature, allowing the use of any ligand/homodimer receptor as well as therapeutic antibodies. In addition, MFLI-FRET has a potential for multiplexing by simultaneously using two target receptors, e.g., TfR and HER2 to monitor and quantify drug internalization. Multiplexing, of course, depends on a development and validation of additional FRET pairs. Since MFLI-FRET is not limited to breast cancer or other cancer types, it can be applied toward imaging of any disease-targeted drug delivery, such as inflammatory disorders.

MFLI FRET can be applied only to antibody- or ligand/receptor-dependent drug delivery. In addition, for homo-dimerized receptors, MFLI-FRET has an inherent limitation to quantify only a fraction of ligand/receptor interactions, up to 50%. Potential receptor clustering has the opposite effect possibly allowing the FRET signal to increase. Protease-based drug targeting can also benefit from FRET since the FRET behavior would reverse. There are also a few studies using FRET to monitor drug released from liposomes that could take advantage of MFLI for *in vivo* imaging studies. FRET is versatile, yet tricky. Another limitation of this approach right now is a limited resolution in the order of 1 mm. Increasing resolution is a current focus of the Intes-Barroso team. To this end, they have pioneered another non-invasive optical imaging modality, Mesoscopic Fluorescence Molecular Tomography, that may offer up to a 100 μm resolution in subcutaneous tumor xenografts.

Beyond preclinical studies, MFLI-FRET is also not readily translatable to the clinic for use in humans. This is not a challenge inherent to MFLI-FRET, but a ubiquitous challenge for all new molecular imaging and/or drug delivery methodologies developed over the last decade. However, there are still a large variety of clinical scenarios in which this new technique may find an application. These range from guiding a therapeutic regimen by following drug response in human derived tumor organoids to profiling receptor dimerization status on fresh excised tissue at bed side. Though its main impact is most likely to be on the drug development pipeline at the pre-clinical stage, NIR MFLI-FRET provides unique information, i.e., target-receptor engagement that integrates target selection and *in vivo* intracellular delivery in a direct and quantitative readout. Hence, NIR MFLI-FRET is well positioned to significantly impact the development of targeted therapeutics by facilitating their evaluation in complex but unaltered *in vivo* settings. Beyond assessment on optimization of targeted drug delivery, NIR MFLI-FRET should also find numerous applications in the measurement of protein-protein interactions or protein conformational changes in intact animals.

Reference

[1] A. Rudkouskaya, N. Sinsuebphon, J. Ward, K. Tubbesing, X. Intes, M. Barroso, Quantitative imaging of receptor-ligand engagement in intact live animals, J. Control. Release 286 (2018) 451–459.

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