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Fluorescence imaging technologies for in situ measurement of drug target engagement and cell signaling pathway reprogramming

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Abstract

Successful cancer treatment continues to elude modern medicine and its arsenal of therapeutic strategies. Therapy resistance is driven by tumor heterogeneity, complex interactions between malignant, microenvironmental and immune cells and cross talk between signaling pathways. Advances in molecular characterization technologies such as next generation sequencing have helped unravel this interaction network and identify therapeutic targets. Tyrosine kinase inhibitors (TKI) are a class of molecularly targeted therapeutics seeking to inhibit signaling pathways critical to sustaining prolifierative signaling, resisting cell death, and the other hallmarks of cancer. While tumors may initially respond to TKI therapy, disease progression is inevitable due to mechanisms of acquired resistance largely involving cellular signaling pathway reprogramming. With the ultimate goal of improved molecularly targeted therapeutic efficacy, our group has developed intracellular paired agent imaging (iPAI) to quantify drug target intereactions and oligonucleotide conjugated antibody (Ab-oligo) cyclic immunofluorescence (cycIF) imaging to characterize perturbed signaling pathways in response to therapy. iPAI uses spectrally distinct, fluorescently labeled targeted and untargeted drug derivatives, which correct for untargeted uptake and facilitate quantitatve in situ assessment of drug target engagement. Ab-oligo cycIF exploits in situ hybridization of complementary oligonucleotides for biomarker labeling while oligo modifications facilitate signal removal for sequential rounds of fluorescent tagging. Ab-oligo cycIF is capable of generating multi-parametric images for quantifying dephosphorylated and phosphorylated protein expression to quantify protein activation, expression, and spatial distribution. Together, iPAI and Ab-oligo cycIF can be applied to interrogate drug uptake and target binding along with changes to heterogenous cell populations within tumors that drive variable therapeutic responses in patients. To date, we have successfully performed ratiometric iPAI on tissue sections after systemic iPAI probe administration to xenograft bearing mice and calculated epidermal growth factor receptor (EGFR) concentration with subsequent Ab-oligo cycIF imaging to measure EGFR signaling, cell viability and state on the same tissue.