Imaging molecular dynamics in vivo: from cell biology to animal models

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Advances in fluorescence microscopy have enabled the study of membrane diffusion, cell migration, and signal transduction at the molecular level in cultured cells. In contrast, imaging in living organisms has primarily been restricted to the localization and dynamics of cells in tissues. Recently, imaging of molecular dynamics has begun to progress from cell culture to sophisticated animal models incorporating fluorescent protein-based biosensors. A major driver of this trend is the understanding that many critical aspects of disease initiation, progression, and response to therapy depend on context specific features of the local microenvironment. Intra-vital imaging also has enormous potential to inform drug target validation and the mechanisms of drug efficacy in vivo. Therefore, in addition to cellular level investigation, it is imperative to develop approaches for molecular level investigation of disease and response to therapy in vivo.

We have demonstrated the first in vivo uses of FRAP and FLIM-FRET at the single cell level to study the dynamics of fluorescent protein-labeled probes. Our approach to in vivo imaging is based on the use of intermediate model systems, such as cell derived matrix and organotypic cultures, for the characterization of probes prior to their use in vivo. We have also made use of non-fluorescent protein signals from second harmonic generation and QuantumDots to provide contextual information about the tumor environment including collagen matrix density and the proximity of cells to local vasculature. We first used FRAP to show that the binding and diffusion of GFP-labeled eCadherin were significantly different in the same cells cultured either on coverslips or subcutaneously in living mice. More importantly, GFP-eCadherin responded differently to anti-invasive treatment with Dasatinib, a clinically approved Src inhibitor, as assessed by FRAP in cells grown subcutaneously. On the basis of this work we have made a mouse in which tissue specific expression of GFP-eCadherin is driven by Cre-Lox technology, and begun using this strain to investigate eCadherin dynamics in mouse cancer models. We subsequently used FLIM-FRET to investigate activation of Rho in an invasive mouse model of pancreatic ductal adenocarcinoma (PDAC) driven by p53. Our results showed that Rho was active at the poles of invasive cells both in vitro and in vivo, and that treatment of subcutaneous tumors with Dasatinib, at a concentration known to inhibit metastasis in this model, led to selective reduction of Rho activity at the cellular poles, rather than globally throughout the cell body. More recently we have used a Src FRET reporter to investigate the spatial and temporal dynamics of Dasatinib treatment in the same murine PDAC model. Our results show that combinational therapy with Cyclopamine, a hedgehog inhibitor which reduces the stromal content of tumors, increases the efficacy of Src targeting by Dasatinib.

Collectively our results present a new paradigm for the molecular level investigation of disease and response to therapy in animal models.