Intravital microscopy of cancer: New insights into the spatiotemporal dynamics of the disease

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Background

Cancer progression and metastasis occur in a complex three-dimensional microenvironment with reciprocal feedback from the surrounding host tissue governing cancer cell behavior. In vitro two-dimensional cancer models combined with immunohistochemistry analysis of patient tissues have been extensively employed to characterize cancer progression. Three-dimensional in vitro platforms have also been designed to mimic the tumor microenvironment and to model the interactions between cancer cells and stromal components (1). However, these static and fixed approaches are unlikely to be appropriate for the comprehensive assessment of events driving cancer, as they do not fully recapitulate the dynamics, heterogeneity, and intricacy of cancer biology. Recent advances in intravital microscopy have offered alternative approaches to study and embrace the complexity of biological events driving cancer development (reviewed in (2–4)). State-of-the-art intravital imaging technologies and genetically engineered fluorescent animal models allow us to monitor live events at the single cell and subcellular level in vivo and represent accurate and reliable tools to analyze the spatiotemporal regulation of key events in cancer progression such as cell proliferation, survival, metastasis, angiogenesis, drug delivery, and chemoresistance. Here, we provide a brief insight into intravital microscopy approaches used to elucidate cancer biology and to improve the discovery of therapeutics in this disease.

Discussion

FRET biosensors to monitor the dynamics of molecular signaling

Förster resonance energy transfer (FRET) biosensors have enabled us to dissect molecular mechanisms in two-dimensional in vitro cultures and have recently been adapted for live imaging in three-dimensional models to dynamically read out key events in cancer. For example, Van Rheenen and colleagues used a caspase-3 FRET biosensor combined with a H2B-Dendra marker to monitor cell apoptosis and mitosis following docetaxel treatment in vivo (5), while spatially localized Akt FRET-biosensors developed by Matsuda and colleagues have improved our understanding of Akt regulation at the subcellular level during cell survival (6). Cell metabolism, which is deregulated in a number of cancers, can also be monitored using FRET biosensors. For instance, subcellular ATP FRET-biosensors are used to visualize intracellular energy transport following inhibition of glycolysis (7), while oxygen FRET-based biosensors can

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monitor intracellular oxygen transport and consumption (8). Wang and colleagues have developed a simultaneous multi-parametric analysis of distinct FRET biosensors including Src, RhoA and FAK and, thereby, demonstrated their interdependent regulation in response to mechanorecipricity, which is known to drive tissue dysplasia and metastasis in a variety of cancers (9, 10). In addition, the development of Raichu Rho GTPase FRET-biosensors has significantly improved the understanding of actomyosin cytoskeletal remodeling, cell proliferation and motility in cancer (11). Analysis of the small Rho GTPase family using Raichu biosensors demonstrated for instance an intercellular gradient of their activity during parenchymal and perivascular invasion in glioblastoma tissue (12). As an extension of this work, genetically modified FRET mice have enabled us to analyze protein activity in live tissues and primary cells without disturbing their biological properties. The Rac-FRET mouse represents a reliable tool to unravel the regulation of Rac1 in various healthy and diseased organs (13). Similarly, the Erk and PAK-FRET biosensor mice have permitted us to untangle the molecular mechanisms governing extracellular signal transduction, proliferation, and differentiation in vivo (14–16). Interestingly, the Erk FRET mouse has recently been used to study Erk activity in distinct subpopulations of breast cancer cells to monitor chemotherapy pharmacokinetics in breast cancer (17). FRET biosensors are, therefore, an emerging preclinical tool for cancer research and help us probe dynamic events in vivo rather than the current in vitro static and fixed endpoints discussed earlier. For a detailed and non-exhaustive list of FRET biosensors and other fluorescent techniques such as FRAP, photo-activation and photo-switching used to image cancer, see (2–4) and representative schematic (Fig. 1).

**Imaging the tumor microenvironment to embrace the complexity of cancer**

Intravital techniques to image stromal components and functional environments have enabled us to visualize the complexity of tissue structures and functions (2–4). Second and Third Harmonic Generation (SHG-THG) imaging have provided insights into tissue texture and organization in relation to cancer development. Correlated imaging of the extracellular matrix (SHG), adipose tissue (THG), stromal and cancer cells has generated a three-dimensional map of tissue tracks and barriers that guide melanoma cell invasion (18) and has identified distinct patterns of cell migration in patient-derived xenograft models (2, 19). Visualization of angiogenesis has also been achieved using quantum dot imaging of tumor vessels. Klemke and colleagues used a transparent zebrafish model to directly visualize the dynamic remodeling of existing blood vessels by cancer cells during angiogenesis and extravasation (20, 21) and to elucidate the molecular mechanisms driving metastasis in breast cancer and melanoma (22). Metastasis is a dynamic process, whose spatiotemporal regulation remains poorly characterized. Optical imaging windows enable continuous and repeated imaging of tissues of interest and have recently provided unprecedented insights into the late stages of metastasis. In particular, Van Rheenen and colleagues used abdominal optical windows to identify a pre-metastatic step during colonization of secondary sites such as liver in colon cancer (23) and to look at normal intestinal stem cell regeneration and competition which, in the future, could give insights into the deregulation of this process in cancer (24). Intravital imaging of the tumor microenvironment, therefore, allows us to integrate the contextual complexity of cancer into our analysis of the disease.
A multi-modal imaging approach to improve drug discovery

Integrating complementary imaging technologies provides a complete and detailed picture of cancer response to preclinical therapy (2, 3, 5). In a multi-modal imaging study, pancreatic adenocarcinoma cell response to the anti-invasive drug dasatinib has been mapped and revealed the benefits of dual targeting in this disease (25). Similarly, intravital imaging has been used to monitor the intracellular pharmacokinetics of PARP-1 inhibitors and of microtubule inhibitors and underlined the heterogeneity of tumor response to chemotherapy (26, 27). These studies demonstrate how intravital microscopy can facilitate therapeutic discovery (2–4).

Future Directions

Intravital microscopy techniques allow us to image the hallmarks of cancer and increase our understanding of the intricacy of the disease (4). We have identified promising areas in which we believe intravital imaging will provide much needed insights.

• Post-intravital imaging analysis of fixed sections through immunohistochemistry (IHC) techniques can be used to study tissue structure and molecular characteristics. Combining...
the advantages of both intravital microscopy and IHC will increase our contextual understanding of tumor behavior in vivo and correlative microscopy approaches are being developed in order to identify and retrace areas post-intravital imaging for further analysis (28).

- Dissecting the dynamic regulation of molecules in live tissue and primary cells has been achieved using new FRET biosensor mouse models (13, 14). Crossing FRET-biosensor mice with genetically modified mouse models that recapitulate cancer biology will allow us to decipher the role of proteins of interest in cancer development and help us identify therapeutic targets in these models to improve the process of drug discovery.
- Genomic instability is known to drive cancer development, and intravital microscopy can support the understanding of genomic deregulation in cancer as suggested by Ellenbroek, et al. (4). Fluorescent reporters detecting various modes of DNA repair have recently been developed and intravital analysis of these biosensors can shed light on the acquisition of cancer-driving mutations (29).
- Mapping of tumor tracks, architecture, and response to drug treatments has been achieved via intravital microscopy and has enabled the identification of chemoresistant cells that may repopulate the tumor (5, 18, 25, 26, 27). We, therefore, envisage that intravital imaging will increase our understanding of the mechanisms of chemoresistance, which remains a key challenge for cancer research.
- Cancer is a highly heterogeneous disease and personalized, patient-derived xenograft approaches have recently been used in preclinical studies. Using intravital imaging in patient-derived cancer models can facilitate the identification of therapeutic targets and the development of patient-specific treatments.

Intravital imaging has significantly increased our understanding of the dynamics of cancer cell biology and has provided a much-needed insight into cancer response to drug treatment. As such, intravital imaging is set to play an important and continuous role in cancer research and will help in the establishment of new anticancer treatments to improve patient outcome in the long term.

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