Cell Stem Cell
Forum

Stem Cells in the Wild: Understanding the World of Stem Cells through Intravital Imaging

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The limitations of canonical imaging techniques have hampered our abilities to study stem cells in their native environment. Here, we discuss some of the exciting ways stem cells are being visualized by intravital imaging, the intriguing discoveries that have resulted from these endeavors, and the future of this stimulating field.

Just as zoologists stress the importance of studying animal behavior in the wild, stem cell biologists have much to gain from studying stem cells in their native environment, the so-called niche. Stem cells and their niche are central for the development and regeneration of all our organs, and their deregulation leads to a disease state. Despite the key relevance of stem cell niches and their conserved features, we still fail to understand the intermediate steps required for stem cells to properly regenerate a tissue, including the critical cellular mechanisms that sustain regeneration as well as the functional interactions with the niche. The major challenge in studying stem cell behaviors is the lack of accessibility to stem cell niches and consequently the inability to visualize the same stem cells over time to address their functional contribution to growth. Thus, while much knowledge has been attained from studying stem cell properties through static analysis, those techniques fail to capture live cells in action and therefore are limited to only the inference of individual stem cell contributions to regeneration.

The development of live imaging has been revolutionary to various fields of study; however, the issue of accessibility is a constant challenge. Cell culture can overcome this hurdle, making virtually all cells accessible in a Petri dish. Much has been learned about biological cellular processes through live imaging in vitro, but this approach is limited by our inability to accurately reproduce the in vivo microenvironment. In contrast, the transparency and easy manipulation of invertebrate model systems such as *Caenorhabditis elegans* and *Drosophila melanogaster* or vertebrates such as zebrafish embryos have proved useful for the study of stem cells in an intact organism, leading to major steps forward in the regeneration field (reviewed in Morrison and Spradling, 2008). The discoveries range from the identification of the relevant cellular mechanisms and cell interactions that drive the development of a fully functional organism from a single cell to an understanding of how cells coordinate their functional contribution during tissue formation, homeostasis, and repair. Despite these scientific advancements in lower model organisms, the study of adult mammalian stem cells in live animals still proves to be a challenge.

Accessing the Mammalian Stem Cell Niche

Major breakthroughs have emerged from utilizing various methods to overcome the challenges of imaging adult stem cell populations in live mammals. In 2007, the Yoshida group established the ability to visualize spermatogonia stem cells by anesthetizing mice for days, pulling the testis out from a small incision, and maintaining it outside the animal in a temperature controlled chamber in order to study the process of differentiation within the spermatogonia stem cell niche. Given that undifferentiated spermatogonia lay at the edges of the organ, visualization was possible using a simple upright fluorescent microscope.

However, many stem cell niches are too deep in the tissue for such resolution. With the development of multiphoton technology, scientists could penetrate tissues much more deeply than before, up to 500 μm in some tissues. For studies requiring more long-term monitoring of cellular dynamics, such as disease progression or fate mapping studies of internal organ regeneration, imaging "windows," which were pioneered by Konrad Messemer and John Condeelis among others, have been adapted by several labs to be surgically installed without tissue dehydration or side effects due to prolonged anesthesia (Kedrin et al., 2008, Ritsma et al., 2014). An advantage of this clever system is that it allows easy revisits of the same region of interest within a tissue. Alternatively, known anatomy, like the blood vessels, can provide additional references for identifying the same regions over time. Much of the work on hematopoietic stem cells has been done in the femur, but the cortical bone is too thick, making the underlying bone marrow inaccessible for intravital imaging. However, the calvarium is a region of the mouse skull that can be exposed with minimally invasive surgery, and it is thin enough to be imaged by both confocal and two-photon microscopy (Figure 1A; work pioneered by Lo Celso, Lin, and Scadden, an extension of which was recently shown in Rashidi et al., 2014). These studies provided, for the first time, the ability to understand how bone marrow stem cells home and behave after irradiation within the niche and how the hematopoietic stem cell-niche interactions are intrinsically different in a pathological state (Rashidi et al., 2014).

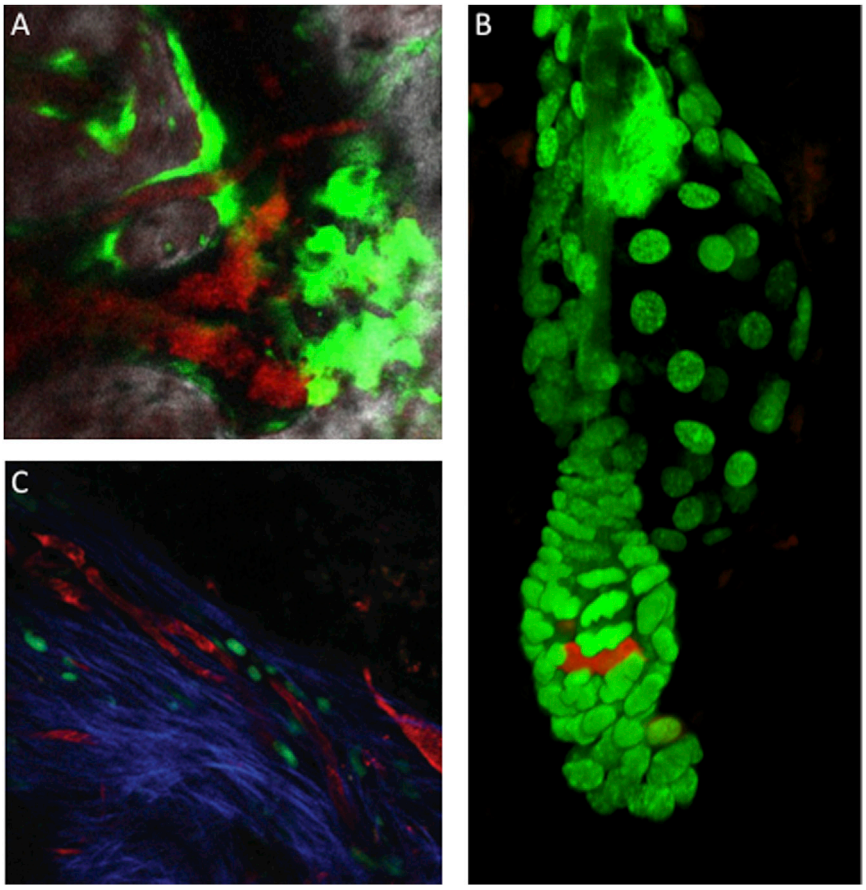

Figure 1. Tracking Stem Cells, Niches, and Disease State by Intravital Live Imaging
(A) Intravital microscopy image of mouse bone marrow, showing components of the stem cell niche such as bone (white, second harmonic generation from 2P excitation), osteoblasts (green, GFP signal from col2.3GFP osteoblast reporter mice, confocal excitation), and vasculature (red, Cy5 dextran, confocal excitation). Image by Edwin Hawkins and Olufolake Akinduro.
(B) Single-cell labeling within a quiescent hair follicle imaged by two-photon microscopy. Green: K14H2BGFP nuclei; red: tdTomato labeling. Image by Samara Brown.
(C) Orthotopic murine glioma (GL261-H2B Dendra2) imaged through a cranial imaging window showing tumor cells migrating along blood vessels. Green: Gl261 nuclei; blue: type i collagen fibers; red: dextran-labeled vasculature. Image by Maria Krasheninnikova.

A unique feature of the skin and its appendages, with respect to other tissues, is its accessibility. Hair follicles and their reservoir of stem cells, for instance, reside just below the skin surface and provide an ideal organ for studying stem cells in their niche in an uninjured live mouse. The extensive molecular and morphological characterization through static analysis allows ready identification of the hair follicle stem cells, the undifferentiated layers, and differentiated layers based on the shape and position of the compartments where they reside. Thus, the hair follicle structure, which has a self-contained organization and predictable cycle of growth and regression, makes these miniorgans an ideal model to study stem cell dynamics in their niche during tissue growth (Figure 1B; Rompolas et al., 2012).

Revealing the Power of the Niche: Novel Understanding through Live Imaging

A cell's niche is highly complex, not only containing many different cell types, but also signals from various sources that are difficult to recapitulate in vitro. Through intravital imaging, however, we have come a long way in making several important advances in the field, from understanding how the position a cell occupies within a niche can affect cell fate decisions during regeneration to capturing behaviors such as cellular migration that are otherwise not possible by static analysis. Thus, it was through pioneering intravital imaging that the Yoshida group revealed the unexpected dynamic behaviors of the spermatogonia GFRα1-positive stem cell pool, which interconvert between single-cell and syncytium states through continuous fragmentation of syncitia of different lengths. Additionally, Hara and coauthors revealed an active migratory behavior of the GFRα1-positive stem cells, which is in contrast to the immotile surrounding spermatogonia support cells known as Sertoli cells (Hara et al., 2014). Alternative imaging approaches include the use of organ culture, which the group led by Erika Matunis developed for studying *Drosophila* testes in culture (reviewed in Morrison and Spradling, 2008). Their results challenged the understanding derived from static analysis of the germline relying exclusively on asymmetric cell divisions and illustrate that a proportion of the germline stem cells also divide symmetrically, giving rise to daughters that follow the same fate.

In the case of hematopoietic stem cells, only intravital imaging into the calvarium could illuminate both the lack of motility and synchronicity in cell proliferation upon bone marrow engraftment but also capture hematopoietic stem cells' acquired migratory behaviors during infection of *Trichinella spiralis* (Rashidi et al., 2014). These dynamic behaviors of stem cells and their niche would be missed in static analysis. Canonical approaches in this field have rightfully invested in the identification of the long-term stem cells for which dissociation of the tissue and Fluorescent Activated Cell Sorting (FACS) was necessary. As a consequence, information about stem cell spatial organization and interactions within the niche are lost. Through intravital imaging, we have a new understanding of the hematopoietic stem cell niche: that it is not merely a rigid framework in which the stem cells reside, but an architecture that is much more dynamic and malleable than previously understood.

By studying the short-term dynamics of the interaction between stem cells and their niche, intravital studies have also revealed invaluable information as to how stem cells behave and why. While the molecular heterogeneity of stem cells in the niche has long been observed in several adult stem cell populations, including

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blood, hair follicles, and intestines, whether this heterogeneity translates to different functional behaviors had been largely elusive. These questions have been extremely difficult to address because of our inability to track the same cells over time within the niche of an intact animal. Instead, thanks to intravital imaging approaches, it has been shown that the stem cell's location within its niche dictates its function and fate, in both the hair follicle and the intestinal crypt niches (Rompolas et al., 2013, Ritsma et al., 2014). These studies have also provided new insight into the surprising degree of plasticity of cell fates provided by the niche. Much of the last several decades of research about hair follicle regeneration have revolved around a pool of stem cells that reside in a region called the bulge. However, recent experiments show that when bulge stem cells are ablated, other epithelial cells that are normally not thought to contribute to hair follicle regeneration can change their fate and move into the empty niche, repopulating it and contributing to hair regeneration (Rompolas et al., 2013). These experiments establish that the bulge hair follicle stem cells are dispensable for hair regeneration. In contrast, their niche, a cluster of mesenchymal cells called the dermal papilla, has been shown to be necessary for hair follicle growth, thus revealing different functional requirements in regeneration for two central components, the stem cells and their niche (Rompolas et al., 2012, 2013).

Illuminating Disease Progression and Therapy

Intravital imaging has also provided important insight into the role of the niche and stem cells in the progression of diseases such as cancer. Through the use of photoswitchable proteins to label subpopulations of cancer cells, it was demonstrated that different niches within the tumor influence different behaviors, such as migration or proliferation. While the heterogeneity of niches within a tumor was already known, its striking implications in disease progression could not be revealed without the long-term revisits made possible by intravital imaging (Figure 1C; Kedrin et al., 2008). It has also been key to capturing the early stages of cancer emergence and revealing the mechanisms utilized by stem cells bearing oncogenic hits to develop tumoral growths. This was demonstrated in the recent study of the ectopic growths that develop when hair follicle stem cells receive a typical oncogenic hit, a stabilized form of a Wnt pathway key effector, beta-catenin. By following a large set of ectopic growths and following their progression over subsequent revisits, Deschene et al. (2014) made the surprising revelation that these developing tumors are made up of both mutant and wild-type cells.

Live imaging has also provided an opportunity for us to track the cellular responses during clinical treatments of disease, such as radiation as a form of cancer therapy. Clinical radiation treatments for leukemia and multiple myeloma have been known to result in the unfortunate side effect of retinal injury. With intravital imaging, the loss of resident microglia in the retina and the delayed engraftment of bone-marrow-derived cells can be tracked within the same individual over an extended period of time, providing great insight into the ramifications of such clinical treatments (Alt et al., 2012).

The Future of Intravital Imaging

As technology makes it easier to access and visualize stem cells in live animals, much remains to be explored with different challenges still to be overcome. Several tissues still lack specific labeling that can unequivocally identify the stem cell pool. For instance, we have gained an extremely powerful understanding of the hierarchical organization of the hematopoietic stem cells through the expression of combination of key surface markers by FACS approaches (through the work of several laboratories including those of Irving Weissman and Sean Morrison; reviewed in Morrison and Spradling, 2008). Yet, we are still limited in our ability to translate this hierarchical organization into the imaging system, as current fluorescent reporters are expressed under the direct control of a single promoter, or at most the overlap of two promoters (one driving the Cre reporter and one driving the Cre-fluorescent reporter). The development of fluorescent reporters that can utilize the overlapping expression domains of different genes will be key to identifying the different functional stem cell pools within the niche and tracking them in vivo in real time. Furthermore, it will enable the field to follow endogenous stem cell populations, such as hematopoietic stem cells, thereby expanding our understanding of tissue physiology in addition to the currently used bone marrow irradiated/grafting models.

The ability to understand how signaling pathways operate and control stem cell function in real time in vivo is still limited. Presently, the largest tool set available to manipulate signaling pathways in the mammalian system is based on the inducible Cre-recombinase system. These mouse lines have the limitation that they induce permanent changes (either constitutively overexpressing a protein or inducing a permanent loss of function) and are variable in their efficiency. Doxycycline-dependent mouse models (TET ON/OFF systems) are more flexible because they offer the reversibility option but are still limited by the inherent variability in promoter expression and the scarce number of promoters available when compared to inducible Cre systems. In the future, moving toward the spatiotemporal control of signaling pathways with light will provide us with high-precision activation of chosen pathways in specific cells, in the way that Miesenböck and Deisseroth creatively established an optogenetics system in the brain (Deisseroth et al., 2006). The spatiotemporal control of different signaling pathways and integration of their activity will be key for the understanding of the signaling mechanisms that control stem cell fate decisions during regeneration.

Understanding the insurgence of diseases such as cancer at the single-cell level is paramount to understanding the way disease really arises from a few mutated cells. Presently as a field, we study cancer utilizing the aforementioned Cre and TET systems, which are canonically driven by promoters that are expressed in large subsets of cells within a tissue. In this regard, live imaging will be key to understanding the early, decisive events that lead to malignancy, compared to what instead are just aberrant behaviors that do not evolve into the disease.

It is the combination of utilizing current approaches while developing new advanced technologies that will ensure a more comprehensive understanding of biological processes. Nevertheless, inspired by the tremendous advances in

several other fields, the field of live imaging has already demonstrated a strong contribution to the basic understanding of fundamental principles of organ regeneration and beyond.

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