Cyto-Tracers™: Novel lentiviral-based molecular imaging tools

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Introduction
Fluorescent protein technology has revolutionized cell biology by permitting visualization of a wide range of molecular events within living cells (1). With the continued expansion of stem cell research and developmental biology, there is greater demand for tools to perform real-time monitoring of protein expression and dynamics during reprogramming and lineage commitment. Currently, most plasmid-based technologies in this class are ineffective due to poor transfection efficiencies and narrow tissue expression capabilities (1,2). To overcome these limitations, System Biosciences (SBI) has created a line of lentiviral-based Cyto-Tracers™ (Figure 1A) for effective transduction and stable expression of fluorescent fusion proteins in any mammalian system, including dividing/nondividing cells or whole-model organisms. Using Cyto-Tracers, cellular structures, such as nuclei and mitochondria, can be visualized in a spatial and temporal manner by fluorescence microscopy. In addition, SBI’s Cyto-Tracers enable researchers to monitor the dynamic movement of a target protein in relation to a given subcellular compartment by fusing the target protein of interest with different fluorescent proteins. In this report, we present the new Cyto-Tracers product line and demonstrate the powerful and unique applications for their use to gain mechanistic insights into cellular processes that otherwise cannot be performed using standard biochemical or immunological assays.

Materials and Methods
Construction of Cyto-Tracers
Each Cyto-Tracer construct encodes a fusion protein consisting of a unique targeting peptide and either a copGFP or RFP protein sequence (Figure 1A). The fused targeting peptide directs the fluorescent protein to the appropriate subcellular location or even into secretory vesicles like exosomes. SBI also offers an untagged vector for creating customized fusion constructs that are not currently available. Making the fusions with a small, monomeric fluorescent protein sequence is key to allow the appropriate targeting of the protein of interest with minimal disruption. We have chosen two bright monomeric fluorescent proteins to include in the Cyto-Tracer lentivectors for this purpose (CopGFP and ruby RFP).

Results and Discussion
How Cyto-Tracers work
The newly synthesized proteins have intrinsic sequences and structures, termed “signal peptides” or “address tags,” which govern their transport and localization in the cell. We have built a collection of Cyto-Tracers that have fusions of specific protein-targeting sequences with fluorescent proteins.
proteins that can highlight various intracellular compartments, organelles, structures (Figure 1B), or extracellular secreted vesicles such as exosomes (Figure 1C). In addition, some of our Cyto-Tracers are able to capture a number of critical biological events with additional natural or artificial sensor sequences, such as BAX-GFP or luciferase circularized with a caspase-3 substrate peptide. The migration of BAX-GFP from cytosol to mitochondria or the activation of the inactive cyclic luciferase upon cell apoptosis allows a real-time monitoring of cell death (3).

Performing the transient transfection experiment with Cyto-Tracers

The Cyto-Tracers plasmid DNA can be transfected into target cells for use in pilot experiments. As shown in Figure 1, A and B, human HEK293 cells were transfected with either intracellular compartment or organelle-directed Cyto-Tracers vector DNA. Following transfection, the Cyto-Tracers expressed fusion-GFP proteins and within 24 h the cellular compartments, organelles, or structures of the transfected HEK293 cells were specifically marked with copGFP fluorescence as directed by the particular fusion expressed.

Performing transduction experiments with Cyto-Tracers

The lentiviral-based Cyto-Tracers are the most effective vehicles for transduction under various conditions, including hard-to-transfect mammalian cell lines, whole-animal models, and stem cells. As shown in Figure 1C, HT1080 cells stably transduced and expressing the CD63-Cyto-Tracer (an exosome marker) specifically highlighted intracellular secretory vesicles and a number of the secreted exosomes can also be observed in the living HT1080 cells. The results are glowing exosome vesicles to allow for tracking studies.

Lighting up pluripotent stem cells with Cyto-Tracers

Pseudotyped lentiviruses are effective tools to transduce embryonic stem (ES) and induced pluripotent stem (iPS) cells. However, expression of transgenes in stem cells varies in a promoter-dependent manner (2). Consistent with previous reports (2), we observed the inactivation of CMV-driven GFP transgenes in human H9 ES cells (Figure 2, left panel). In contrast, murine stem cell virus promoter (MSCV)—driven GFP expresses at high levels in iPS cells (Figure 2, right panel). Therefore, the MSCV promoter is particularly useful for driving Cyto-Tracer transgene expression in stem cells to enable powerful tracking of protein dynamics.

Conclusions

In response to a growing demand for reliable and high-throughput imaging tools, we have developed a lentiviral-Cyto-Tracers platform that can accurately capture various molecular events in living cells. Cyto-Tracer constructs combine the lentiviral delivery system with robust fluorescent fusion protein technologies to enable long-term and in-depth studies in virtually any cell type. For reliable expression in stem cells, our MSCV-driven Cyto-Tracers will meet the expanding need for stem cell researchers. More information on Cyto-Tracers can be found online at www.systembio.com/cyto-tracers, or call SBI at 1 (650) 968-2200.

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