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Review Article

Development of a Human Pluripotency Sensor for Real-Time Cell Imaging and Biomedical Applications

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Abstract

Human Pluripotent Stem Cells (hPSCs) arean essential cell source for regenerative medicine.With the increasing importance of hPSCs for cell-based therapy, the need for hPSCs in basic and clinical research is to develop live-cell imaging systems that monitor hPSCs during reprogramming or differentiation processes.For applying live-cell imaging systems, small fluorescent molecules have been developed as BODIPY-based library compounds by the Diversity-Oriented Fluorescent Library (DOFL) approach.Recently, we identified a new pluripotency sensor (SHI5) from the BODIPY-based library compounds by the DOFL approach. We demonstrated that SHI5 was able to detect live cells that gain the pluripotency in the reprogramming process without any effect on their viability. We also observed the internalization of SHI5 through the clathrin-mediated endocytosis pathway. This article aims to overview of PSC-specific fluorescent probes designed by the DOFL approach, and discusses the potential of cell-specific small fluorescent molecules as a tool for understanding the mechanism of a biological process as well as clinical application.

Keywords: BODIPY; DOFL; Human pluripotency sensor; Real-time imaging

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Introduction

Reprogramming somatic cells into patient-specific human induced Pluripotent Stem Cells (hiPSCs) is considered to be a powerful strategy for regenerative medicine and drug development [1]. Human Pluripotent Stem Cells (hPSCs), including Embryonic Stem Cells (ESCs) and iPSCs, are essential cell sources for regenerative medicine due to their ability to self-renew and to differentiate into many cell types. For application in cell-based therapy and drug screening, hPSCs require additional differentiation step for the desired cells [2]. Although precise isolation of pure hPSCs is inevitable for the treatment of complex diseases and drug development, there still remains no effective way to monitor somatic cells being reprogrammed as stem cells or differentiated into desired cells. Conventional methods of detecting hPSCs use antibodies that recognize stem cell-specific markers [3], or genetically modified cells that express fluorescent proteins by activation promoters of hPSC-specific genes [4,5]. These methods may not be suitable for the clinical approach, even if demonstrated in several studies.

Fluorescent-conjugated small molecules have been utilized as sensors and probes for live-cell imaging [6,7]. The fluorescent probes are highly permeable, sensitive to specific targets, such as molecules and cells, and biologically safe.Recently, for real-time visualization of PSCs, PSC-specific fluorescent molecules have been developed from a Diversity-Oriented Fluorescence Library (DOFL), which consists of structural diversity of fluorescent compounds synthesized by combinatorial chemistry [7-13]. In our previous study, we demonstrated a highly selective to pluripotency sensor (named as selective human pluripotency indicator 5, SHI5) in human PSCs developed by the DOFL approach [13]. This review will discuss the DOFL approach to develop PSC-specific fluorescent molecules and the study of biological processes, as well as the utilization of cell-specific small fluorescent molecules in clinical applications.

Diversity-Oriented Fluorescence Library

Previous studies discussed two main methods for the development of fluorescent compounds for live-cell imaging [14]. The Target-Oriented Approach (TOA) was widely used to develop small fluorescent molecules, such as probes and sensors as one of the methods. The TOA is applied to construct a fluorescent molecule by binding a fluorophore to a target recognition motif through a linker. The target recognition motif is designed based on knowledge of target identity and structure. For this reason, the TOA for fluorescent molecule development is interrupted due to a lack of target information.

In contrast to TOA, the "diversity-driven approach" builds fluorescence libraries developed for unbiased screening. The Diversity-Oriented Fluorescence Library (DOFL) approach is utilized for high-throughput screening of unique sensors and probes that synthesized various fluorescent scaffolds linked with diversity target motifs by combinatorial chemistry. In this section, a summary of the PSC-specific fluorescence molecules developed by the DOFL approach is provided in table 1.

Probe name	Chemical class	Optical wavelength (λex/λem)	Target cell type	Reference
CDy1	Rosamine	535/570	mESC, miPSC, hESC, hiPSC	[8]
CDb8	Xanthone	369/487	mESC	[9]
Cdg4	Chalcone	430/560	mESC	[10]
CDy9	BODIPY	563/578	mESC, miPSC	[11]
KP-1	Rosamine	515/529	hESC, hiPSC	[12]
SHI5	BODIPY	595/656	hESC, hiPSC	[13]

Table 1: Summary of PSC-specific probes from DOFL.

mouse Embryonic Stem Cell, mESC; mouse induced Pluripotent Stem Cell, miPSC; human Embryonic Stem Cell, hESC; human induced Pluripotent Stem Cell, hiPSC

Discovery of a Human Pluripotency Sensor from BODIPY-Based Library via DOFL Approach

For clinical application, hPSCs needs anon-invasive live-cell imaging tool to monitor their pluripotency in the reprogramming process. hPSCs need to be precisely isolated during the differentiation steps of desired cells to provide cells for transplantation or drug test [2]. Previously, the DOFL approach developed PSC-specific fluorescent molecules from various fluorescent molecule libraries for real-time visualization of PSCs [7-13]. Several studies have demonstrated that the modification of these compounds eliminates the risk of tumorigenicity of hPSCs after transplantation [15,16].

Among the fluorescent molecules from the DOFL, hPSC-specific fluorescent molecules that based on BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) scaffolds are relatively rare. BODPY dyes show a wide range of optical properties and are highly stable and permeable fluorescent scaffold [17]. BODIPY-conjugated fluorescent molecules have been commonly used to monitor intracellular organelles, including mitochondria [18], endoplasmic reticulum, Golgi apparatus [19] and lipid droplets [20,21].

In our study, the DOFL compounds synthesized from the BODIPY scaffolds were screened using a high-throughput in vitro screening platform as mentioned in previous studies [22]. Compared to TOA, the DOFL approach was able to perform an unbiased screening by incubating BODIPY-based compounds with hPSCs to develop human pluripotency sensors.

From the BODIPY-based compound library, we discovered SHI5, which is highly selective to hPSCs. Previously, Im and colleagues discovered a mouse and human ESC-specific compound, which was named compound of designation yellow 1 (CDy1) [8]. This rosamine-based probe showed selective detection in the early stage of the reprogramming process and was also suitable for live-cell imaging. Compared with CDy1, SHI5 is strictly selective to hPSCs but not to mESCs, fibroblasts, or differentiated hPSCs [13].

Real-time cell imaging using fluorescence-molecules, compared with conventional methods of detecting PSCs in the reprogramming process, can be used for future application of the labeled cells because they do not require fixationof the PSCs or genetic modification of the parent cells [8]. SHI5 enables rapid, efficient, and selective monitoring of live-cells that gain pluripotency in the reprogramming process without any effect on their viability [13]. The DOFL approach is a powerful tool for developing fluorescent probes to monitor and isolate PSCs in the reprogramming and differentiating processes, but it is noteworthy to understand the mechanism of the selective to PSCs [14].

Several studies have demonstrated specific target molecules of PSC-specific probes [8,12,13]. CDy1 is selectively localized to mitochondria within the mouse and human PSCs [8]. Hirata and colleagues have demonstrated that KP-1 is selective to the mitochondria of hPSCs due to the distinct efflux activity of ABC transporter in hPSCs [12]. SHI5 is rapidly internalized by Clathrin-Mediated Endocytosis (CME) and selectively localized to lipid droplets in hPSCs [13]. In previous studies, genetically modified CME has revealed a relationship between the endocytic activity and cell fate specification mechanism inhESCs [23,24]. Loss of CME by siRNA reduced the activity of alkaline phosphatase, which is a pluripotencymarker, by altering the extracellular signal-regulated kinase signaling in ESCs [24]. Therefore, we assume that the early steps of CME in the cells that gain pluripotency during the reprogramming process may play a crucial role in the internalization of SHI5.

Conclusion

The development of PSC-specific imaging probes makes it possible to isolate and monitor live PSCs during the reprogramming or differentiation processes. For real-time imaging, the small molecules, such as probes or sensors should be biocompatible and permeable without disrupting cellular viability. In this paper, we discussed the efficiency of the DOFL approach to develop a highly selective pluripotency sensor for monitoring the live state of hPSCs. Therefore, the DOFL approach will be utilized for the development of cell fate-specific probes to understand the mechanism of the biological process, as well as to expedite clinical applications.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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