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rBC2LCN, a new probe for live cell imaging of human pluripotent stem cells

Yasuko Onuma [a], Hiroaki Tateno [b], Jun Hirabayashi [b], Yuzuru Ito [a,*], Makoto Asashima [a]

[a] Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 4, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan
[b] Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 2, 1-1-1 Umezono, Tsukuba, Ibaraki 305-8568, Japan

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ABSTRACT

Cell surface biomarkers have been applied to discriminate pluripotent human embryonic stem cells and induced pluripotent stem cells from differentiated cells. Here, we demonstrate that a recombinant lectin probe, rBC2LCN, a new tool for fluorescence-based imaging and flow cytometry analysis of pluripotent stem cells, is an alternative to conventional pluripotent maker antibodies. Live or fixed colonies of both human embryonic stem cells and induced pluripotent stem cells were visualized in culture medium containing fluorescent dye-labeled rBC2LCN. Fluorescent dye-labeled rBC2LCN was also successfully used to separate live pluripotent stem cells from a mixed cell population by flow cytometry.

1. Introduction

Both positive and negative selection of pluripotent stem cells are key steps for practical applications, such as reducing risk of teratoma formation in transplantation therapy, and maintaining a homogeneous pluripotent cell population in continuous culture. Recent studies have focused on cell surface markers, which are specifically expressed on human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), for visualizing and sorting stem cells by flow cytometry or magnetic cell separation. The conventionally used markers are cell surface glycans specific for pluripotent stem cells, and antibodies for those glycans are well established, such as stage-specific embryonic antigens-3, -4, and-5 (SSEA-3, SSEA-4, SSEA-5), and tumor rejection antigens-1–60 and -1–81 (TRA-1–60, TRA-1–81) [1–5]. Several lectin proteins, which bind to unique glycans, are also useful probes for identifying pluripotent stem cells. UEA-I, which is a lectin isolated through array-based glycomic analysis, has high efficiency to detect human pluripotent stem cells [6]. We also have identified that rBC2LCN, which is a recombinant peptide corresponding to the N-terminal domain of BC2L-C protein, specifically detects undifferentiated iPSCs and ESCs in a lectin microarray system [7,8]. rBC2LCN binds specifically to Fucα1–2Galβ1–3GlcNAc (GalNAc)-containing glycans, such as H type1 (Fucα1–2Galβ1–3GlcNAc), H type3 (Fucα1–2Galβ1–3GalNAc), Lewis b (Fucα1–2Galβ1–3(Fucα1–4)GlcNAc) and Globo H (Fucα1–2Galβ1–3GalNAcβ1–3Galα1–4Galβ1–4Glc) (Supplementary Fig. S1) [7,8]. Globo H is expressed on the surface of undifferentiated hESCs [5], and anti-SSEA-5 antibody is reported to bind to H type1 antigen [4]. In addition, we have demonstrated the presence of α-1–2 linked fucose in both N- and O-linked glycans of iPSCs [9].

In this study, we demonstrate the potential of rBC2LCN as a probe for pluripotent ESCs and iPSCs in both imaging and flow cytometry. Fluorescent dye-labeled rBC2LCN stained human ESCs and hiPSCs and exhibited higher sensitivity upon iPSC differentiation, compared to the established cell surface marker antibodies. Fluorescent dye-labeled rBC2LCN was also capable of visualizing and separating live pluripotent hESCs and hiPSCs by flow cytometry. rBC2LCN is a useful tool for evaluating human ESCs and hiPSCs and holds promise for improving cell sorting efficiency in medical and industrial applications of pluripotent stem cells.

2. Materials and methods

2.1. Cell culture

KhES-1 and KhES-3 cells were maintained as previously described [10]. Human ES cell line, H1, was maintained according to WiCell Feeder Independent Pluripotent Stem Cell Protocols provided by the WiCell Research Institute (http://www.wicell.org). Human iPS cell lines, 201B7 [11] and 253G1 [12], were maintained in DMEM-F12 medium (Invitrogen) supplemented with 20% of KSR (Invitrogen), 0.1 mM of 2-mercaptoethanol (Sigma–Aldrich), MEM non-essential amino acids (Invitrogen), and 10 ng/ml of recombinant human basic FGF (Wako) on mitomycin C-treated mouse embryo fibroblast feeder cells. The 201B7 cell line was cultured in mTeSR1 (STEMCELL Technologies) on BD Matrigel hESC-qualified

* Corresponding author. Fax: +81 29 861 2987.
E-mail address: yuzu-itou@aist.go.jp (Y. Ito).

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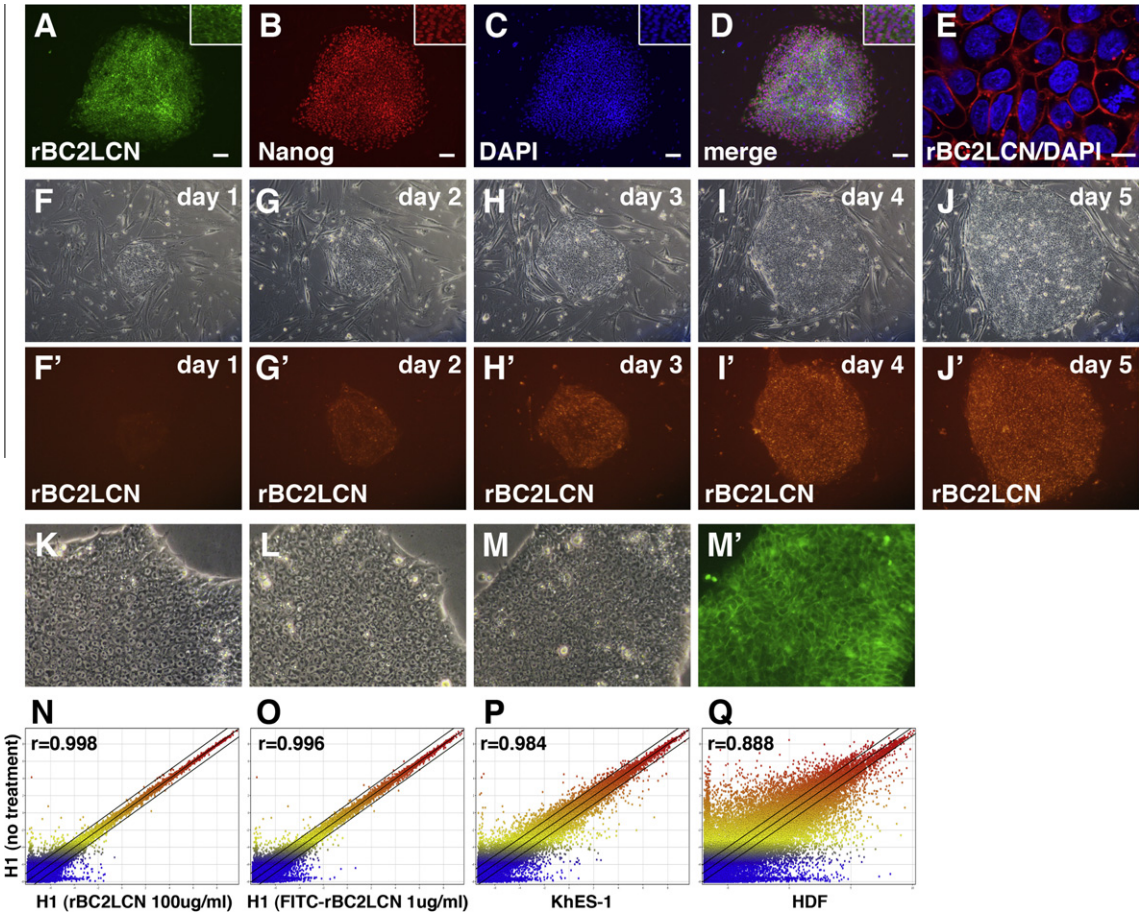

Fig. 1. Fluorescent dye-labeled rBC2LCN directly stains hiPSC colonies. (A–D) A 253G1 iPSC colony stained with FITC-conjugated rBC2LCN (A), anti-Nanog antibody (B) and DAPI (C), and the overlay of A–C images (D). (E) Confocal image of 201B7 cells stained with Cy3-conjugated rBC2LCN and DAPI. rBC2LCN signal can be observed on the cell membrane. (F–J, F′–J′) Chronological live cell imaging of 201B7 iPSC using Cy3-conjugated rBC2LCN in 0.1 μg/mL of final concentration. (K-Q) H1 ESCs were treated with 100 μg/mL rBC2LCN or 1 μg/mL of FITC-rBC2LCN for 3 days. (K–M) Cell morphologies of H1 ESCs without treatment (K), 100 μg/mL of rBC2LCN treatment (L), and 1 μg/mL of FITC-rBC2LCN treatment (M and M′). Treatment with rBC2LCN caused no significant morphological changes. (N–Q) Scatter plot showing $\log_2$ transformed average expression values from gene expression profiles between untreated H1 cells and H1 cells treated with 100 μg/mL of rBC2LCN (N) or 1 μg/mL of FITC-rBC2LCN (O), KhES-1 ESCs (P), or HDF (Q), using arrayed 60 k probe sets. Pearson correlation coefficients (r), using all probes, between untreated and treated cells are indicated. One typical data set of two independent, duplicate examinations is shown. The full array data set was deposited in the GEO databank (GSE42976). Black lines indicate 2-fold up-regulation and down-regulation. Scale bar: 10 μm in (E). 100 μm in others.

matrix (BD) for confocal laser microscopy observation. Normal human adult dermal fibroblast (HDF) cells were cultured in fibroblast basal medium (ATCC) supplemented with fibroblast growth kit-low serum (ATCC).

2.2. Lectin labeling and staining

rBC2LCN protein was prepared according to a previous paper [8]. For fluorescence labeling, rBC2LCN was labeled withCy3-*N*-hydroxysuccinimide (NHS) ester (GE Healthcare), fluorescein-4-isothiocyanate (FITC, Dojindo), and HiLyte Fluor 647 Labeling Kit (Dojindo), respectively. Fixed cell staining was performed as described previously [13]. Briefly, hESCs and hiPSCs were fixed with 4% paraformaldehyde for 10–60 min at 4 °C or room temperature. After rinsing with PBS, cells were incubated with 10 μg/mL of Cy3- or FITC-conjugated rBC2LCN diluted in 1% BSA containing PBS for 1 h at room temperature. The cells were counterstained with DAPI (Dojindo), and then images were collected with a BIOREVO BZ-9000 fluorescence microscope (Keyence) and a FluoView FV1000 confocal laser scanning microscope (Olympus).

Live cell imaging was performed in the regular hESC or iPSC culture medium (with phenol red) by addition of Cy3- or FITC-conjugated rBC2LCN in 0.1–1 μg/mL of final concentration. After 2 h incubation under typical culture conditions, images were collected with an Axiovert 40 CFL inverted microscope (Carl Zeiss Microscopy), without washing. For chronological observations, the same concentration of fluorescent dye-labeled rBC2LCN was added with daily medium changes.

2.3. Immunocytochemistry

Immunocytochemical analysis was performed as described previously [14–16]. Primary antibodies used in this study were the following: anti-SSEA4 (MC-813–70, 1:300 dilution; Millipore), anti-TRA-1–60 (1:300 dilution; Millipore), anti-TRA-1–81 (1:300 dilution; Millipore), anti-Oct-3/4 (1:300 dilution; Santa Cruz Biotechnology), and anti-human-Nanog (1:800 dilution; Cell Signaling Technology). Cells were incubated with a primary antibody diluted in 1% BSA and 5% serum containing PBS at 4 °C overnight. Secondary staining was performed with an appropriate secondary antibody conjugated to Alexa Fluor 488 or Alexa Fluor 594 (1:300; Invitrogen) for 1 h at room temperature. Cells were counterstained with DAPI (Dojindo). Images were collected with a BIOREVO BZ-9000 fluorescence microscope (Keyence).